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Review

Methods for bladder cancer diagnosis – The role of autofluorescence and photodynamic diagnosis

Kamil Bochenek[a,b], David Aebisher[c], Anna Międzybrodzka[a,d], Grzegorz Cieślar[a], Aleksandra Kawczyk-Krupka[a,*]

[a] School of Medicine and Dentistry in Zabrze, Department of Internal Diseases, Angiology and Physical Medicine, Center for Laser Diagnostics and Therapy, Medical University of Silesia in Katowice, 15 Batory St., 41-902 Bytom, Poland
[b] Urovita- Silesian Center of Urology, 11 Strzelców Bytomskich St., 41-500 Chorzów, Poland
[c] Department of Photomedicine and Physical Chemistry, Faculty of Medicine, University of Rzeszów, Tadeusza Rejtana Avenue 16 C, 35-310 Rzeszów, Poland
[d] Non-Public Health Care Institution, Katowice Str. 3, 43-426 Dębowiec, Poland

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ABSTRACT

Bladder cancer is one of the most common Genito-urinary malignant tumors in humans. Improved diagnostic and therapeutic methods that aim to reduce rates of recurrence and progression of bladder cancer are needed. In current publications, one can find information on such methods as Raman spectroscopy, ultraviolet autofluorescence microscopy, confocal laser endoscopy, photoacoustic imaging, molecular imaging, multi-photon microscopy and many other new diagnostic techniques. These methods do not show significant adverse effects and are procedures well tolerated by patients as they use mostly physical phenomena that are neutral towards the human body. This review highlights the techniques of autofluorescence (AF) or laser induced fluorescence (LIF) and photodynamic diagnostics (PDD) which have been widely clinically studied for many years as a complement to cystoscopy. These methods can be performed during standard cystoscopy and they can be used in routine practice. This review shows that Autofluorescent and Photodynamic diagnostics are effective and have great potential in enhancing the diagnosis of bladder cancer. However, more research should be performed to help realize their full potential.

1. Introduction

1.1. Epidemiology

Bladder cancer is one of the most common malignant tumors in humans. It is the 9th most common type of cancer in the world, and in Europe it ranks 7th among men and 11th among women. Statistically, bladder cancer is rarely diagnosed in people less than 50 years of age, and most often it is diagnosed in patients at about 70 years of age. Ninety percent of cases are urothelial carcinomas and less common types are squamous and glandular cancers. At the time of diagnosis, 75% of cancers do not infiltrate the bladder and are termed non-muscle invasive bladder carcinoma (NMIBC). Of all NMIBC cases, 70% are in the Ta stage, 20% T1 and 10% intraepithelial carcinoma (CIS - carcinoma in situ) according to the TNM Classification of Malignant Tumors. Regardless of the type of histology, the severity of symptoms or the severity of the disease, the standard diagnostic for bladder tumor suspicion for over a century has been classical white cystoscopy (WLC - white light cystoscopy). This test consists of inserting the cystoscope into the lumen of the urinary bladder through the urethra for visual assessment of the bladder mucosa and collection of tissue material for histopathological examination. The whole procedure is carried out by viewing the bladder mucosa under the conditions of white light transmitted through the cystoscope lens. The sensitivity and specificity of this method are estimated to be between 62%–84% and 43%–98%, respectively [1–6]. These values are lower in the case of small flat lesions that are invisible in the light of a white cystoscope, such as dysplasia or cancer in situ [7]. Bladder cancer is a cancer that most often produces scarce, non-specific symptoms, and sometimes develops asymptomatically over an extended period of time. The most characteristic symptom is painless hematuria. The natural course of bladder cancer depends on the location and nature of the cancer; there are low-invasive types with low malignancy and low risk of progression, and high-grade types with high risk of progression and recurrence. Over the last several years, a significant problem has been observed related to the diagnosis and treatment of bladder cancer.

* Corresponding author.
E-mail address: akawczyk@sum.edu.pl (A. Kawczyk-Krupka).

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1.2. Diagnosis

The diagnosis of bladder cancer is based on a patient's medical history, symptoms, and laboratory test results, imaging investigation, cytology, biomarker examination and cystoscopy, which is a gold standard procedure. Imaging procedures include ultrasonography (USG) (transabdominal, transrectal/vaginal and transurethral) especially simple transabdominal ultrasonography with 63%−98% sensitivity and 99% specificity. Using USG, it is also possible to check the kidney, ureter, prostate and lymph nodes in addition to the bladder. Furthermore, Color Doppler ultrasound examination is used to estimate tumor blood flow. Some researchers emphasize that while transurethral USG may estimate and precisely evaluate tumor stage, a computed tomography (CT) examination is also recommended for assessing tumor infiltration, lymph node status and metastasis to surrounding tissue and distant organs include bones, in addition to estimating tumor stage [8]. To estimate bladder cancer staging and bladder wall, magnetic resonance imaging (MRI) is recommended with an accuracy of tumor grading between 72%−96%. Dynamic enhanced MRI is especially useful and provides images of muscle invasion with accuracy estimated at 85% [8]. For the specialist, other, albeit invasive, urological imaging procedures are: urography (CTU), magnetic resonance urography (MRU) and intravenous urography (IVU), which are performed to assess the entire urinary tract for patients with contraindications to contrast agents (allergy, renal insufficiency, ureterohydronephrosis). For analysis of surrounding tissue and tumor invasion, apparent diffusion coefficient (ADC) and diffusion-weighted imaging (DWI) are useful methods. During bladder cancer diagnosis, estimation of lung metastases using Chest plain X-ray film or CT plain scan should be routinely performed, as well as bone scintigraphy, CT scan on MRI to exclude bone metastases. In case of any diagnostic CT or MRI doubts, execution of Positron emission tomography-CT (PET-CT) remains. Also, urine tests are of importance in bladder cancer diagnosis, which includes cytology and tumor markers, such as nuclear matrix protein 22 (NMP22), and fluorescence in situ hybridization (FISH), bladder tumor antigen (BTA), fibrinogen degradation product FB/FDP and immunocytochemistry (ImmunoCyt) [8]. Diagnostic transurethral resection of bladder tumors (TURBt) provides an opportunity to obtain pathological diagnosis, especially in grading and staging of the disease to introduce target therapy. Cystoscopy and biopsy are the most trustworthy gold standard methods for identifying bladder cancer. This technique, enriched with various possibilities of optical biopsy, gives much greater possibilities of early detection of bladder cancer and early detection of cancer recurrence. Bladder tumor classification includes urothelial tumors, squamous cell tumor, glandular tumor, urachal cancer, neuroendocrine tumor, melanoma and mesenchymal tumor. Bladder cancer mainly includes urothelial carcinoma (90%), squamous cell carcinoma (SCC) (3–7%) and adenocarcinoma (2%). Immunohistochemistry detection gives opportunity to assess bladder cancer markers, such as uroplakin III, GATA3, CK7, CK20, CK34Be12, and P63 S-100 P. Molecular classification of urothelial carcinoma is classified into basal-like (Basal), luminal-like (Luminal) and wild-type P53-like forms according to the expression of CK5/6, CD44, CK20 and P53. It is associated with prognosis and the basal-like classification has the worst prognosis while wild-type P53-like has the best [8].White light cystoscopy has limitations and a high rate of recurrence, especially in the case of small and flat lesions or carcinoma in situ (CIS) of NMIBC.

Diagnostic information obtained in real time utilizing optical biopsy methods has great potential as an aid in diagnosis noninvasively. Various spectral and imaging technologies include: fluorescence, Raman scattering, elastic light scattering and vibrational spectroscopies, and biophotonic methods [9]. Also, a most interesting Stokes Shift Spectroscopy provides opportunity for bladder cancer diagnosis by an in vivo analysis of urine and blood samples using a mathematical nonnegative matrix factorization algorithm, reflectance and fluorescence spectral analysis, and spectral changes of metastases using combination of both absorption and emission and in vivo real time diagnosis [10]. Fluorescent cystoscopy is performed by adding photosensitizers which selectively accumulate in the abnormal bladder mucosa and emit red fluorescence after excitation by blue light, while normal healthy mucosa emits green fluorescence. The white light invisible flat and small tumors especially in situ could be detected with higher recognition rates, increasing up to nearly 25%. The disadvantage of this method is connected with a lower specificity estimated to be 63% due to inflammation, and bleeding vessels which gives false positive results, while the specify of bladder cancer recognition in white light cystoscopy (WLC) reaches almost 81%. A novel technique, which uses blue-green light at wavelengths of 415 nm and 540 nm is termed narrow band imaging (NBI). The light is absorbed by hemoglobin, thus accenting contrast between tumorous and healthy areas that are accentuated in appearance respectively as brown or green. Researchers emphasizes that NBI is a very useful method for detecting tumors overlooked by WLC. Drejer et al. revealed that the use of NBI has aided in finding bladder cancers that were overlooked by using WLC only, and additional information obtained by NBI changed the clinical decision [11]. Although Seung Bin Kim et al. described differences in 1-year recurrence-free rate that showed a trend for higher recurrence in the NBI group regardless of the benefits of the method, in comparison to WLC [12]. Dimitar et al. described NBI devices (Olympus Corp, Tokyo, Japan) which are approved to be integrated with standard cystoscope systems in clinical use in the United States allowing for rapid and real-time evaluation of malignant suspected lesions [13]. In a meta-analysis of studies including 1022 patients, the detection of bladder cancer was higher by NBI compared to WLC (94% vs. 85%) but the specificity of NBI was lower compared to WLC (55% vs. 72%) [14]. Other randomized clinical studies have confirmed that LIFE/PDD expands detection of papillary bladder tumors, and flat-appearing CIS in comparison to WLC (87% vs. 75%) [15–17].

Another optical biopsy technology is confocal laser endomicroscopy (CLE). This method uses the dye fluorescein and light from a 488-nm laser fiber source. Video obtained at 12 frames per second gives the real-time dynamic images of different tissue processes [18]. Optical Coherence Tomography (OCT) is also an optical biopsy technology which uses near-infrared light (890–1300 nm) and measures the backscatter of assessed tissue layer to deliver a tissue image with 2 mm depth of penetration and 10–20 μm spatial resolution. Bladder cancer OCT cystoscopy is estimated to have a sensitivity of between 84%–100% and specificity between 65%–89% [19]. In the early diagnosis of bladder cancer, multimodal imaging is also introduced for enhancement of imaging specificity and sensitivity. Combination imaging modalities include: PDD and NBI, CLE and OCT, PDD and CLE; intraoperative tumor grading was also estimated by the combination of imaging methods. Schmidbauer et al. evaluated 232 lesions from 66 patients with suspected bladder cancer using WLC, PDD alone, and PDD + OCT, obtained an increase of specificity from 62% to 87% [20]. Other emerging technologies include Raman Spectroscopy (RS), and surface-enhanced Raman scattering (SERS) nanoparticles. These methods provide, after illumination with near infrared light (785–845 nm), spectra that give the structural fingerprint of the treated tissue. Other techniques are ultraviolet (UV) autofluorescence, Multiphoton Microscopy, Scanning Fiber Endoscopy (SFE), molecular imaging using labeled CD47 antibody (anti-CD47), and imaging bladder lesions by PDD or CLE [21–23].

As mentioned previously, a high number of relapses have been noted, as well as a high risk of overlooking flat, low-level lesions in the bladder wall such as dysplasia or CIS [24] that are invisible in the picture of classic cystoscopy [25] and often detected accidentally as a result of the so-called blind biopsy. According to analyses worldwide, in cases of NMIBC, the recurrence of the disease after standard therapy in the Ta stage approaches 80% and disease progression, the main risk in the presence of T1 stage cancer and in situ cancer occurs in 45% of patients [2]. For many years, research has been carried out in centers

around the world aimed at finding a diagnostic method allowing early detection of the smallest neoplastic lesions that minimizes risk of not detecting developing tumors. Often, recurrence or progression of the disease are a consequence of maladaptive tumor removal caused by an inability to accurately visualize the tissues involved in the neoplastic process at an early stage of development [26,27].

Another motivation for the search for alternative detection methods is the fact that the current diagnostic and therapeutic procedures of bladder cancer are among the most expensive medical procedures in the world. Frequent relapses and a high risk of disease progression necessitates performing regular control cystoscopies, taking multiple samples for histopathological examination, and often performing resections or eventually implementation of radical surgery combined with chemotherapy and/or radiotherapy. Modern procedures generate tremendous costs, reaching billions of dollars annually [2,28,29]. Tumor treatment consisting of transurethral resection of the tumor (TURBT) is performed in stages Ta - T1, whereas in the case of T2 - T4, complete bladder resection is performed. The extent and scope of surgery is dependent on the severity of the disease and the presence of metastases and is often performed with complementary chemotherapy and sometimes radiotherapy [30]. These operations are associated with a significant reduction in the quality of life of patients, therefore, an extremely important task for the medical community is to find methods and algorithms that allow for detection of cancer in the early stages of development and, above all, minimizing the risk of leaving a tumor undetected during routine, commonly approved procedures.

The techniques of autofluorescence (AF) or laser induced fluorescence (LIF) and photodynamic diagnostics (PDD) have been widely studied for many years as a complementary and to cystoscopy [16–19]. These methods can be performed during standard cystoscopy and they can be used in routine practice. Most importantly, AF and PDD demonstrate an undeniably higher sensitivity for detecting neoplastic changes as compared to routine cystoscopy in white light [31]. They also allow effective monitoring of treatment effects, including the ability to perform a biopsy in a more precise manner [32]. They are characterized by a lower rate of relapse as compared to classical cystoscopy [33,34].

Autofluorescence cystoscopy (AFC)/PDD are well tolerated, with minimal side-effects. Photosensitizers administered intravesically allow for avoidance of the intravenous route and systemic side-effects, such photosensitivity and allergies. Other side effects are: recurrent acute cystitis, or recurrent urinary tract infection (UTI), contracted bladder or macroscopic hematuria [35]. Rarely, AF/PDD cystoscopy can introduce microorganisms into the urinary tract, causing an infection, especially in patients of advanced age, or in the cases of smoking patients, or extraordinary anatomy in the urinary tract. AFC/PDD might cause some blood in urine, although blood clots in the urine and serious bleeding seldom occurs. During the procedure, abdominal pain and a burning sensation are observed, but these signs are generally mild and decrease after the procedure. After the AF/PDD cystoscopy, very rarely is observed an inability to urinate, ulcerations inside the urethra or bladder, abdominal or pelvic pain, nausea, fever, painful urination, interstitial cystitis, urinary retention, recurrent bladder infections, urethral strictures and frequent urination. These all adverse effects are normally observed during a standard cystoscopy procedure, no more often when using AFC/PDD.

Either in previous 5-aminolevulinic acid (ALA) or specifically designed hexaminolevulinic acid (HAL) studies, side effects following bladder instillation or bladder-wall illumination- for example, dysuria, hematuria, bladder pain, and bladder spasm-have been rarely reported, are nonspecific, and are probably not drug related [36]. Only one report of anaphylactic shock after HAL instillation in one patient demands further consideration for specific drug involvement [37].

Fluorescence imaging is one of the most important research tools, but due to the optical properties of the tissue, is limited by the depth of the light excitation, and is also influenced by the vascularity and texture of the mucosa, as well as the volume of urine. These limitations lead to a decrease in fluorescence of hemoglobin, enzymes, and collagen.

Additional AFC/PDD limitations are related to methodical aspects (operator ability, divergent illumination), anatomical conditions dependent on the time and forms of previous oncological treatments (scar, blood vessel, bleeding, and inflammation) or mucosa properties [38,39]. The limitations of LIF/ PDD are also connected with false red fluorescence illuminations from nonmalignant, sometimes inflammatory tissue or from blood vessels which, as highlighted by some authors, is a problem with AFC/PDD specificity connected with false positives [39] or true positive diagnosis results [40]. It is possible to avoid this diagnostic inconvenience by the combination of AFC/PDD procedure with other diagnostic methods, such as OCT or tissue image magnification (TIM) or spectral analysis. Unfortunately, up to a 30% false-positive rate was described during the PDD studying curve, especially with prior Bacillus Calmette–Guérin (BCG) vaccine. Despite some unevenness connected with AFC/PDD specificity, the method sensitivity in CIS finding reaches 90% and allows a target biopsy and precise tumors resection of the bladder.

A special issue of concern during PDD is the quality of luminosity associated with damage of the cystoscope and light fiber because of the reduction of the instrumental light output and worse tissue visibility. Correct instrument conservation is also essential to the quality of images obtained with PDD. Other limitations involve preoperative catheterization and reduced visualization with inadequate hemostasis [41,42].

It should be remembered when taking into account the most perfect optical techniques and new imaging systems, they never do replace histopathological analysis. These diagnostic modalities hold great potential for more target localization and classification of bladder lesions with more precise properties for goal biopsy and resection. To assess their real and practical value, these techniques require randomized studies to recognize and understand their potential and accuracy in bladder cancer diagnosis and to establish objective standard protocols.

2. Autofluorescence diagnosis (LIF - laser induced fluorescence) and autofluorescence cystoscopy (AFC)

Autofluorescence diagnostics or LIF is based on the observation of tissue fluorescence which occurs as a result of irradiation of tissue with a specific wavelength. It has been demonstrated that there are differences in chemical structure at the cellular level that affect the observed fluorescence spectra due to processes occurring in cells involved in tumor transformation that alter cell metabolism resulting in visible changes between healthy cells and cancer cells. Researchers have proposed algorithms that increase the effectiveness of detecting cancerous cells using these processes. For about a decade, AFC has been commonplace in everyday clinical practice as a complementary tool of classical cystoscopy. Different lasers are used to excite the fluorescence of tissues, e.g. ionic krypton and argon lasers, dye lasers, and diode lasers. Thanks to continuous advances in technology and optics, selectable optical filters were developed that enable simultaneous white and fluorescent light testing, and algorithms that transform images into spatial maps of autofluorescence intensity. This advance, among others, has significantly improved diagnosis and an increased precision of sampling during tissue biopsy for histopathological examinations [32].

A particular example of autofluorescence imaging equipment is the Onco-LIFE system (Light-Induced Fluorescence Endoscopy) (Xillix, Richmond, Canada). In this system blue light is used to excite native fluorophores in tissue. This light source consists of a 150 W super-high-pressure mercury (Hg) arc lamp with a backup halogen lamp. Two kinds of light- red at wavelength 650–700 nm and green at wavelengths between 470–560 nm emitted from the autofluorescence image are filtered, analyzed and presented on a monitor. This system allows for an objective comparison between green and red autofluorescence by the calculation of a ratio, which is named as a numerical color value (NCV).

Table 1
Tested fluorophores and wavelengths of excitation and emitted light.

Fluorophore	Exciting light [nm]	Light emitted [nm]
NADH	365	490
FAD	365	550
collagen	365	420
porphyrin	633	710

2.1. Milestones in the development of bladder cancer fluorescence diagnosis

In 1984, one of the first studies demonstrating the possibility of using fluorophores naturally occurring in tissues was published by Alfano et al. Researchers exposed healthy and cancerous kidney, rat prostate cells, and mouse urinary bladder cells to 488 nm blue light. The observed fluorescence spectra of healthy tissue were found to differ significantly from tumor tissue. Two peak measurement points were observed that were assigned to flavin adenine dinucleotide (FAD) and porphyrins - two tissue fluorophores [43]. This report provided the basis for a deeper analysis of this phenomenon and the search for the possibility of its use in diagnostics. For many years, scientists have conducted research to understand the contribution of various fluorophores to complex fluorescence spectra.

In the case of the bladder, the most clinically important fluorophores are reduced nicotinamide adenine dinucleotide (NADH), flavin adenine dinucleotide (FAD) and collagen [44,45]. Table 1 lists several tested fluorophores and their wavelengths of excitation and emission.

D'Hallewin et al. state in their studies that the differences in fluorescence intensity between healthy and cancer tissue are mainly the result of increased thickness of the epithelium, increased blood flow, reduced collagen excretion in the submucosa and a reduced amount of NADH in cancer cells. According to these assumptions, the limitation of this method is the lack of a technical possibility of diagnosing in situ flat cancerous lesions due to the lack of thickening of the epithelium and increased blood circulation [44].

Palmer et al. Studied the contribution of intracellular porphyrins in shaping fluorescence spectra and the metabolic activity of tissues based on oxidation-reduction ratio (ORR). In a study from 2016, they demonstrated that bladder cancer cells have a reduced oxidoreductive ratio, increased intracellular porphyrin content, and a reduced NADH/porphyrin ratio compared to healthy tissue. These results stand in opposition to the results from the same team in 2015, when they observed an increased ORR. They explained these discrepancies as a possible effect of metabolic disorders in various types of cells, including cancerous and surrounding healthy fibroblasts. In addition, they concluded that the increased porphyrin content in cancer tissue can be explained by the accumulation of porphyrins in cancer cells in a selective manner, which can be used as in PDD, but without the need for external substances [45,46].

In a study conducted by Aboumarzouk et al., 67 biopsies from 21 patients were obtained for fluorescence analysis [47]. Histopathological examination showed benign lesions in 33 biopsies (49.3%), cancer (including CIS) in 30 biopsies (44.8%), and 4 others described as atypical where dysplastic changes were excluded from the study. Comparing the data of fluorescence spectra between benign and malignant lesions, statistically significant differences in fluorescent wavelengths between these groups were observed. Similarly, differences in fluorescence intensity were compared. A much lower intensity of fluorescence from malignant tissue was recorded, which was accompanied by an increased fluorescence signal of protoporphyrins.

In 2013, French scientists reported a diagnostic method based on tissue fluorescence used in fourteen cases of non-invasive bladder cancer. The developed method was based on the excitation of fluorescence in naturally occurring molecules in bladder tissue with pulses of ultraviolet radiation. The study was carried out under endoscopic control. The feedback signal received was transformed into an intensity factor of emitted light, which was closely related to the histopathological status of the tissue being assessed. Light at 360 and 450 nm was used. A simple, color-coded image was obtained, where green marked healthy bladder tissue and red tumor tissue. There were clear differences in the shape of the spectrum of tumor tissues, including in situ cancer compared to the spectra of inflamed mucosal tissue. The red images in each case corresponded to tumor tissue, so in this particular case the sensitivity of the method was 100%. A convincing case was made about the effectiveness of this method in the detection of non-invasive bladder cancer (NMIBC) [48].

In turn, Kriegmar et al. conducted a pilot study evaluating the utility of wide field AF imaging in during transurethral resection of the bladder tumor (TURBT) to detect bladder cancer. They performed spectral fluorescence measurements of tissues using a spectrometer and light of wavelength 440 nm was used. A significantly lower fluorescence intensity of the tissue involved in the neoplastic process was observed compared to the tissue without the characteristics of malignant transformation. The normal mucous membrane was visible as green areas, while the neoplastic lesions were areas of brown-red color. The sensitivity and specificity of this method was 96.7% and 53.8%, respectively, and for cystoscopy in white light, 86.7% and 69.2%, respectively. The study concluded that AF imaging during transurethral resection may increase the detection rates of bladder tumors, which would make this method more effective than standard cystoscopy in white light [49].

3. Photodynamic diagnosis and fluorescence cystoscopy

Photodynamic diagnosis and has found a wide application in medicine. Decades of research have demonstrated the effectiveness of this method in diagnostics in many areas, including dermatology, gastroenterology, urology, and oncology [32]. Photodynamic diagnosis requires the introduction of an exogenous substance into the body, called a photosensitizer, which accumulates in the cells. Photosensitizers are chemical compounds that have the ability to absorb light energy of appropriate wavelength promoting them into an excited state. Decay of the excited state to the ground state is accompanied with emission fluorescence of a specific wavelength. Fig. 1 depicts excitation of protoporphyrin IX with blue light and resulting red fluorescent emission.

The results of numerous studies have shown that photosensitizers accumulate selectively in high concentration in tumor cells, and to a much smaller extent in healthy cells. The tissue in which the cells have accumulated photosensitizer is subjected to light with a wavelength defined for a given compound, which results in the emission of radiation usually giving an image of red fluorescence of the tumor tissue in comparison with the normal green color of the surrounding healthy tissue. Exemplary photosensitizers that are used in fluorescence cystoscopy are listed in Table 2.

The diagnostic problem in the case of bladder cancer is low-level lesions, such as dysplasia and intraepithelial carcinoma. Diseases of this type are burdened with a very high risk of progression and are difficult to detect using white light cystoscopy. Biopsies carried out in WLC in the case of dysplasia and CIS are performed "blindly" because these changes are invisible to the naked eye; in a light white mucosa tissue appears to be normal. However, under blue light, after the administration of a photosensitizer, neoplastic and pre-cancerous lesions are visible as red fields among the green areas of healthy tissue, which allows precise sampling for histopathological examination and subsequent removal of the entire lesion with an appropriate margin. However, fluorescence diagnostics are also unreliable in the case of flat, low-level lesions, especially when using a rigid cystoscope. Using a rigid cystoscope diseased tissue located in areas that are difficult to access may remain undetected. Therefore for diagnostic purposes a flexible cystoscope is recommended [50]. A limitation of PDD is the high percentage of false positives. This is due to accumulation of the

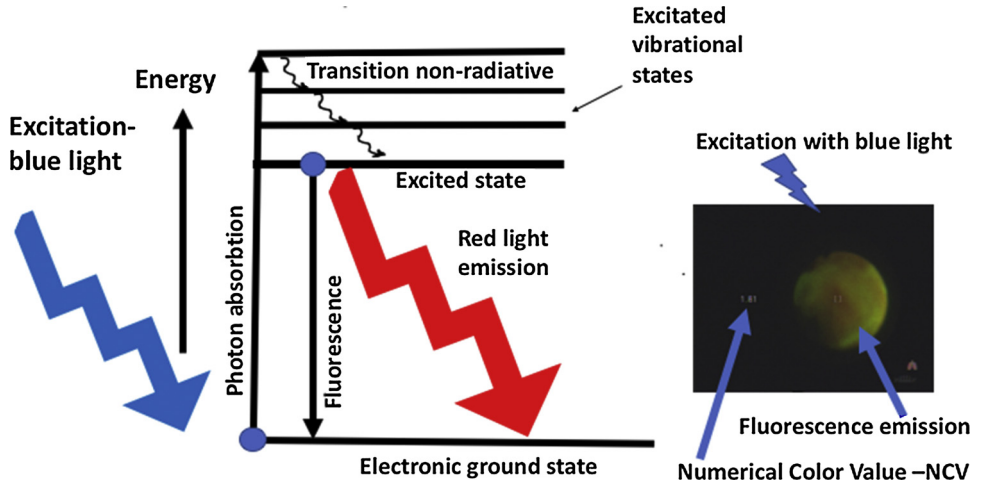

Fig. 1. Excitation of protoporphyrin IX with blue light and resulting red fluorescent emission. The simplified Jablonski diagram and its impact on the auto-fluorescence image using Onco-LIFE system in patient with bladder cancer.

Table 2
Application of exemplary photosensitizers in fluorescence cystoscopy.

PS	Concentration or weight of PS	Application time	λ (nm)	Ref.
5-ALA	1.5 g	2-3 h	406.7	[53]
	3% r-r /50ml	2-3 h	380 - 440	[54]
HAL	85 mg	1 – 2 h	D-Light® System (STORZ)	[55]
hypericin	8 μmol/l /40 ml	2 - 4 h	375 - 400	[57]
Pirarubicin	30 mg	15 min	D-Light® System (STORZ)	[59]
	30 mg	15 min	375-450	[60]

PS - photosensitizer; 5 - ALA - delta-aminolevulinic acid; HAL - hexaminolevulinic acid; THP ((2′R) -4′-O-tetrahydropyranyl doxorubicin, Pirarubicin).

photosensitizer not only in cancer cells, but also in the tissue involved in the inflammatory process. For example, in a reported fluorescence study, a high percentage of false positives were observed less than 3 months after intravesical administration of BCG and after recent bladder resections [47].

3.1. How photodynamic diagnosis was developed using the different photosensitizers- a survey review

Seminal work on PDD was presented by J. F. Kelly, M.E. Snell and M. C. Berenbaum in 1975 on the use of hematoporphyrins to diagnosis urothelial cancer in vitro [51]. One day after intravenous administration of 2 mg of a hematoporphyrin derivative, samples taken during cystectomy were observed under UV light. Neoplastic CIS and exophytic changes as well as dysplasia were characterized by bright red fluorescence whereas normal tissues showed no fluorescence. Common exogenous fluorophores used in clinical photodynamic diagnostics studies on the usefulness in photodynamic diagnostics include 5-aminolevulinic acid (5-ALA), its ester derivative - hexylaminolevulinate (HAL) and hypericin. There are also few reports using the fluorophore pirarubicin. In the medical database, there are many articles describing fluorescence diagnostics using 5-ALA, HAL and hypericin. The results of these tests, regardless of the photosensitizer used, undoubtedly places photodynamic diagnosis over WLC as a diagnostic method [52]. Photodynamic diagnosis has a higher sensitivity than WLC resulting in a demonstrated reduction in recurrence rate [33]. The chemical structures of 5-ALA, HAL, hypericin, and pirarubicin are shown in Fig. 2.

3.1.1. Delta-aminolevulinic acid (5-ALA)

Researchers in Munich presented studies using PDD that demonstrated extremely high sensitivity using 5-aminolevulinic acid (5-ALA) in patients with bladder dysplasia and early stage cancer in 1996 [53]. Before the planned bladder biopsy, 104 patients were given a 3% solution of 5-aminolevulinic acid directly into the bladder. Cystoscopy was performed wavelength of 406.7 nm. The results showed a much higher sensitivity of this technique (96.9%) than classic cystoscopy in white light (72.7%). There were no significant differences in the specificity of both techniques. Therefore, it was concluded two decades ago that due to the high sensitivity of PDD in the detection of neoplastic bladder lesions, biopsies under fluorescence cystoscopy are recommended. In 2002, Zaak et al. published a paper presenting the results of a 5-year clinical trial on the effectiveness of PDD using 5-ALA in detecting dysplastic changes and in situ bladder cancer. A large cohort of 713 patients participated in the study and 3834 biopsies from the bladder were collected after administration of 5-ALA. Of the sample biopsies analyzed, 1250 were diagnosed with malignant lesions, including 304 s stage dysplasia and in situ carcinoma. In contrast, using WLC, 30.3% of dysplastic stage II and 52.8% of cases of in situ carcinoma were undetected [54].

3.1.2. Hexaminolevulinic acid

A team of researchers from Romania presented a report based on the results of their studies using hexaminolevulinic acid (HAL) in the diagnosis of non-invasive bladder muscle cancer [55]. A cohort of 44 patients participated in the study in which PDD in 22 subjects was compared to 22 diagnosed with classical cystoscopy. Fluorescence cystoscopy PDD revealed 25.8% more tumors. A significant reduction in relapse was also reported, and FC control tests were carried out after 3, 6, 9 and 12 months. Burgues et al. [56] also conducted PDD of NMIBC using HAL. The procedure was performed in 305 patients during transurethral resection. In this study, 1659 suspicious changes were recorded, 522 biopsies were diagnosed using FC and WLC, 237 with FC only, 19 with WLC only and 881 biopsies were diagnosed randomly. Of the 600 tumors, PDD revealed 563, WLC 441 and 29 random biopsies which included 20 cases of CIS. Photodynamic diagnostic sensitivity was 93.8%, WLC 78.2%, with specificity of 81.5% and 90.5%, respectively. The study showed that FC using HAL improves the detectability of bladder cancer, especially in the case of CIS.

3.1.3. Hypericin

The next photosensitizer tested in terms of clinical utility during PDD was hypericin. Sim et al. [57] included 41 patients who underwent transurethral resection for bladder cancer. Before cystoscopy, patients

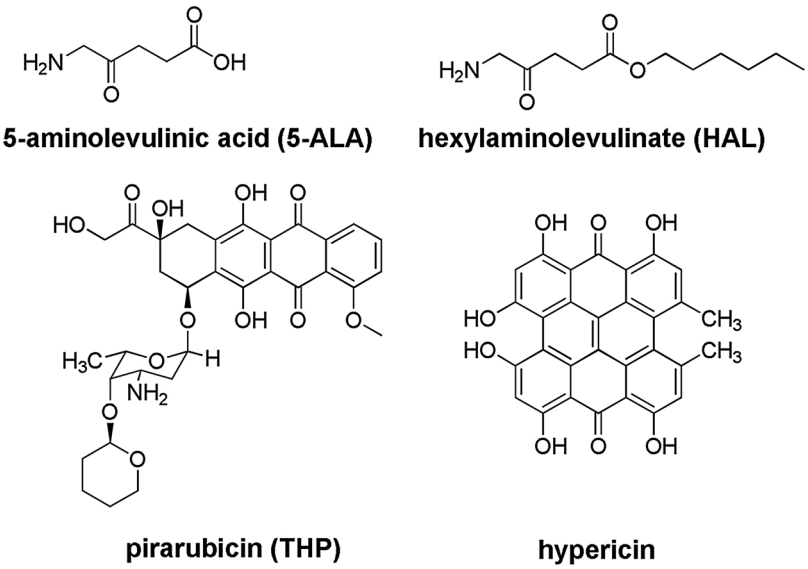

Fig. 2. Chemical formulas of fluorophores used in photodynamic diagnosis.

were given a solution of hypericin to the bladder, followed by WLC and immediately followed by FC. A total of 179 biopsies were collected from 41 patients. Urothelial carcinoma was present in 41% of samples, and 80% of patients had macroscopically visible tumors. From biopsies performed during FC, 40% were positive, of which 86% showed cancer. The sensitivity and specificity of PDD with hypericin in this study was 82 and 91%, respectively. For comparison, WLC these values had a lower sensitivity (62%) but equivalent specificity (98%).

A study with hypericin was also conducted by Kubin et al. [58]. After administration of hypericin, WLC and FC was performed in 57 patients with suspected or recurrent bladder cancer. A total of 163 biopsies from suspect sites were analyzed by FC and WLC. The sensitivity of FC in this study was 100%, whereas classical cystoscopy was much lower at 33%. In the case of CIS, these values were 85% and 31% respectively.

3.1.4. Pirarubicin

Studies on the clinical usefulness of pirarubicin (THP) in photodynamic diagnostics were performed in China between the years 2008–2009 in 48 patients with diagnosed or suspected bladder cancer [59]. The THP solution was intravesically infused for 15 min followed by cystoscopy. Under white light, changes were observed as orange color, and bright red under blue light. A total of 238 biopsies were performed, of which 84 were diagnosed with cancer, 20 with dysplasia, and 134 with non-malignant lesions. The sensitivity of FC was 74.7%, and the percentage of false positive results was 32.5%. There were no significant adverse reactions to pirarubicin. A similar study was conducted in China from 2012 to 2015 in 25 patients with painless hematuria [60]. In 18 patients, it was the first episode of hematuria, and in 7 it was recurrent. Before administration of pirarubicin, cystoscopy was performed in white light. Pirarubicin solution was introduced into the bladder through the catheter and after 15 min the bladder was emptied, cystoscopy was performed in white and blue light. A total of 109 biopsies were taken for histopathological examination which showed cancer in 26 samples and non-malignant lesions in 2 samples. The sensitivity of cystoscopy in white and blue light after THP administration was both 100%, while the specificity of method was 96.15% and 84.74%, respectively. Table 3 lists comparative percent sensitivity and selectivity between FC and WLC for several of the photosensitizers previously discussed.

Table 3
Comparison of sensitivity and specificity of bladder cancer detection in classical and fluorescent cystoscopy based on selected examples.

WLC		FC		PS	Ref.
Sensitivity [%]	Specificity[%]	Sensitivity [%]	Specificity [%]		
86.7	69.2	96.7	53.8	–	[49]
78.2	90.5	93.8	81.5	HAL	[56]
62.0	98.0	82.0	91.0	Hyp	[57]
100	84.74	100	96.15	THP	[60]

WLC – white light cystoscopy; FC - fluorescent cystoscopy.
PS - photosensitizer; HAL - hexaminolevulinic acid.
Hyp - hypericin; THP ((2′R) -4′-O-tetrahydropyranyl doxorubicin, Pirarubicin).

4. Summary

Research is underway on diagnostic and therapeutic methods that aim to reduce rates of recurrence and progression of bladder cancer. In current publications, one can find information on such methods as Raman spectroscopy, ultraviolet autofluorescence microscopy, confocal laser endoscopy, photoacoustic imaging, molecular imaging, multiphoton microscopy and many other diagnostic techniques. Most of these methods do not show significant adverse effects and are procedures well tolerated by patients as they use mostly physical phenomena that are neutral towards the human body. Modern advances in technology allow for the use of sophisticated methods and devices to improve the detection and effectiveness of cancer treatment thanks to the combination of advanced technologies, including enabling transformation of different signals coming from the tissue into images. Physical phenomena also prove useful in clinical practice, such as the fluorescence diagnostics discussed herein, which, apart from cystoscopy, can also be used in conjunction with multitron microscopy (MPM - multiphoton microscopy). This method, also in the case of bladder cancer, enables the visualization of various biological processes at the cellular level in vivo, and has the ability to provide real-time three-dimensional images of unstained tissue in vivo at the subcellular level [61,62]. Multiphoton microscopy, like PDD, has a positive effect on bladder cancer detection rate. Another advantage of modern methods and devices is associated with the advancing technology of miniaturization that increases practicality and portability of tools as well as easier access due to global commercialization [61]. The results of research on

fluorescence diagnostics around the world have led to the introduction of these methods into everyday practice. There is a need for further improvement of tools, and a search for unified algorithms that strive to increase detection and improve the specificity index of these methods. Autofluorescent and photodynamic diagnostics are undeniably effective and have great utility in diagnosing bladder cancer, so more research should be performed to realize their full potential.

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