***CHAPTER (I):***

***BLADDER CANCER***

**Introduction:**

Bladder cancer (BC) is a heterogeneous disease with a variable natural history. It is one of the most prevalent urologic malignancies worldwide ***(Antoni et al., 2017)***. At one end of the spectrum, low-grade tumors have a low progression rate and require initial endoscopic treatment and surveillance but rarely present a threat to the patient. At the other extreme, high-grade tumors have a high malignant potential associated with significant progression and cancer death rates approximately 30% of all newly diagnosed patients present with muscle-invasive BC (MIBC), of them 50% will develop distant metastasis ***(Witjes et al., 2014)****.*

There is a critical need to implement new tests into clinical practice to improve the quality of clinical care, decrease unnecessary invasive therapies and ultimately save costs. BC detection requires unpleasant and expensive cystoscopy and biopsy, which are often accompanied by several adverse effects. Currently, no molecular or genetic biomarker has been widely integrated into daily clinical practice. So, there is an urgent need to develop novel diagnostic methods for initial detection and surveillance in both MIBCs and NMIBCs ***(Santoni et al., 2018)***.

**Incidence and Epidemiology:**

Bladder cancer is the 10th most common cancer worldwide. It is considered the second most common malignancy of the genitourinary tract. For the year 2019, the American Cancer Society estimates 80,470 new bladder cancer cases in the United States, and 17,670 bladder cancer related deaths. It has been demonstrated that urinary bladder cancer accounts for >5% of newly diagnosed tumors in European countries ***(***[***Siegel***](https://onlinelibrary.wiley.com/action/doSearch?ContribAuthorStored=Siegel%2C+Rebecca+L) ***et al., 2019).***

It is the fourth most common cancer in men, occurring less frequently in women. The incidence of urinary bladder cancer is increased in developed countries compared with less developed regions, accounting for 5% of all new cancer cases in the United States ***(Bray et al., 2018).*** BC prevalence increases with age. About 90% of bladder cancer occurs at age 55 years or older; whereas only 1.8% of bladder cancer is developed at age younger than 40 years ***(Ferlay et al., 2018)***.

In Egypt, bladder cancer constituted about 7.2% of all new cancer cases discovered in 2018, and it was reported to be the second most common malignancy in males after hepatocellular carcinoma (HCC). Over the past three decades, a shift from SCC to UC and an increase in the mean age at diagnosis have been reported ***(Ibrahim et al., 2014)***. According to the report of the Global Cancer Observatory in 2018, Egypt has been ranked among the top 20 countries with the highest incidence rates of bladder cancer. Moreover, Egypt has shown the highest mortality rates of bladder cancer along with 3 countries in the Middle East- North Africa region, namely Lebanon, Turkey and Armenia ***(Bray et al., 2018).***

On the other hand, in Egypt a changing pattern of bladder cancer has been observed throughout the years. In the past (1920- 1990), squamous cell carcinoma; the malignancy associated with the blood fluke Schistosoma hematobium, was the predominant bladder tumor in Egypt, due to the high infection rates (70–80%) ***(Khaled, 2013).***

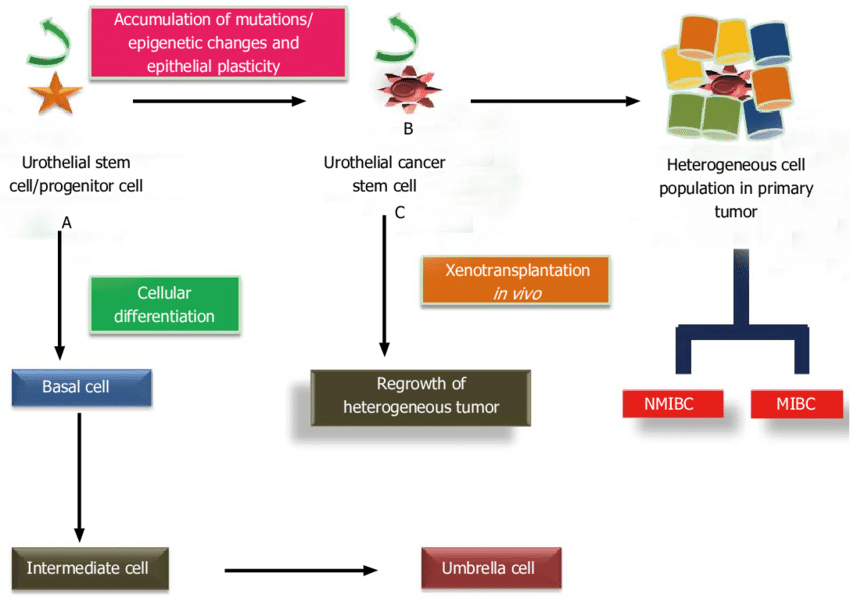
However, recent data suggest that this profile has been dramatically changed over the past 25 years due to introduction of efficient anti bilharzial drugs and public awareness campaigns to eradicate schistosomiasis, which has led to a significant decline in the incidence of squamous cell cancer. Nevertheless, the high prevalence of tobacco smoking in cigarettes, as well as waterpipe smoking have triggered a shift towards UCC, thus minimizing the differences between its features in Egypt and that in western countries ***(Antoni et al., 2017).***

**Molecular Basis of Bladder cancer*:***

The development of bladder carcinoma is postulated to occur via different and complex mechanisms that have not been yet fully elucidated. However, the molecular pathways underlying the two main distinct types of UC, low-grade non-muscle invasive UC and high-grade muscle invasive UC ***(Jebar et al., 2005)*** have been investigated to identify new potential markers for diagnosis, disease monitoring, prognosis and the development of new targeted therapies ***(Castillo-Martin et al., 2010)***.

The most common genetic alteration of BC associated with low-grade and low-stage is deletion of chromosome 9 and an activating mutation of the Fibroblast growth factor receptor 3 (FGFR3) gene ***(Castillo-Martin et al., 2010)***.

As a result of mutational insults, some of the normal urothelial stem cells undergo transformation into a tumor subpopulation known as urothelial cancer stem cells /tumor-initiating cells. These subsets of cells possess the capacity of self-renewal and the ability to generate cellular tumor heterogeneity via differentiation ***(Garg, 2016).*** These cells have been documented to have upregulated levels of various oncogenes and transcription factors to help them sustain the pluripotent properties of stem cells and aggressiveness of tumor invasion (figure 1). ***(Moad et al., 2013; Garg, 2016)***.



**Figure (1):** **Cellular differentiation and mutational transformation of urothelial stem cells and dual pathways of carcinogenesis**. A: Cellular differentiation of UroSCs (present in form of clonal patches in basal layer) generate basal cellsw which further differentiate to intermediate cells and then into single layer of umbrella cells; B: Mutational insults including epithelial plasticity result in malignant transformation of UroSCs into self-renewing UroCSCs which undergo aberrant activation and differentiation to form papillary NMIBCs and MIBCs; C: In vivo xenotransplantation of a small number of UroCSCs that have the potential for the regrowth of heterogeneous tumor cells ***(Garg, 2016)***.

Papillary tumors develop, when the hyperplastic urothelium starts to grow towards the lumen of the bladder, which is triggered by genetic alterations in several proto-oncogenes, including fibroblast growth factor receptor-3 (FGFR3) and Harvey rat sarcoma viral oncogene (HRAS) ***(Castillo-Martin et al., 2010).***

Although such tumors are non-muscle invasive and often associated with a positive prognosis, they tend to have a high recurrence rate ***(Thompson et al., 2015).*** Moreover, 20%-30% of such tumors can progress to more aggressive, invasive and metastatic bladder tumors with an overall survival rate of 5% ***(Garg, 2016)***.

On the other hand, invasive urothelial cancers are thought to emerge from either severe dysplasia or carcinoma in situ (CIS). Often they are a result of inactivation of tumor suppressor genes p53, Retinoblastoma (RB1) and Phosphatase and tensin homolog (PTEN) genes. MIBCs have less favourable prognosis with five-year survival ***(Pandith et al., 2013)***.

**Risk Factors:**

Several factors have different impacts on the incidence and pathophysiology of BC. This phenomenon is called etiologic fraction or attributable risk ***(Burger et al., 2013)***.These factors include:

**1. Smoking:**

Tobacco smoking is the most well-established risk factor for BC, causing 50-65% of male cases and 20-30% of female cases ***(van Osch et al., 2016)***. The incidence of BC is directly related to the duration of smoking and the number of cigarettes smoked per day ***(Freedman et al., 2011)***.

Cigarette smokers have a 2- to 4-fold increased risk of bladder cancer compared to non-smokers ***(Kirkali et al., 2005)***, and the risk increases with increasing intensity and/or duration of smoking. Upon cessation of cigarette smoking, the risk of bladder cancer falls >30% after 1–4 y and >60% after 25 y but never returns to the level of risk of non-smokers ***(Colombel et al., 2008)***.

Tobacco smoke contains aromatic amines, such as-naphthylamine, and polycyclic aromatic hydrocarbons known to cause BC. These are renally excreted and exert a carcinogenic effect on the entire urinary system ***(Burger et al., 2013)***.

**2. Occupational risk:**

Occupational exposure to urothelial carcinogens is the second most important risk factor, accounting for 5–20% of all bladder cancers ***(Kogevinas et al., 2003)***.

The most important risk factor for developing BC after smoking is exposure to aromatic amines. Current or historical exposure to aromatic amines (e.g., benzidine, 2-naphthylamine and 4-aminobiphenyl) have all been associated with the development of bladder cancer. These compounds are found in dyes, chemical compounds, rubber, hair coloring dyes, cigarette smoke, plastics, vehicle smoke, paint products, and fungicides ***(Letašiová et al., 2012).*** An increased risk of non-smoking BC in individuals exposed to aromatic amines, including people working in different industries such as rubber and leather producers, weavers, colored product workers, and printing companies ***(Carreón et al., 2010)***.

The chemicals involved have contributed minimally to the current incidence of BC in Western countries because of strict regulations. Importantly, in recent years, the extent and pattern of occupational exposure have changed because awareness has prompted safety measures, and population-based studies established the occupational attribution for BC in men to be 7.1%, while no such attribution was discernible for women ***(Burger et al., 2013)***.

**3. Medical conditions:**

Medical conditions may predispose individuals to bladder tumorigenesis through direct causation or as a side effect of treatment. Examples of direct causative roles include (a) chronic urinary retention and upper tract dilation increasing urothelial exposure to carcinogens and (b) carcinogenesis associated with chronic inflammation or schistosomiasis ***(Burger et al., 2013)***. Squamous cell carcinoma of the urinary bladder has been known for many years to be associated with Schistosoma haematobium infection ***(Kirkali et al., 2005)***.

With regard to treatment, BC may arise as a consequence of exposure to ionizing radiation and pharmaceutical agents ***(Burger et al., 2013)***. ***Abern et al. (2013)*** found an increased age-standardized incidence rate of BC following external-beam radiotherapy for prostate cancer.

Two pharmacologic agents have also been related to BC; cyclophosphamide is an alkylating agent mainly applied in lymphoma and leukemia, and a long-term use increases BC incidence ***(Burger et al., 2013)***. Pioglitazone, an antidiabetic drug of the thiazolidinedione class, has been found to have a weak relation to BC incidence with longer-term use ***(MacKenzie et al., 2011)***.

For diabetes mellitus, an increased BC incidence has been reported, which was greater with longer duration and use of oral hypoglycemic medication ***(Lewis et al., 2011)***.

**4. Dietary factors:**

Lack of adequate fluid intake, especially water, increases the risk of BC. Bladder vacancy seems to cause chemical accumulation due to delay in the removal of bladder waste, leading to increased risk of BC ***(Ros et al., 2011).***

Vitamin D is one of the complementary ingredients which may reduce cancer incidence. The risk of BC in subjects receiving vitamin D and those exposed to ultraviolet was significantly lower than in control group ***(Brinkman et al., 2010).***

Although it has been suggested that coffee consumption and artificial sweeteners may be associated with an increased risk of bladder cancer, results from epidemiologic studies investigating these agents have been inconclusive ***(Colombel et al., 2008)***. A major problem in evaluating the independent effect of coffee consumption on the development of bladder cancer is its relationship to cigarette smoking ***(Murta-Nascimento et al., 2007)***.

**5. Genetic susceptibility:**

People with family members having BC are at greater risk for BC since BC risk is twice as high in first degree relatives than in other. Inherited genetic factors, such as the genetic slow acetylator N-acetyltransferase 2 (NAT2) variants make it difficult to decompose some of the toxins in the body and thus lead to BC. However, factors such as slow acetylation may not intrinsically lead to BC but may confer additional risk to exposure of carcinogens such as tobacco products ***(Burger et al., 2013)***.

**6. Age, sex, ethnicity, race, and socio-economic status:**

BC risk increases in the elderly; in fact, 90% of BC cases occur in people over the age of 55 while the average age for BC diagnosis is 73 ***(Erlich and Zlotta, 2016).***

The incidence of BC in men is higher than in women; some studies have reported the incidence in men to be 4 times higher. However, mortality rates in women are higher than in men ***(Ranasinghe et al., 2012).*** It seems that one main reason for the lower incidence of BC in women is the lower prevalence of smoking and lower occupational risk in women ***(Burger et al., 2013).*** The reasons for the higher mortality rate in women, however, remains unclear.

The risk of BC is two times higher in Whites than in African Americans. In the United States, the age-standardized rates for African Americans are 13 per 100,000 and for Whites are 22 per 100,000 ***(Yee et al., 2011).***

There is a relationship between low socio-economic status and the unsustainable survival of BC ***(Koroukian et al., 2010).*** It seems that lack of access to healthcare and health services, as well as the increased risk of smoking in groups with a low socioeconomic status, account for the connection.

**Pathology:**

**1. Major pathologic subtypes**:

Transitional cell carcinoma (TCC) is the most common primary pathologic subtype of bladder cancer and is observed in >90% of tumors. Squamous cell carcinoma and adenocarcinoma are less common and occur in approximately 5% and 1% of bladder cancers, respectively ***(Colombel et al., 2008)***.

The predominant types include urothelial carcinoma, formerly known as transitional cell carcinoma, which develops in the innermost cells, and squamous cell carcinoma (SCC), which occurs in thin, flat cells forming after long-term infection or irritation.

UC is presented in male more than female cases and occurs at older ages (≥60 y) than SCC. It accounts for more than 90% of bladder malignancies in industrialized countries but for <80% of cases in Southeast Asia and <50% in some areas of Africa. SCC constitutes up to 75% of cases in some Middle Eastern and African countries ***(Felix et al., 2008).***

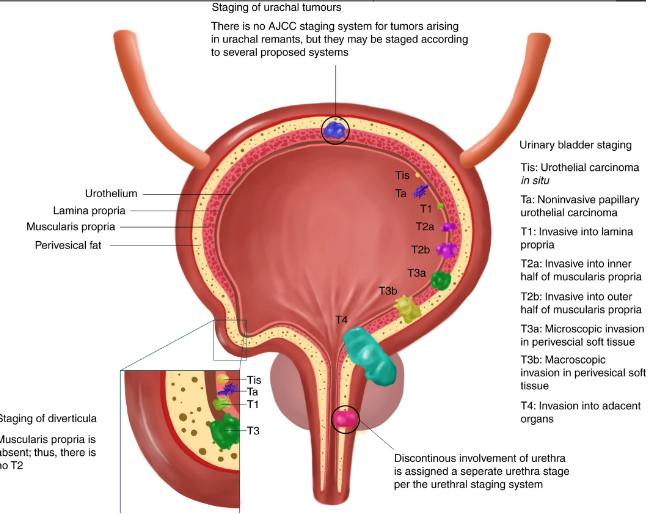
At the time of diagnosis, approximately 75 % of the patients present with non-muscle-invasive bladder cancer (NMIBC), which is defined as disease confined to the mucosa (stage Ta and CIS) or submucosa (stage T1). Although these three types of lesions indeed are non-muscle-invasive, T1 and CIS lesions are distinct from Ta lesions since they have a high potential to become invasive (≈50 % of CIS lesions progress if left untreated). Thus, accurate histopathologic assessment and diagnosis is crucial for correct clinical management which differs drastically between MIBC and NMIBC ***(Bray et al., 2018)***.

**2. Staging:**

The most widely used and universally accepted staging system is the 2009 tumor-node-metastases (TNM) system approved by the Union for International Cancer Control (UICC), which was updated in 2017 but no change in relation to bladder tumors, shown in (table 1) (figure 2) ***(Magers et al., 2019).***

**Table (1):** 2009 TNM classification of urinary bladder cancer: from The American Joint Committee on Cancer (AJCC) Staging System (8th edition, 2017):

|  |  |  |
| --- | --- | --- |
| T - Primary Tumor | | |
| Tx | | Primary tumor cannot be assessed |
| T0 | | No evidence of primary tumor |
| Ta | | Non-invasive papillary carcinoma |
| Tis | | Carcinoma in situ: “flat tumor” |
| T1 | | Tumor invades subepithelial connective tissue |
| T2 | | Tumor invades 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, or presacral) | |
| N2 | Metastasis in multiple lymph nodes in the true pelvis (hypogastric, obturator, external iliac, or presacral) | |
| N3 | Metastasis in common iliac lymph node(s) | |
| M – Distant Metastasis | | |
| M0 | No distant metastasis | |
| M1 | Distant metastasis | |

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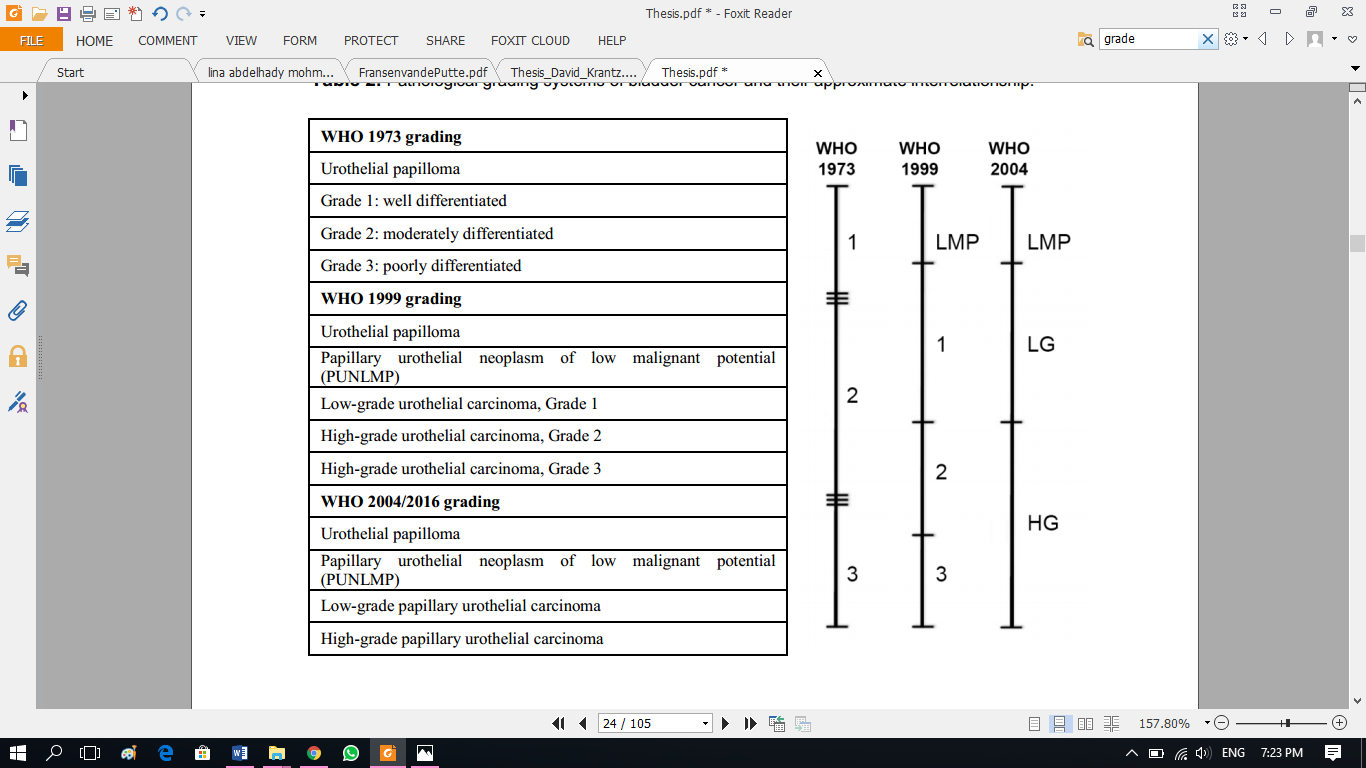
**Figure (2): Overview of staging of tumors arising from the urinary bladder, diverticulum and urachal remnants *(Magers et al., 2019).***

It should be stressed, that there are major ongoing efforts to refine the subgrouping of patients in order to better predict the most suitable treatment for a given patient. In this pursuit, five new distinct MIBC subtypes (luminal-papillary, luminal infiltrated, luminal, basal/squamous, and neuronal) have been identified. These subtypes do not necessarily reflect the histopathological appearance of BC but are rather associated with specific pathway alterations and other biological features ***(Magers et al., 2019).***

**3-Grading:**

The tumor is also given a histologic grade based on the degree of cellular atypia, growth pattern, and mitotic activity. The heterogeneous behavior of non-muscle invasive bladder tumors is main reason for the importance of grading in clinical decision-making ***(Compérat et al., 2018).***

The most frequently used grading system is the WHO/ISUP 2004/2016 (International Society of Urological Pathology) grading system first proposed in 1998 and updated in 2004 and 2016. The WHO/ISUP system introduced the papillary urothelial neoplasm of low malignant potential (PUNLMP) category also used in the WHO 1999 system, but differs from the 1973 and 1999 systems by separating tumors into only two categories; low grade (LG) and high grade (HG), with “high grade” largely equating to those of grade 2 and 3 in the WHO 1999 system ***(Soukupet al., 2017)***.

**Table (2):** World Health Organization (WHO) grading of urinary tumors in 1973 and 2004***.***

|  |
| --- |
| ***WHO 1973 grading*** |
| Urothelial papilloma |
| Grade 1: (G1) Well differentiated |
| Grade 2: (G2) Moderately differentiated |
| Grade 3: (G3) Poorly differentiated |
| ***WHO 1999 grading*** |
| Urothelial papilloma |
| Papillary urothelial neoplasm of low malignant potential (PUNLMP) |
| Low-grade urothelial carcinoma, Grade 1 |
| High-grade urothelial carcinoma, Grade 2 |
| ***WHO 2004/2016 grading*** |
| Urothelial papilloma |
| Papillary urothelial neoplasm of low malignant potential (PUNLMP) |
| Low-grade papillary urothelial carcinoma |
| High-grade papillary urothelial carcinoma |

**Diagnosis of Urinary bladder cancer**

1. **Clinical picture:**

Bladder cancer commonly presents with intermittent or persistent microscopic or macroscopic haematuria; rates may be as high as 78.3% in patients with macroscopic haematuria and 13.7% in patients with  
microscopic haematuria ***(Ramirez et al., 2016).*** Macroscopic haematuria in bladder cancer patients is associated with advanced pathological stage. Unfortunately, many patients with microscopic haematuria suffer from inadequate evaluation especially in the absence of active screening for bladder cancer ***(Elias et al., 2010).***

The symptom complex of bladder irritability and urinary  
frequency, urgency and dysuria is the second most common presentation  
of bladder cancer and is usually associated with diffuse CIS or invasive bladder cancer ***(Kirkali et al., 2005)***.

Other symptoms and signs of bladder cancer include flank pain  
caused by ureteral obstruction, lower extremity edema and a palpable  
pelvic mass. Very rarely, patients present with symptoms of advanced  
disease, such as weight loss and abdominal or bone pain from distant  
metastases. However, these symptoms almost never occur without  
microscopic or macroscopic hematuria ***(Kirkali et al., 2005)***.

1. **Investigations:**

***A. Urine Cytology (UC):***

In which exfoliated cells of the urothelium are extracted and microscopically examined shows a high diagnostic specificity for BC cells (90–100%). In G3 tumors, UC promotes a high sensitivity whereas only low sensitivity in G1 lesions is shown ***(Turco et al.,***[***2011***](https://08102dwbg-1105-y-https-link-springer-com.mplbci.ekb.eg/referenceworkentry/10.1007/978-3-319-42623-5_15#CR25)***).*** Therefore, UC should be utilized with cystoscopy in high-risk tumors of the bladder, since positive UC can indicate urothelial tumors anywhere in the urinary tract. Nevertheless, negative UC does not exclude the presence of malignancy of the bladder. Accuracy of UC is limited by examiner’s experience and can be impeded by local urinary infections, nephrolithiasis, and intravesical instillation therapy ***(Brimo et al., 2009).***

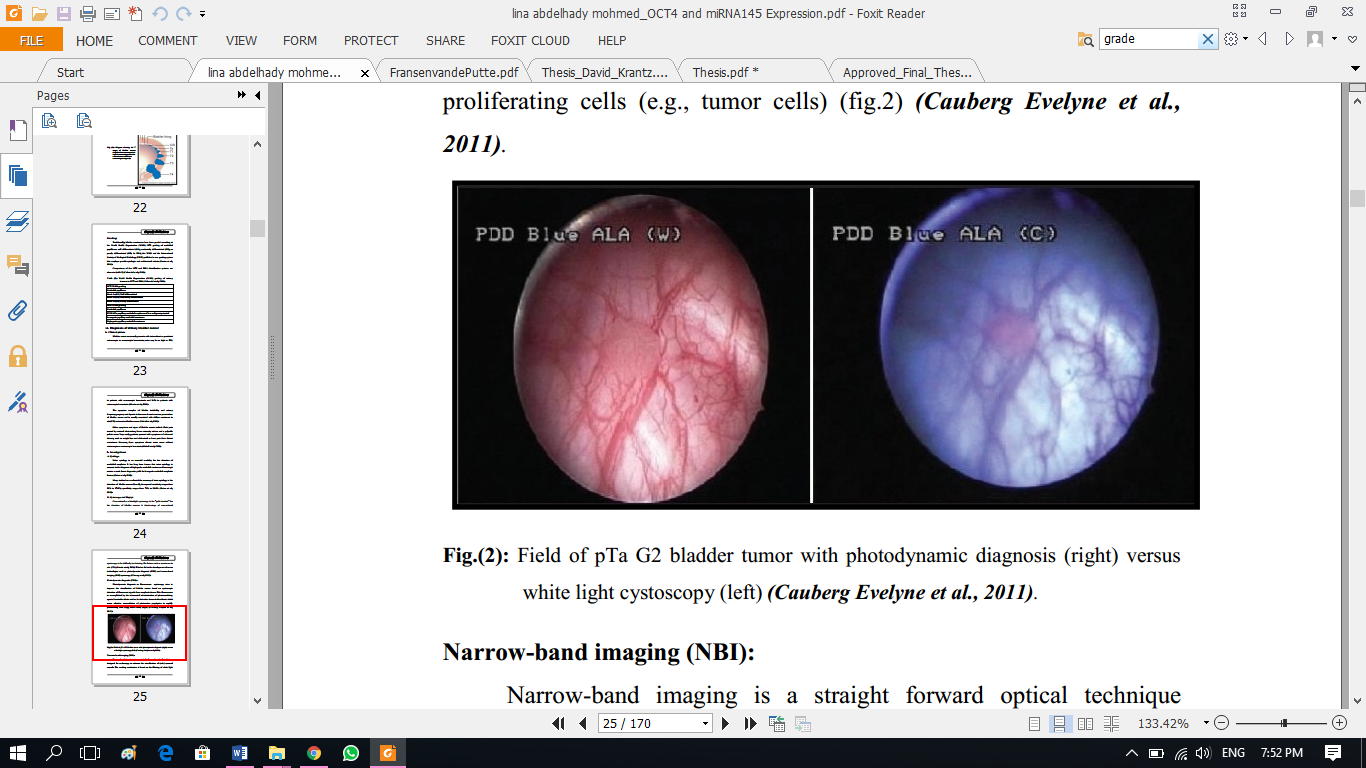
In order to improve sensitivity of UC, numerous different urinary marker tests were developed. Different marker systems such as NMP22, ImmunoCyt/uCyt+, BTA stat, BTA TRAK, cytokeratins, and FISH (UroVysion) have been admitted by the US Food and Drug Administration (FDA) ***(Tritschler and Scharf,***[***2007***](https://08102dwbg-1105-y-https-link-springer-com.mplbci.ekb.eg/referenceworkentry/10.1007/978-3-319-42623-5_15#CR23)***).***

***B. Cystoscopy and biopsy: the rigid or flexible******white light cystoscopy (WLC)*** presents the diagnostic gold standard for NMIBC and MIBC. Sensitivity and specificity in WLC in detection varies between 6–84% (sensitivity) and 43–98% (specificity) ***(Jocham et al.***[***2008***](https://08102dwbg-1105-y-https-link-springer-com.mplbci.ekb.eg/referenceworkentry/10.1007/978-3-319-42623-5_15#CR11)***)***. If the presence of a BC is evident, a transurethral resection of the tumor (TURB) is mandatory. The procedure of TURB is the basis for both: histopathological diagnosis and the complete resection of the lesion. TURB should therefore be performed systematically and in individual steps ***(Babjuk et al.***[***2015***](https://08102dwbg-1105-y-https-link-springer-com.mplbci.ekb.eg/referenceworkentry/10.1007/978-3-319-42623-5_15#CR1)***).***

A disadvantage of conventional cystoscopy is the difficulty in detecting flat lesions such as carcinoma in situ (CIS) ***(Jacobs et al., 2010)***. This has led to the development of newer technologies such as photodynamic diagnosis (PDD) and narrow-band imaging (NBI) cystoscopy ***(Cheung et al., 2013)***.

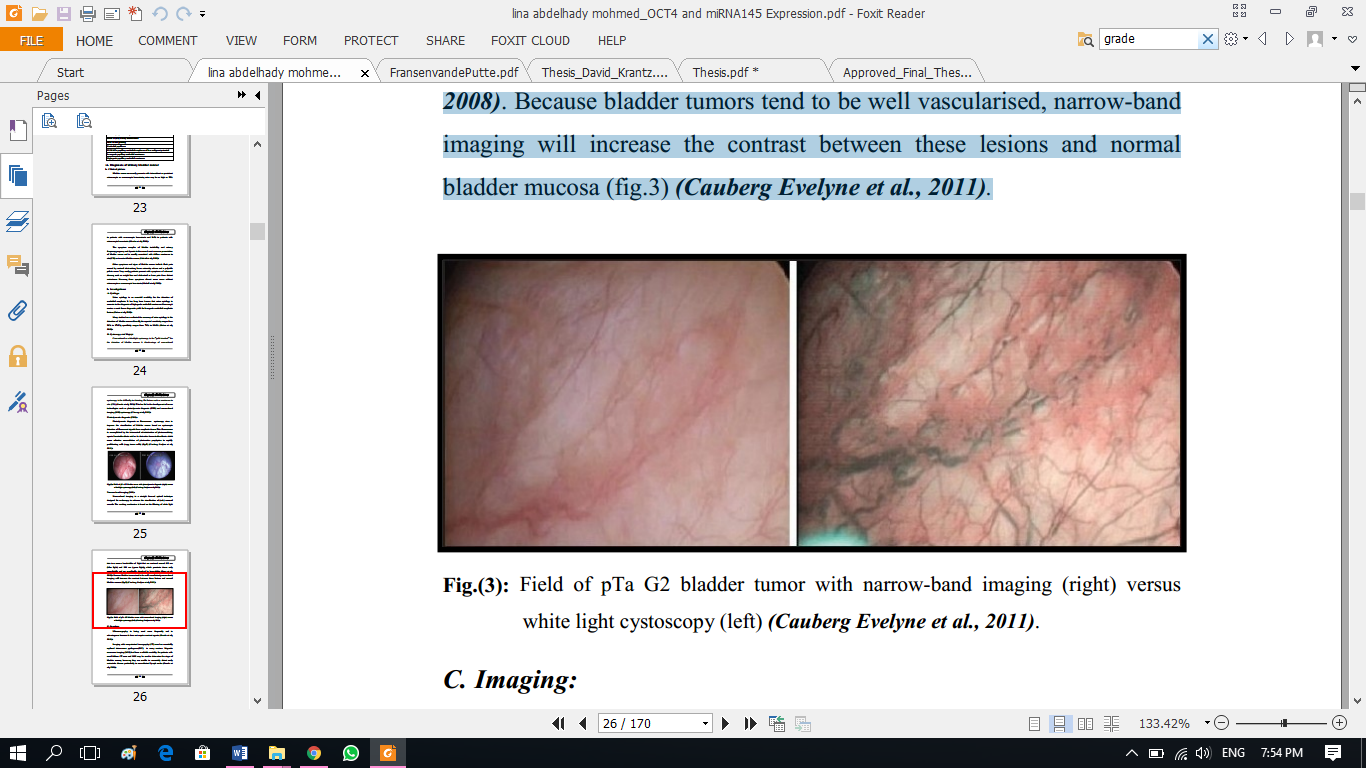
***-******Fluorescence cystoscopy (photodynamic diagnosis (PDD):*** Photodynamic diagnosis or fluorescence cystoscopy aims to  
improve the visualization of bladder cancer based on cystoscopic  
detection of fluorescent signals from neoplastic tissue. This fluorescence  
is accomplished by the intravesical administration of photosensitizing  
agents 5-aminolevulenic acid or its derivative hexaminolevulinate which  
cause selective accumulation of photoactive porphyrins in rapidly  
proliferating cells (e.g., tumor cells) (figure 3) ***(Cauberg et al.,***  
***2011).***

PDD has a significant higher detection rate compared to white light. The detection rate for CIS lesions is considered to be up to 40% higher using PDD. Therefore, PDD in the initial TURB is highly recommended. Additionally PDD should be performed in patients with multifocal tumors, high-grade tumors in patient’s history, and suspected CIS ***(Babjuk et al.***[***2015***](https://08102dwbg-1105-y-https-link-springer-com.mplbci.ekb.eg/referenceworkentry/10.1007/978-3-319-42623-5_15#CR1)***).***



**Figure (3):** Field of pTa G2 bladder tumor with photodynamic diagnosis (right) versus white light cystoscopy (left) ***(Cauberg Evelyne et al., 2011)****.*

***- Narrow-band imaging (NBI):***is a straight forward optical technique  
designed for endoscopy to enhance the visualization of (sub) mucosal  
vessels. The working mechanism is based on the filtering of white light into two narrow bandwidths of light that are centered around 415 nm  
(blue light) and 540 nm (green light), which penetrate tissue only  
superficially and are specifically absorbed by hemoglobin ***(Song et al.,***  
***2008)***. Because bladder tumors tend to be well vascularised, narrow-band  
imaging will increase the contrast between these lesions and normal  
bladder mucosa (figure 4) ***(******Cauberg Evelyne et al., 2011)****.*



**Figure (4):** Field of pTa G2 bladder tumor with narrow-band imaging (right) versus white light cystoscopy (left) ***(Cauberg Evelyne et al., 2011).***

***C. Imaging:*** Ultrasonography is being used more frequently and is  
advantageous because it does not require contrast agents. Imaging with computerised tomography (CT) scan has essentially  
replaced intravenous pyelograms (IVP) in many centers. Magnetic  
resonance imaging (MRI) had been a reliable modality for patients with  
renal failure. CT scan and MRI may be used to determine the stage of  
bladder cancer; however, they are unable to accurately detect early  
metastatic disease particularly in normal-sized lymph nodes ***(Jacobs et***  
***al., 2010)***.

***D. Molecular markers:*** since the metabolic environment of bladder tumor cells is urine, their direct or induced metabolites will remain in the urine. Some of the metabolites such as telomerase, nuclear matrix protein and bladder tumor antigen have been identified as biomarkers of bladder tumor ***(Duquesne et al., 2017)***. This would provide a new way for the painless and noninvasive diagnosis of bladder cancer ***(Siravegna et al., 2017).***  Numerous urinary markers have been investigated, with the aim of reducing frequency of cystoscopy ***(Yutkin et al., 2010*)**. Several are commercially available, but none has been adopted into routine standard of care, owing to poor sensitivity and/or expense. These markers may serve as an adjunctive diagnostic test in cases where urine cytology is equivocal ***(Cheung et al., 2013)***.

1. **Fluorescence in situ hybridization(FISH):**

FISH can be used to detect urinary cells that have chromosomal  
abnormalities consistent with a diagnosis of bladder cancer. For example,  
The UroVysion Bladder Cancer Kit (UroVysion Kit) uses fluorescently  
labeled DNA probes to detect aneuploidy in chromosomes 3, 7, and 17  
and loss of the 9 p21 locus of the P16 tumor suppressor gene ***(Cheung et***  
***al., 2013)***.

1. **Microsatellite analysis (MSA)**

Microsatellites are highly polymorphic, short, tandem DNA repeats  
found in the human genome. Two types of microsatellite alterations can  
be found in many cancers: loss of heterozygosity (LOH), an allelelic  
deletion, and somatic alteration of microsatellite repeat length ***(Vrooman***  
***and Witjes, 2008)***. In bladder cancer, most mutations are in the form of  
LOH ***(Turyn et al., 2006)***. Microsatellite alterations in exfoliated urine  
are detected by a polymerase chain reaction (PCR) using DNA primers  
for a panel of known microsatellite markers ***(Vrooman and Witjes, 2008)***.

1. **ImmunoCytTM**

Immunocytology is based on the visualisation of tumour associated  
antigens in urothelial carcinoma cells using monoclonal antibodies. Three  
fluorescently marked antibodies label two mucin like proteins and a high molecular weight form of carcinoembryonic antigen. After this process  
the cells are examined under a fluorescent microscope ***(Vrooman and***  
***Witjes, 2008)***

1. **Telomerase:**

Telomeres are repetitious sequences at the end of chromosomes  
that protect genetic stability during DNA replication. There is loss of  
telomeres during each cell division, which causes chromosomal  
instability and cellular senescence. Bladder cancer cells express  
telomerase, an enzyme that regenerates telomeres at the end of each DNA  
replication and therefore sets the cellular clock to immortality.  
Determination of telomerase activity is a PCR-based technology and must  
be performed in specialized laboratories ***(Vrooman and Witjes, 2008)***.

1. **Bladder tumor antigen (BTA) BTA-TRAKTM and BTA-statTM:**

BTA-TRAK and BTA-stat (Alidex Inc, Redmond, WA, USA) are  
both versions of the bladder tumour antigen assay that measures  
complement factor H–related protein in urine. BTA-stat is an  
immunoassay that can be performed ‘‘on bench’’ within several minutes.  
BTA-TRAK is a quantitative test that is performed in a laboratory  
***(Vrooman and Witjes, 2008)***.

1. **Hyaluronic acid and hyaluronidase**

Hyaluronic acid (HA) is a glycosaminoglycan and a normal  
component of tissue matrices and body fluids. In tumour tissues, elevated  
HA is mostly localised to tumour stroma. In bladder carcinoma HA is  
found in tumour cells, and elevated HA levels have been shown in  
urinary samples of bladder cancer patients ***(Lokeshwar et al., 2005)***. The  
concentration of HA is also associated with tumour metastases  
***(Lokeshwar et al., 2001)****.* Hyaluronidase (HA-ase) is an enzyme that cleaves HA into fragments. HA-ase levels are elevated in bladder tumour  
tissue, and an increase is correlated with tumour grade ***(Vrooman and***  
***Witjes , 2008)***.

1. **Nuclear matrix protein 22(NMP22):**

NMP22 is a nuclear matrix protein and is an important regulator of  
mitosis. In tumour cells the nuclear mitotic apparatus is elevated and  
NMP22 is released from cells in detectable levels ***(Vrooman and Witjes,***  
***2008)***.

1. **Cytokeratins:**

Cytokeratins are intermediate filaments; their main function is to  
enable cells to withstand mechanical stress. In humans 20 different  
cytokeratin isotypes have been identified. Cytokeratins 8, 18, 19, and 20  
have been associated with bladder cancer. The Urinary Bladder Cancer  
test detects cytokeratin 8 and 18 fragments in urine ***(Vrooman and***  
***Witjes, 2008)***.

Cytokeratin 19 fragment (CYFRA 21-1) is a soluble fragment of  
cytokeratin 19, is analysed with Enzyme-linked immunosorbent assay  
(ELISA), and is measurable in serum and urine ***(Pariente et al., 2000)***.

1. **Survivin:**

Survivin is a member of the family of proteins that regulate cell  
death, the so-called inhibitor of apoptosis family. Its overexpression  
inhibits extrinsic and intrinsic pathways of apoptosis ***(Moussa et al.,***  
***2006)***. Survivin is expressed during foetal development but not in  
terminally differentiated adult tissues ***(Dabrowski et al., 2004)***.  
However, it is one of the most commonly overexpressed genes in cancer  
***(Black et al., 2006)***. In bladder cancer, survivin is expressed in urine, and  
its expression is associated with disease recurrence, stage, progression and mortality ***(Shariat et al., 2007)***. Reverse transcription polymerase  
chain reaction (RT-PCR) provides a diagnostic tool to detect survivin  
messenger RNA (mRNA) in urine ***(Vrooman and Witjes, 2008)***.

1. **microRNA:**

microRNAs are receiving growing attention because of numerous reports on their dysregulation in human diseases and their potential as diagnostic and therapeutic targets. Because of their stability and presence in almost all body fluids, miRNAs constitute a novel class of noninvasive biomarkers ***(Brase et al., 2010)****.*

Some miRNAs have been reported to be up-regulated in bladder cancer tissues. For example, miR-129 was the most commonly upregulated and its up-regulation was associated with poor outcome **(*Dyrskjøt et al., 2009)***; the expression of miR-96 and miR-183 in urine were significantly correlated with tumor stage and grade, and their expressions were significantly decreased after radical surgery ***(Yamada et al., 2011)***; miR-133b and miR-518c were also strongly up-regulated in bladder cancer tissues **(*Dyrskjøt et al., 2009)***.

Meanwhile, some miRNAs were reported to be downregulated in  
cancer tissues and might function as tumor suppressors. miR-200 family  
members were lower in urine sediment of bladder cancer patients and  
increased significantly following surgery which suggested this  
microRNA family could be used as diagnostic and prognostic markers of  
bladder cancer ***(Wang et al., 2012)***. These aberrant expression miRNAs  
in bladder cancer are attractive as potential biomarkers and new targets  
for bladder cancer therapy ***(Feng et al., 2014)***.

***E. Metastatic work-up:*** For invasive bladder tumors, metastatic evaluation should include chest radiography, liver function tests, and alkaline phosphatase. Abdominal and pelvic imaging (MRI or CT) is not accurate for staging of the primary bladder tumor but may be useful when metastatic disease is suspected. A bone scan is unnecessary in all cases, but it should be performed in the presence of bone pain or elevated alkaline phosphatase ***(Kirkali et al.,*** ***2005***).

Briefly, the diagnostic work-up includes, but is not limited to, physical examination, imaging and transurethral resection of the bladder tumor (TURBT) with subsequent histological evaluation. In patients with confirmed MIBC, Computer tomography (CT) of the chest, abdomen and pelvis is currently used for staging ***(Bray et al., 2018)***.

**Treatment of bladder cancer:**

Because of the unpredictable disease course, high recurrence rate, and risk of progression, patients with bladder cancer require continuous, costly, follow-up monitoring which poses a burden both to the patient and the healthcare system. This makes bladder cancer one of the most expensive malignancies per patient ***(Yeung et al., 2014***).

The tumor stage is closely related to patient outcome and is the crucial factor for treatment selection. For patients with stage Ta, Tis, and T1 tumors the preferred treatment choice is local resection of the tumor, paired most commonly with intravesical immunotherapy in the form of Bacillus Calmette-Guerin (BCG) instillations or intravesical cytostatic chemotherapy using mitomycin C, a DNA crosslinking drug, or DNA intercalating agents such as epirubicin or pirarubicin ***(Svatek et al., 2014***). Intavesical therapy is particularly well suited for NMI bladder cancers because of their superficial confinement as well as the anatomical properties of the bladder, and aims to reduce the risk of recurrence and progression ***(Svatek et al., 2014***). Chemotherapy has been shown to significantly reduce the rate of recurrences, but shows little effect on the rate of progression ***(Sylvester et al., 2016***).

BCG was developed as a live attenuated vaccine against tuberculosis in the beginning of the 20th century. In the 1930s it was noted that tuberculosis patients had a lower cancer incidence ***(Pearl, 1929***), and BCG was proposed as a potential cancer therapy. The first use of BCG for the treatment of bladder cancer was reported in 1976 ***(Morales et al., 1976)***.

Intravesical chemotherapy and BCG both significantly reduces the rate of tumor recurrences, but the reduction is greater with the use of BCG. BCG also has the advantage of reducing the rate of disease progression, and is considered the superior treatment of choice both for high and intermediate risk NMI bladder cancer ***(Spencer et al., 2013)***.

The mode of action of BCG immunotherapy is complex and not fully understood, depending on molecular interactions between the patient, the immune system, and the tumor ***(Redelman-Sidi et al., 2014)***. While BCG is effective, the treatment fails in up to 30-40% of patients ***(Nepple et al., 2010)***. It has been reported that the survival rate for patients progressing from NMI to muscle invasive disease may be worse than for patients with MIBC without a history of NMIBC ***(van den Bosch and Alfred, 2011)***.

The most appropriate treatment method for high risk T1 and operable primary or progressed muscle invasive tumors (stage ≥T2) is radical cystectomy (RC). This is a surgery with curative intent involving removal of the urinary bladder and pelvic lymphadenectomy and is always accompanied by prostatectomy in males or usually hysterectomy in females. If the patient is eligible, the cystectomy may be paired by systemic neoadjuvant chemotherapy (NAC) to target potential micrometastatic disease prior to surgery, which has been shown to provide a 5-8% increase in overall survival (OS) and a 9% increase in cancer specific survival (CSS) ***(Yin et al., 2016)***. The chemotherapy mainly consists of cisplatin-based combination e.g., gemcitabine and cisplatin (GC), or methotrexate, vinblastine, adriamycin, and cisplatin (MVAC). These regimes are sometimes also used in the adjuvant setting ***(Zargar et al., 2018)***.

Open radical cystectomy (ORC) is the current gold-standard  
treatment for MIBC and for high-risk recurrent NMIBC. Ideally, all  
patients with MIBC should receive platinum-based neo-adjuvant  
chemotherapy (***Grossman et al., 2003***). The 5-year survival after cystectomy is about 50%. Advanced bladder cancer metastasizes to lymph nodes, bones, lungs or liver and these patients can be treated with cisplatin systemically ***(Shah et al., 2011)***.

ORC has a peri-operative complication rate of 25 to 62%. Therefore, minimally invasive techniques such as laparoscopic radical cystectomy (LRC) have been explored ***(Cheung et al., 2013)***. The advantages of LRC include decreased blood loss, reduced postoperative pain, early return of bowel function and shorter hospital stay. Furthermore, LRC has good early oncologic outcomes with low morbidity in large cohorts with up to 5 years followup ***(Shah et al., 2011)***.