International Journal of Clinical Oncology (2019) 24:1075–1080
https://doi.org/10.1007/s10147-019-01447-5

ORIGINAL ARTICLE

High diagnostic efficacy of 5-aminolevulinic acid-induced fluorescent urine cytology for urothelial carcinoma

Gaku Yamamichi[1] · Wataru Nakata[1] · Masaru Tani[1] · Go Tsujimura[1] · Yuichi Tsujimoto[1] · Mikio Nin[1] · Akihiro Mimura[2] · Hideaki Miwa[3] · Masao Tsujihata[1]

Received: 29 November 2018 / Accepted: 4 April 2019 / Published online: 11 April 2019

Abstract

Background In general, urine cytology is often problematic because of its low sensitivity, especially for low-grade urothelial carcinoma (UC) in clinical practice. To improve the sensitivity, we focused on 5-aminolevulinic acid (5-ALA), because recent studies suggested that 5-ALA-induced urine cytology can be used for photodynamic diagnosis. In this study, we evaluated the diagnostic efficacy of 5-ALA-induced fluorescent urine cytology for UC.

Methods We included in this study 318 patients comprising 158 non-cancer patients, 84 bladder tumor patients, and 76 upper urinary tract urothelial carcinoma (UUT-UC) patients treated in our institution from March 2013 to September 2018. Using the same voided urine sample, we compared sensitivity and specificity between conventional urine cytology and 5-ALA-induced fluorescent urine cytology.

Results Overall, the sensitivity of 5-ALA-induced fluorescent urine cytology was significantly higher than that of conventional urine cytology (86.9% vs. 69.4%; $p = 0.0002$), and the specificity was equivalently high (96.2% vs. 95.6%; $p = 1.0$). In subgroup analysis, the high sensitivity of 5-ALA-induced fluorescent urine cytology was also detected regardless of age, sex, and tumor type. However, in terms of stage and grade, differences were only detected in patients with less than pTa stage (89.2% vs. 52.1%; $p = 0.0001$) and low-grade tumor (91.5% vs. 51.1%; $p < 0.0001$).

Conclusions 5-ALA-induced fluorescent urine cytology was significantly more effective for UC diagnosis when compared with the conventional cytology, especially in patients with low-stage and low-grade tumors. These findings indicate that 5-ALA-induced fluorescent urine cytology may potentially be a very useful tool for clinical use.

Keywords 5-Aminolevulinic acid · Urothelial carcinoma · Cytology · Sensitivity · Specificity

Introduction

In general, a bladder tumor (BT) is diagnosed and followed up by cystoscopy and cytology of voided urine in clinical practice. Cystoscopy is a relatively invasive but highly sensitive and essential examination for BT detection. However, flat lesions, such as carcinoma in situ, and tiny tumors are difficult to detect visually. In 2006, the European Association of Urology approved the use of 5-aminolevulinic acid (5-ALA) for photodynamic diagnosis (PDD) to identify BT by cystoscopy more precisely. 5-ALA is a precursor in heme biosynthesis, and protoporphyrin IX, which is a metabolic product of 5-ALA, accumulates in mitochondria following its administration to the cell [1]. It accumulates more in tumor cells than in healthy cells due to varied causes such as the decreasing ferrochelatase activity of tumor cells [2]. Protoporphyrin IX emits red fluorescence when excited with blue light at a wavelength of 405 nm, has a peak at a wavelength of 635 nm, and is used to specifically visualize cancer cells [3]. The sensitivity of PDD with oral 5-ALA for transurethral resection of bladder tumor (TURBT) is higher than that of the conventional white light endoscopic diagnosis [4], but cystoscopy with 5-ALA has a higher false-positive rate

✉ Wataru Nakata
nakata_wa@osakah.johas.go.jp

Gaku Yamamichi
mm6085yg@yahoo.co.jp

[1] Department of Urology, Osaka Rosai Hospital, Nagasone-cho 1179-3, Kitaku, Sakaishi, Osaka, Japan

[2] Department of Laboratory Medicine, Osaka Rosai Hospital, Osaka, Japan

[3] Department of Diagnostic Pathology, Osaka Rosai Hospital, Osaka, Japan

than white light cystoscopy [5]. In contrast, urine cytology is a non-invasive examination, but its sensitivity is as low as 60–70%. Especially, low-grade BTs have a sensitivity as low as 45% for urine cytology [6]. Recently, one study of fluorescent urine cytology staining with 5-ALA using voided urine reported that the sensitivity of this method was good at 82% [7]. However, this study included only a small number of patients with BT ($n=61$).

Upper urinary tract urothelial carcinoma (UUT-UC) is a relatively uncommon disease accounting for only about 5% of urothelial cancers [8]. The standard diagnosis of UUT-UC includes the assessment of computed tomography urography (CTU) and voided urine cytology. CTU is useful for detecting and staging UUT-UC, because its sensitivity for UUT-UC is 82.6% and specificity is 94.8% [9, 10]. However, we are often annoyed by the clinical problem of whether the tumor is a malignant tumor or a benign tumor when CTU shows a tumor, but urine cytology is negative. In this situation, further invasive examination is required, such as selective upper tract urinary cytology and ureteroscopic biopsy. However, selective upper tract urinary cytology also has low sensitivity of 53.1% [11], and ureteroscopic biopsy is reported to increase the recurrence of intravesical tumors [12]. Clinically, therefore, improvement in the sensitivity of urine cytology for diagnosis is strongly required. To improve the sensitivity, we focused on 5-ALA. In terms of UUT-UC, there are no reports of 5-ALA-induced fluorescent urine cytology to our knowledge.

In this study, therefore, we evaluated the efficacy of 5-ALA-induced fluorescent urine cytology in both BT and UUT-UC.

Patients and methods

Patients

This study comprised 160 UC patients, 84 patients with BT and 76 with UUT-UC, who underwent TURBT and nephroureterectomy and were confirmed pathologically to have UC between March 2013 and September 2018 in Osaka Rosai Hospital, and 158 non-tumor patients who did not have a cancer history and were hospitalized due to benign prostatic hyperplasia (BPH), urinary stone, urinary tract infection (UTI), pelviureteric junction (PUJ) stenosis, radiological cystitis, and scrotal swelling. Tumors were staged according to the AJCC TNM staging system (2010) and graded according to the WHO Classification (2004). This study was performed in accordance with the ethical principles of the 2013 Declaration of Helsinki and was approved by the ethics committee of Osaka Rosai Hospital (approval number 30-71).

Collection of urine samples and treatment with 5-ALA

All samples were collected on the day before surgery or any other invasive urological examinations from > 150 mL of voided urine, and the following procedures were performed within 1 h for pathological examination. First, we separated these samples for the conventional cytology and ALA-induced fluorescent cytology. We centrifuged the urine samples for ALA-induced fluorescent cytology at 1500 rpm for 5 min and decanted the supernatant. The pellets were suspended in Minimum Essential Medium (MEM) with 5-aminolevulinic acid hydrochloride (Wako Pure Chemical Industries, Osaka, Japan), and the concentration was adjusted 200 μg/mL. Then, the suspension was stored in the dark at 37 °C for 2 h. After that, the samples were centrifuged again at 1500 rpm for 5 min, and the pellet was resuspended in MEM. Finally, these urine samples were tested for protoporphyrin IX fluorescence using a spectrophotometer (Nikon ECLIPSE Ni; Nikon Corporation, Tokyo, Japan) at appropriate settings (excitation wavelength of 405 nm and emissions wavelength of 600–650 nm) (Fig. 1).

Evaluation of conventional cytology and 5-ALA-induced fluorescent cytology

Conventional cytology and 5-ALA-induced fluorescent cytology were evaluated by the same examiner using the same urine sample. In the conventional urine cytology, negative and suspicious cytology such as class 1, 2, and 3 were evaluated as negative, and positive cytology such as class 4 and 5 were regarded as malignant. We classified 5-ALA-induced fluorescent cytology showing no red light or dark red as negative and that showing clear red as positive (Fig. 2). The final judgment was confirmed by two pathologists.

Statistical analysis

Data were compared using the Wilcoxon test or Chi square test. Differences were considered statistically significant when $p<0.05$. Statistical analyses were performed using JMP14 (SAS Institute Inc., Cary, NC, USA).

Results

Table 1 shows the patients' clinicopathological characteristics. The group with UC was older (median age 75 years old) than the non-cancer group (median age 72 years old) ($p<0.01$). The UC group included 84 patients with BT

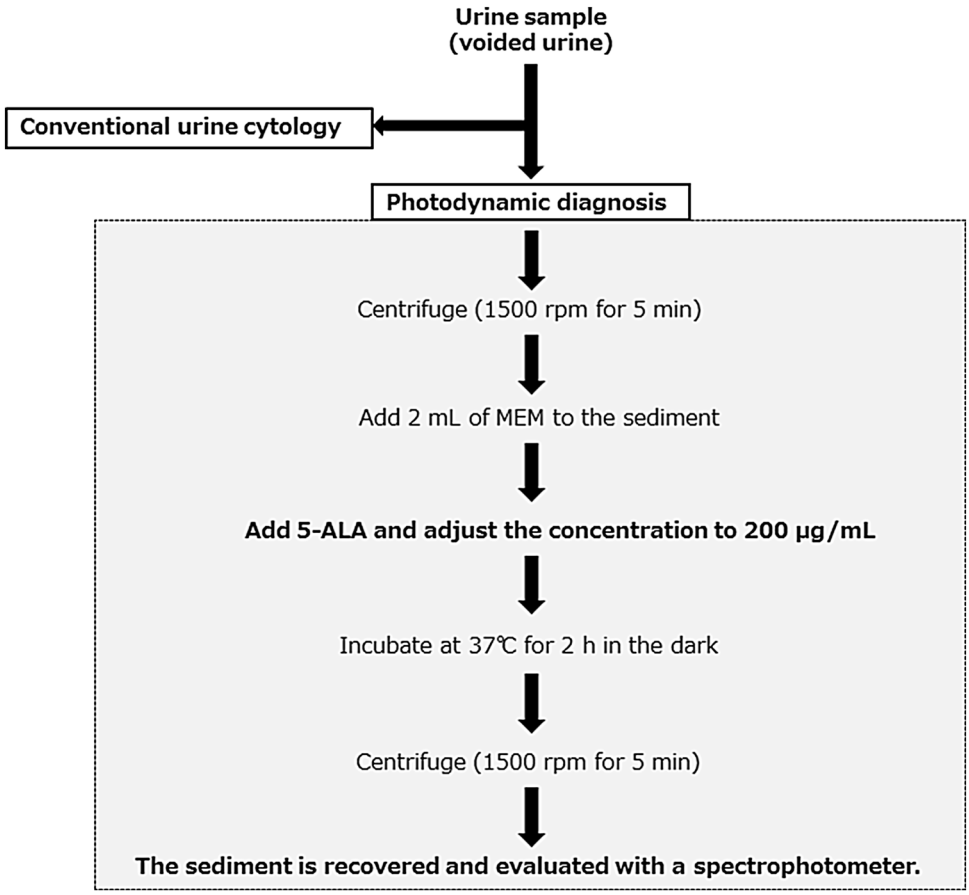

Fig. 1 The protocol of 5-ALA-based fluorescent detection assay. *MEM* minimum essential media, *ALA* aminolevulinic acid

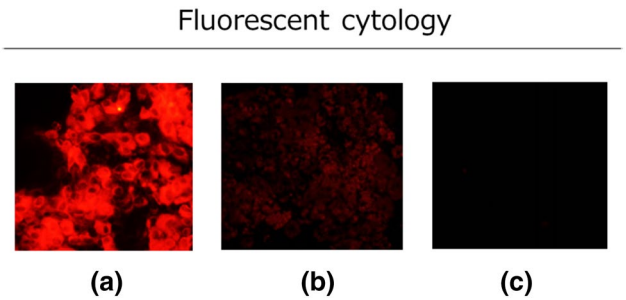

Fig. 2 5-ALA-induced fluorescent cytology. **a** Clear red, **b** dark red, and **c** no red

and 76 with UUT-UC. The non-cancer group included 117 patients with BPH, 18 with urinary stones, 17 with UTI, 3 with PUJ stenosis, 2 with radiological cystitis, and 1 with scrotal swelling. In the cancer group, 110 patients had cancer of pT1 or less (48, 28, and 34 patients with pTa, pTis, and pT1, respectively), 50 had cancer of pT2 or more, 47 had a low-grade tumor, and 113 had a high-grade tumor.

The sensitivity and specificity of conventional cytology and 5-ALA-induced fluorescent cytology in all specimens are presented in Table 2. The sensitivity (86.9% vs. 69.4%,

Table 1 Patients' characteristics

Characteristic	Urothelial carcinoma ($n=160$)	Non-cancer ($n=158$)	p value
Age (years)	75 (50–90)	72 (27–90)	0.0001
Sex (M:F)	130:30	126:32	0.78
Diagnosis			
BT	84		–
UUT-UC	76		
BPH		117	
Urinary stone		18	
Infection		17	
Other		6	
pT stage			
pTa	48	–	–
pTis	28		
pT1	34		
≥ pT2	50		
Tumor grade			
Low grade	47	–	–
High grade	113		

BT bladder tumor, *UUT-UC* upper urinary tract urothelial carcinoma, *BPH* benign prostatic hyperplasia

Table 2 Sensitivity, specificity, PPV, and NPV of conventional cytology and 5-ALA-induced fluorescent cytology in BT and UUT-UC

Variables	Cancer type	Conventional	5-ALA	*p* value
Sensitivity	All UC	69.4% (111/160)	86.9% (139/160)	0.0002
	BT	67.9% (57/84)	83.3% (70/84)	0.030
	UUT-UC	71.1% (54/76)	90.8% (69/76)	0.0033
Specificity	All UC	95.6% (151/158)	96.2% (152/158)	1.0
	BT	95.6% (151/158)	96.2% (152/158)	1.0
	UUT-UC	95.6% (151/158)	96.2% (152/158)	1.0
PPV	All UC	94.1% (111/118)	95.9% (139/145)	0.57
	BT	89.1% (57/64)	92.1% (70/76)	0.57
	UUT-UC	88.5% (54/61)	92.0% (69/75)	0.56
NPV	All UC	75.5% (151/200)	87.9% (152/173)	0.0023
	BT	84.8% (151/178)	91.6% (152/166)	0.067
	UUT-UC	87.3% (151/173)	95.6% (152/159)	0.010

PPV positive predictive value, *NPV* negative predictive value, *ALA* aminolevulinic acid, *BT* bladder tumor, *UUT-UC* upper urinary tract urothelial carcinoma

$p = 0.0002$) and negative predictive value (87.9% vs. 75.5%, $p = 0.0023$) were significantly higher for 5-ALA-induced fluorescent cytology versus that for conventional cytology, with a similar trend shown in both BT and UUT-UC, respectively. However, the specificity (96.2% vs. 95.6%, $p = 1.0$) and positive predictive value (95.9% vs. 94.1%, $p = 0.57$) were equivalently high between 5-ALA-induced fluorescent cytology and conventional cytology. In conventional cytology, false positives were found in seven patients: three with BPH, three with UTI, and one with radiation cystitis. In 5-ALA-induced fluorescent cytology, false positives were found in six patients: two with BPH, two with UTI, and two with urinary stones. In cases judged as false positive by the conventional cytology or 5-ALA-induced fluorescent cytology, transurethral bladder random biopsy, selective upper tract urine cytology, and contrast-enhanced CT were performed to examine the urinary tract tumor. There were no findings to indicate UC either by additional imaging or pathological examinations, and in the subsequent follow-up of all patients, urine cytology was reexamined every 3 months for over 1 year and confirmed to be negative. These results suggested that ALA-induced fluorescent cytology may be a superior tool to conventional cytology in clinical practice.

Table 3 shows the sensitivity of conventional cytology and 5-ALA-induced fluorescent cytology for UC by subgroup analysis. The 5-ALA-induced fluorescent cytology tended to be more sensitive than conventional cytology regardless of patient age (classified by the median age of 75 years old) and sex. However, the sensitivity of 5-ALA-induced fluorescent cytology was significantly better than that of conventional cytology and in tumors of pTa stage (89.6% vs. 52.1%, $p = 0.0001$) and in low-grade tumors (91.5% vs. 51.1%, $p < 0.0001$). For tumors of more than pT2 stage (82.0% vs. 74.0%, $p = 0.47$) and high-grade tumors (85.0% vs. 77.0%, $p = 0.17$), however, the sensitivity of 5-ALA-induced fluorescent cytology and conventional cytology were equal. These results indicated that even in tumors of pTa stage and in low-grade tumors, where sensitivity is low in conventional cytology, 5-ALA-induced fluorescent cytology can maintain high sensitivity as in tumors of more than pT2 stage and high-grade tumors.

Table 3 Sensitivity of UC for conventional cytology and 5-ALA-induced fluorescent cytology in the subgroup analysis

Variables	Conventional	5-ALA	*p* value
≤ 75 years	70.4% (57/81)	85.2% (69/81)	0.04
> 75 years	68.4% (54/79)	88.6% (70/79)	0.01
Male	70.0% (91/130)	86.2% (112/130)	0.01
Female	66.7% (20/30)	90.0% (27/30)	0.06
≤ pT1	67.3% (74/110)	89.1% (98/110)	0.0001
pTa	52.1% (25/48)	89.6% (43/48)	0.0001
pTis	78.6% (22/28)	85.7% (24/28)	0.73
pT1	79.4% (27/34)	91.2% (31/34)	0.30
≥ pT2	74.0% (37/50)	82.0% (41/50)	0.47
Low grade	51.1% (24/47)	91.5% (43/47)	< 0.0001
High grade	77.0% (87/113)	85.0% (96/113)	0.17

UC urothelial carcinoma, *ALA* aminolevulinic acid

Discussion

The sensitivity of voided urine cytology in BT and UUT-UC is low (55% and 53%, respectively) [11, 13]. Especially, conventional cytology is limited by its low sensitivity for low-grade tumors because of the weakness of nuclear atypia, nuclear enlargement, and hyperchromasia. In addition, urine cytology is affected by inflammation or urinary calculi. Therefore, it is difficult to distinguish metamorphic urine cells, which are not malignant, from UC with low nuclear heterozygosity. We, thus, require a more accurate examination for detecting UC.

The feasibility of diagnosing BT with 5-ALA was first reported in 1994 [14]. Currently, 5-ALA is approved as a photosensitizer of PDD for carcinoma around the world. For example, ALA as an optical imaging medicine was approved to enhance intraoperative detection of malignant glioma and also to detect bladder cancer [15]. 5-ALA is a precursor in heme biosynthesis: protoporphyrin IX, a metabolic product of 5-ALA, accumulates in mitochondria following its administration to the cell [1], and protoporphyrin IX accumulates more in tumor cells than in healthy cells because of the decreased ferrochelatase activity of tumor cells [2]. How 5-ALA induces the accumulation of protoporphyrin IX in tumor cells has not been completely identified, but

it was recently reported that peptide transporter 1 (PEPT-T1) and ATP-binding cassette transporter (ABCG2) play pivotal roles in porphyrin accumulation. Bladder cancer showed a high level of PEPT-T1 and a low level of ABCG2, and this dysregulation resulted in greater accumulation of protoporphyrin IX in cancer cells than in normal cells [16]. Because tumor selectivity of protoporphyrin IX is increased 9–16-fold in urothelial cells [17], UC is a good use for PDD enhanced by 5-ALA.

A recent report on 5-ALA staining of urine specimens in an extracorporeal exposure showed PDD sensitivity to be effective compared with conventional cytology in BT (82% vs. 49%, respectively), particularly in low-grade and low-stage tumors, and to have comparable specificity (80% vs. 100%, respectively) [7]. However, that paper studied a small number of patients (61 with BT only), and there were some limitations in that conventional cytology for low-grade BT had very low sensitivity (18%), and the details of the non-cancer group were not described. The present study included more patients ($n=318$) as well as patients with UUT-UC. Our results indicated that the sensitivity of 5-ALA-induced fluorescent cytology was significantly higher than that of conventional cytology for BT and UUT-UC, especially for tumors of pTa stage and low-grade tumors, as did the previous study for BT. We speculated that this difference was caused by the diagnostic method used. In conventional urine cytology, diagnosis is by nuclear heteromorphism, and therefore, it is difficult to distinguish UC with low nuclear grade from degenerative urothelial cells. However, in 5-ALA-induced fluorescent urine cytology, diagnosis is based on the difference in metabolism of mitochondria between normal cells and cancer cells.

In this way, 5-ALA-induced fluorescent cytology shows very high sensitivity even in low-grade and low-stage tumors. These findings suggest that it might be possible to follow up using only 5-ALA-induced fluorescent cytology without cystoscopy in low- and intermediate-risk BT. Moreover, for the UUT-UC diagnosis, it is possible that additional invasive selective upper tract urine cytology examinations and ureteroscopic biopsies can be reduced by 5-ALA-induced fluorescent cytology.

Some reports indicated that the false-positive findings of 5-ALA can be induced by several factors such as infection, inflammation, hyperplasia, and inexperience with the use of PDD [3, 18]. There were only six cases of false positives by 5-ALA-induced fluorescent cytology in the present study, probably because of inflammation associated with infection and calculi. However, there were 21 false-negative cases. There are two possible reasons for this, the first being the lack of cellular components in the voided urine specimen, and the second being that urinary cellular components including the cancer cells died out over the passage of time from sample collection to pathological examination. In this case, these cancer cells can substantially lose their mitochondrial metabolic activity, and thus, 5-ALA cannot be metabolized. To prevent the death of these cancer cells in the present study, we treated the extracorporeal 5-ALA culture within 1 h of collecting the urine samples.

The present study showed a significant difference between 5-ALA-induced fluorescent urine cytology and conventional urine cytology only in low-grade UC. Not statistically difference but a tendency toward good sensitivity in high-grade UC was observed by 5-ALA-induced fluorescent cytology, probably because of the small sample size. By further increasing the number of UC samples, the effectiveness of 5-ALA-induced fluorescent urine cytology in high-grade UC as compared with conventional urine cytology might be clarified statistically.

Several urinary biomarkers other than 5-ALA-induced fluorescent cytology have been reported (Table 4). The respective sensitivity and specificity of these markers range from 53–89 to 53–89% for the bladder tumor-associated antigen (BTA) test, 32–92% and 51–94% for the nuclear matrix protein (NMP) 22 test, and 50–71% and 66–72% for the UroVysion test [19–22]. The time taken for the examination of one urine specimen is about 180 min for BTA, 300 min for NMP 22, 4–8 days for UroVysion, and 150 min for 5-ALA-induced fluorescent cytology. In addition, the cost of one test is €38 for both NMP 22 and BTA, €310 for UroVysion, and €0.7 for ALA-induced fluorescent cytology. As described above, 5-ALA-induced fluorescent cytology showed high sensitivities within a short period of time and at much lower cost compared with these other urinary biomarkers.

There are some limitations in this study. Our study is a retrospective study of patients from a single institution, and thus, the present results need to be validated in other cohorts to definitively identify the high diagnostic efficacy of 5-ALA-induced fluorescent urine cytology for UC.

Table 4 Sensitivity, specificity, inspection time, and cost of urinary biomarkers of conventional cytology, BTA, NMP22, UroVysion, and 5-ALA-induced fluorescent cytology

Variables	Conventional	BTA	NMP22	UroVysion	5-ALA
Sensitivity	40–60%	53–89%	32–92%	50–71%	74–86%
Specificity	90–100%	53–89%	51–94%	66–72%	70–100%
Inspection time	30 min	180 min	300 min	4–8 days	150 min
Cost	€0.1	€38	€38	€310	€0.7

BTA bladder tumor-associated antigen, *NMP* nuclear matrix protein, *ALA* aminolevulinic acid

Conclusions

The present study showed that 5-ALA-induced fluorescent cytology has high sensitivity compared to the conventional cytology for UC diagnosis, especially in patients with low-stage and low-grade tumors. Furthermore, it takes a relatively shorter time than other tests and is very low in cost. These findings indicate that 5-ALA-induced fluorescent urine cytology may potentially be a very useful tool in clinical practice.

Acknowledgements We thank the study patients for permitting us to publish this report. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Compliance with ethical standards

Conflict of interest The authors declare no competing financial interest.

References

1. Malik Z, Lugaci H (1987) Destruction of erythroleukaemic cells by photoactivation of endogenous porphyrins. Br J Cancer 56:589–595
2. Nakai Y, Tatsumi Y, Miyake M et al (2016) Expression of ferrochelatase has a strong correlation in protoporphyrin IX accumulation with photodynamic detection of bladder cancer. Photodiagn Photodyn Ther 13:225–232
3. Nakai Y, Ozawa T, Mizuno F et al (2017) Spectrophotometric photodynamic detection involving extracorporeal treatment with hexaminolevulinate for bladder cancer cells in voided urine. J Cancer Res Clin Oncol 143:2309–2316
4. Jocham D, Stepp H, Waidelich R (2008) Photodynamic diagnosis in urology: state-of-the-art. Eur Urol 53:1138–1148
5. Inoue K, Anai S, Fujimoto K et al (2015) Oral 5-aminolevulinic acid mediated photodynamic diagnosis using fluorescence cystoscopy for non-muscle-invasive bladder cancer: a randomized, double-blind, multicentre phase II/III study. Photodiagn Photodyn Ther 12:193–200
6. Raab SS, Lenel JC, Cohen MB (1994) Low grade transitional cell carcinoma of the bladder. Cytologic diagnosis by key features as identified by logistic regression analysis. Cancer 74:1621–1626
7. Nakai Y, Anai S, Onishi S et al (2015) Protoporphyrin IX induced by 5-aminolevulinic acid in bladder cancer cells in voided urine can be extracorporeally quantified using a spectrophotometer. Photodiagn Photodyn Ther 12:282–288
8. Roupret M, Babjuk M, Comperat E et al (2015) European Association of Urology Guidelines on upper urinary tract urothelial cell carcinoma: 2015 update. Eur Urol 68:868–879
9. Metser U, Goldstein MA, Chawla TP et al (2012) Detection of urothelial tumors: comparison of urothelial phase with excretory phase CT urography—a prospective study. Radiology 264:110–118
10. Inoue K, Fukuhara H, Shimamoto T et al (2012) Comparison between intravesical and oral administration of 5-aminolevulinic acid in the clinical benefit of photodynamic diagnosis for nonmuscle invasive bladder cancer. Cancer 118:1062–1074
11. Potretzke AM, Knight BA, Vetter JM et al (2016) Diagnostic utility of selective upper tract urinary cytology: a systematic review and meta-analysis of the literature. Urology 96:35–43
12. Guo RQ, Hong P, Xiong GY et al (2018) Impact of ureteroscopy before radical nephroureterectomy for upper tract urothelial carcinomas on oncological outcomes: a meta-analysis. BJU Int 121:184–193
13. Glas AS, Roos D, Deutekom M et al (2003) Tumor markers in the diagnosis of primary bladder cancer. A systematic review. J Urol 169:1975–1982
14. Kriegmair M, Baumgartner R, Knuechel R et al (1994) Fluorescence photodetection of neoplastic urothelial lesions following intravesical instillation of 5-aminolevulinic acid. Urology 44:836–841
15. Krammer B, Plaetzer K (2008) ALA and its clinical impact, from bench to bedside. Photochem Photobiol Sci 7:283–289
16. Hagiya Y, Fukuhara H, Matsumoto K et al (2013) Expression levels of PEPT1 and ABCG2 play key roles in 5-aminolevulinic acid (ALA)-induced tumor-specific protoporphyrin IX (PpIX) accumulation in bladder cancer. Photodiagn Photodyn Ther 10:288–295
17. Steinbach P, Weingandt H, Baumgartner R et al (1995) Cellular fluorescence of the endogenous photosensitizer protoporphyrin IX following exposure to 5-aminolevulinic acid. Photochem Photobiol 62:887–895
18. Spiess PE, Grossman HB (2006) Fluorescence cystoscopy: is it ready for use in routine clinical practice? Curr Opin Urol 16:372–376
19. Nguyen CT, Jones JS (2008) Defining the role of NMP22 in bladder cancer surveillance. World J Urol 26:51–58
20. Poulakis V, Witzsch U, De Vries R et al (2001) A comparison of urinary nuclear matrix protein-22 and bladder tumour antigen tests with voided urinary cytology in detecting and following bladder cancer: the prognostic value of false-positive results. BJU Int 88:692–701
21. Grossman HB, Soloway M, Messing E et al (2006) Surveillance for recurrent bladder cancer using a point-of-care proteomic assay. JAMA 295:299–305
22. Kojima T, Nishiyama H, Ozono S et al (2018) Clinical evaluation of two consecutive UroVysion fluorescence in situ hybridization tests to detect intravesical recurrence of bladder cancer: a prospective blinded comparative study in Japan. Int J Clin Oncol 23:1140–1147