

Cystoscopic Diagnosis of Bladder Cancer by Intravesical Instillation of 5-Aminolevulinic Acid Induced Porphyrin Fluorescence—The Singapore Experience

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Abstract

Objectives: 1) To determine whether fluorescence cystoscopy after intravesical administration of 5-aminolevulinic acid (5-ALA) is more sensitive in detection of dysplasia and bladder cancer when compared with conventional cystoscopy. 2) To determine the safety of using 5-ALA. **Materials and Methods:** A 3% 5-ALA solution was instilled intravesically before cystoscopy in 41 patients. The 5-ALA-induced porphyrin fluorescence was excited by violet light from a xenon arc lamp (λ375-440 nm). **Results:** Among the 175 biopsies obtained, the sensitivity of the fluorescence cystoscopy was greater than that of conventional cystoscopy (89.1% versus 65.6%, $P < 0.05$). Specificity was at 64.8% and 83.8% respectively with $P < 0.05$. Duration of ALA instillation did not seem to affect the sensitivity and specificity of photodynamic diagnosis. The procedures were well tolerated by all patients with no additional complication. **Conclusions:** Fluorescence-guided biopsies are more sensitive than random biopsies in detecting dysplasia or bladder cancer without additional risk or complication. The duration of ALA instillation did not seem to affect the accuracy of photodynamic diagnosis.

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Introduction

Bladder cancer is the sixth most frequent malignant disease in the world.¹ Superficial stages of the tumour are found in 75% to 85% of cases upon first diagnosis.² More than 70% of patients with superficial tumours suffer one or several recurrences after the first treatment. Progression of the tumour is observed in about one-third of all bladder cancer patients.

Different from papillary urothelial tumours, which are endoscopically visible, flat neoplasms such as dysplasia and carcinoma *in situ*, are hardly visible during regular cystoscopy or may even be concealed in the normal appearing bladder mucosa. Flat neoplastic lesions detected by random biopsy were found to have a significant impact on the recurrence and progression rates of bladder cancer.³ The high recurrence rate is not only connected with dysplasia and carcinoma *in situ* but also with overlooked urothelial neoplasia. Regular white light cystoscopy and random cold-cup biopsies, which are the current methods of

surveillance in patients who are at high risk of recurrent bladder cancer, are insufficient to detect all urothelial neoplasia.

Therefore, methods of labelling flat urothelial neoplasms and also tiny papillary tumours with visible dyes have been sought. Since the late 1970s, with the development of photodynamic diagnosis and therapy, many photosensitive fluorescent dyes are being tested for their use as fluorescent labels of cells and tissues.⁴ Photodynamic diagnosis is a two-step process, where first a photosensitiser that will accumulate selectively and specifically in neoplastic tissue is administered. Subsequent light irradiation of the photosensitiser-bound neoplastic tissue with the appropriate wavelength of light will excite the photosensitiser and cause the abnormal tissue to fluoresce. One such photosensitiser precursor is aminolevulinic acid (ALA). ALA induces protoporphyrin IX (PPIX), an endogenous fluorescent photosensitiser that is an immediate precursor to heme in the biosynthetic pathway of intracellular heme.^{5,6}

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As heme-containing enzymes are required by cells for energy metabolism, all nucleated cells have the intrinsic capacity to synthesize PPIX. In many types of cells, heme biosynthesis is regulated at the cellular level by negative feedback on the rate limiting step of the pathway, which is the synthesis of endogenous ALA from glycine and succinyl CoA. This step may be bypassed by the addition of exogenous ALA, allowing cells to produce PPIX and heme at a rate governed by their individual enzyme profiles. This means exogenous administration of ALA would cause a time-dependent increase in the amount of porphyrins especially PPIX in the tissue in order to offset the imbalance of ALA within the cell. The amount of PPIX synthesis and accumulation will depend on the rate of conversion of PPIX into heme.⁷

There is much evidence that in several malignant cells, there is an altered metabolism of heme resulting in an increase of PPIX synthesis and accumulation due to a change in the enzyme profile of these cells and tissues.⁸ The ratio of PPIX concentration at peak accumulations in tumour cells can be about 15 times higher than their normal counterparts or adjacent stromal cells.⁹ The porphyrin ratio between neoplastic and normal urothelial cells enables tissue selectivity and specificity. ALA induced PPIX is therefore being widely used as an endogenous photosensitiser to detect and treat neoplasms in various organs including the bladder.^{5,10}

During fluorescence cystoscopy, light with a specific spectral composition (400 nm; violet) is introduced into the body endoscopically via an optical waveguide system 2 to 3 hours after the instillation of ALA into the bladder.⁹ The violet light will excite the ALA induced PPIX in the tumour tissue and cause it to fluoresce in the red. In this way tumour tissue can be distinguished from healthy tissue with a high level of contrast. It has been reported that flat neoplastic lesions such as dysplasia and carcinoma *in situ*, which may be hidden in the normal or inflamed mucosa were easily recognised using the fluorescence diagnostic technique.¹¹ Small papillary tumours could also be detected by fluorescence more rapidly and the probability of them being overlooked was smaller.¹¹

Moreover, it has been reported that the advantage of intravesical instillation of ALA is that it is topical and not systemic and therefore prevents any cutaneous adverse photoreactions due to photosensitivity.¹² There is no report of photoreaction using topical ALA.

Photodynamic diagnosis for bladder cancer has never been used in Singapore and there is no published study concerning ALA from Asia. The aims of this study were to assess the efficacy and safety of this diagnostic technique for early malignancies of the bladder in the Asian population and also to correlate the macroscopic findings obtained

from fluorescence cystoscopy with the final histopathology of the biopsied specimens.

Materials and Methods

Patient Selection

Forty-one patients (24M:17F) with a mean age of 66.8 years (range 42 to 89 years), suspected of primary or recurrent bladder cancer (January 1997 to December 1998) were selected for both white light and fluorescence cystoscopy.

Photosensitisation

The ALA solution was made fresh using 1 g of ALA hydrochloride (Merck, Darmstadt, Germany) dissolved in 50 ml of a mixture of 8.4% NaHCO₃ and physiological saline solution (15:85). The pH of the solution should be adjusted to about 6.5. This was instilled intravesically via a size 14-foley catheter after pyrogens were eliminated using a 0.2 µm filter. The solution was kept *in situ* for at least 2 hours before both white light and fluorescence cystoscopy. The patients were asked to alter position at regular intervals to ensure optimal contact between the solution and the bladder wall. The bladder was emptied and catheter removed before cystoscopy.

Photofluorescence Cystoscopy

A Xenon arc lamp (Storz GmbH, Tuttlingen, Germany) with a corresponding band pass filter system was used for photodynamic diagnosis. Blue violet light in the wavelength range between 375 and 400 nm was used to induce PPIX fluorescence. For endoscopic observation, a GG filter (2 mm) was put on the eyepiece of the endoscope (Hopkins II 30°, 70° telescope). This filter reduced the reflected blue excitation light without blocking the red fluorescence light.

The bladder was first examined under white light. The macroscopic cystoscopic findings were documented by means of a colour video camera, then fluorescence cystoscopy was performed. Biopsies were taken from fluorescent and non-fluorescent areas of the bladder under excitation with violet light. The fluorescence, macroscopic white light findings and location were documented for each biopsy. Hypervascularised, erythematous, erosive or oedematous areas of the mucosa were considered non-specifically inflamed bladder mucosa. Both conventional and fluorescence cystoscopies were done swiftly to minimise the effect of photobleaching.

Postoperative Care

Patients were usually discharged on the second or third postoperative day. Any immediate adverse event was documented. Possible complications such as urinary tract infection, bleeding and phototoxicity were advised as well.

Patients were reviewed in the outpatient clinic 3 to 4 weeks after discharge.

Statistical Analysis

The sensitivities and specificities for urothelial neoplasia detection under white light and fluorescence detection were calculated on the basis of 2 x 2 frequency tables. The histology of the biopsies was used as the confirmed diagnosis in both tables. The sensitivities and specificities between white light and fluorescence cystoscopy were later compared using Fisher's exact test with $P < 0.05$ regarded as statistically significant.

Results

Overall 175 biopsies were taken from various sites of the bladder (Table I). The final histological status is shown in Table II. Out of 39 biopsies taken from the papillary lesions, fluorescence was positive for all of them except 1 which was found to be an inverted papilloma (Table III). Within this group, 1 dysplasia and 36 urothelial tumours were detected. One patient demonstrated benign histology despite being papillary and demonstrated positive fluorescence.

TABLE I: LOCATION OF BLADDER BIOPSIES

Site	No. (%)
Posterior wall	38 (21.7)
Trigone	21 (12.0)
Right lateral wall	31 (17.7)
Left lateral wall	34 (19.4)
Dome	16 (9.1)
Others	35 (20.0)
Total	175 (100.0)

Fifty-eight of the 136 biopsies obtained from flat mucosal areas of the bladder (normal and non-specific inflammatory) were determined as being fluorescence positive lesions (Table III). A total of 10 dysplasias and 10 urothelial tumours were found in this group and all these 20 (11%) lesions had been missed by conventional white light cystoscopy.

False positive fluorescence was noted in 38 (22%) of the biopsies. Among these biopsies, 25 were found to have either cystitis, hyperplasia, metaplasia or granuloma. Seventy-eight random biopsies were obtained from area of bladder without any fluorescence. Of the 7 (11%) false negative fluorescence lesions, 5 were dysplasias and 2 were carcinomas *in situ*.

The overall sensitivity for detecting the presence of urothelial neoplasia was significantly greater for fluorescence cystoscopy than white light cystoscopy (89.1% versus 65.6%, $P < 0.05$). The overall specificity was found to be 64.8% and 83.8% for fluorescence cystoscopy and white light cystoscopy, respectively ($P < 0.05$) (Table IV).

TABLE II: HISTOLOGY

Pathology	No. (%)
Benign	111 (63.4)
Dysplasia	16 (9.1)
T _{CIS}	7 (4.0)
T _a G ₁	11 (6.3)
T _a G ₂	9 (5.1)
T _a G ₃	1 (0.6)
T ₁ G ₁	3 (1.7)
T ₁ G ₂	6 (3.4)
T ₁ G ₃	6 (3.4)
≥ T ₂	5 (2.9)
Total	175 (100)

TABLE III: COMPARISON BETWEEN CONVENTIONAL CYSTOSCOPIC FINDINGS IN CONJUNCTION WITH FLUORESCENCE FINDINGS AND PATHOLOGY

			Microscopy					
			Fluorescence (+)	Dysplasia	T _{CIS}	T _a -T ₁	≥T ₂	Benign
M	Normal	41	7	3	5	0	26	
A	Non-specific inflammatory	17	3	1	1	0	12	
C	Papillary	38	1	1	30	5	1	
R								
O								
			Fluorescence (-)	Dysplasia	T _{CIS}	T _a -T ₁	≥T ₂	Benign
M	Normal	74	5	2	0	0	67	
A	Non-specific inflammatory	4	0	0	0	0	4	
C	Papillary	1	0	0		0	1	
R								
O								
Total		175	16	7	36	5	111	

TABLE IV: EFFICACY OF CONVENTIONAL VERSUS FLUORESCENCE CYSTOSCOPY

	All patients (n = 175)		Patients with flat lesions (n = 136)	
	Conventional	Fluorescence	Conventional	Fluorescence
True (+) TP	42	57	5	20
False (+) FP	18	39	16	38
False (-) TN	22	7	22	7
True (-) FN	93	72	93	71
Sensitivity %	65.6 *	89.1 *	18.5 γ	74.1 γ
Specificity %	83.8 β	64.8 β	85.3	65.1
PPV %	70.0	59.4	23.8	34.5
NPV %	80.9	91.1	80.8	91.0

(+): dysplasia/CIS/bladder tumour

PPV: positive predictive value

NPV: negative predictive value

*, γ, β: $P < 0.05$ (Fischer's exact test)

$$\text{Sen} = \frac{\text{TP}}{\text{TP} + \text{FN}}$$

$$\text{Spec} = \frac{\text{TN}}{\text{TN} + \text{FP}}$$

$$\text{PPV} = \frac{\text{TP}}{\text{TP} + \text{FP}}$$

$$\text{NPV} = \frac{\text{TN}}{\text{TN} + \text{FN}}$$

TABLE V: SENSITIVITY AND SPECIFICITY OF FLUORESCENCE CYSTOSCOPY CORRELATED WITH DURATION OF ALA INSTILLATION

Duration	Fluorescence cystoscopy			
	% sensitivity	% specificity	% PPV	% NPV
≤2.5 hours	92.3	66.7	55.8	95.0
>2.5 hours	86.1	64.0	63.2	86.5

PPV: positive predictive value

NPV: negative predictive value

As shown in Table IV, sensitivity was also calculated separately for flat lesions (macroscopically normal bladder mucosa or bladder mucosa with non-specific inflammation). Though the sensitivity for fluorescence detection was reduced, it remained higher than that of white light cystoscopy (74.1% versus 18.5%, $P < 0.05$). The duration of ALA retention within the bladder, on the other hand, did not seem to have any effect on the overall sensitivity and specificity of fluorescence cystoscopy (Table V).

Besides 2 patients who complained of urgency and unable to retain ALA for more than 2 hours, there was no clinically significant short-term side effects such as urinary tract infection and phototoxicity. However, the duration of follow-up in our study was too short to draw any conclusion on long-term effects on bladder capacity and function.

Discussion

Although conventional white light cystoscopy is sensitive in the detection of papillary form of transitional cell carcinoma, it is difficult to visualise flat lesions such as dysplasia and carcinoma *in situ* of the urothelium. Numerous early stage and precancerous lesions escape diagnosis by random biopsy alone. Missing such flat neoplastic lesions, particularly high-grade dysplasia and carcinoma *in situ*,

can have a decisive impact on the recurrence and progression rates of bladder cancer and greatly effect the patient's prognosis.³

Therefore, different methods of labelling such flat urothelial neoplasms with visible dyes have been sought. Developments in the field of photodynamic diagnosis have been in progress since the late 1970s.⁴ Synthetic porphyrin mixture such as photofrin is manufactured outside the body and usually given systemically. None of these substances can avoid a transient sensitisation of the skin. In contrast, endogenous photosensitiser such as PPIX is produced within the body from non-photosensitising precursor, which is ALA. This can be administered topically into the bladder without any phototoxicity.

The ALA-induced PPIX fluorescence is being widely used for detection of neoplasms in various organ including the bladder.^{5,9,12-14} Kriegmair^{9,12} and D'Hallewin¹³ reported improved sensitivity for detecting bladder tumours with minimal side effects using fluorescence cystoscopy following intravesical instillation of 5-ALA.

This photodynamic diagnostic technique has never been used in Singapore. Therefore this report serves to evaluate the efficacy and safety of this diagnostic technique in Asian patients with both primary and recurrent malignancies of the bladder.

The superior sensitivity of the fluorescence cystoscopy over white light cystoscopy in detecting bladder tumours (89.1% versus 65.6%) in this study were similar to those published by Kriegmair et al.^{9,12} and D'Hallewin et al.¹³ However, the specificity of fluorescence cystoscopy seemed to be impaired (83.8% versus 64.8%, $P < 0.05$), which is different from that reported by Kriegmair who showed that violet excitation light had no impact on specificity.¹² This could probably be attributed to the initial period of learning

to differentiate equivocal fluorescent areas from those that were definitely exhibiting strong positive fluorescence.

Kriegmair et al⁹ emphasised that the high sensitivity of ALA fluorescence cystoscopy was not impaired for the detection of flat urothelial lesions. On the contrary, a decrease in the sensitivity of the procedure was noted in this study when the analysis focused only on the detection of flat urothelial tumours. Nevertheless, the sensitivity in diagnosing flat lesions remained higher for fluorescence cystoscopy than conventional white light cystoscopy.

Despite positive fluorescence, benign histology was noted in 39/96 (40.6%) biopsies. This is similar to the result reported in Kriegmair and D'Hallewin's studies^{12,13} which also found a false positive rate of 62% and 45%, respectively. However, it is the improvement in sensitivity rather than specificity, which was clearly seen in all lesions, that allows ALA photodynamic diagnosis to be a useful tool for guiding biopsies.

Similar to the finding in Kriegmair's study,¹² the majority of these false positive biopsies were taken from areas of cystitis, hyperplasia, metaplasia and granuloma. The actual *in vivo* kinetics of ALA-induced fluorescence in these benign tissues and how it is different from neoplastic tissue is still uncertain. Chronic infection, for instance, is a well-known risk factor for the development of bladder cancer. It was proposed that malignant (and premalignant) transformation might be associated with changes in the enzyme profile for the biosynthetic pathway for heme.¹⁴ It is not yet clear whether these false positive areas identified metabolically unstable areas that are at risk of future development of bladder tumour. The follow-up period in this study is too brief to draw any comments in this regard. Prospective analysis of the contribution of such areas to tumour recurrence will be another interesting issue to look into when the follow-up period is longer.

An attempt to modulate the concentration of PPIX by varying the time that the urothelium was exposed to the ALA was performed. However, the duration of ALA instillation did not seem to have any impact on the overall sensitivity and specificity of bladder tumour detection. Datta et al¹⁵ noted tumour fluorescence to peak between 2 to 6 hours after *in vitro* bladder tissue incubation in ALA. In the rat bladder tumour model, Xiao et al¹⁶ have shown the optimal incubation time to be 4 hours. As all the patients in this study had ALA instilled into the bladder between 2 to 5 hours, this may possibly explain for the lack of effect of the duration of ALA instillation on the overall accuracy of tumour detection. Moreover, one can postulate that the difference in concentration of PPIX in the normal and abnormal tissues might already reached its maximum. Further increase in the concentration of exogenous ALA or the duration of ALA exposure might have little additional

effect in tissue sensitivity and specificity unless they act to emphasise the pre-existing differences between normal and abnormal cells.

Animal study showed that there was a dose dependent increase in both tumour and urothelial PPIX concentrations after intravesical instillation of ALA.¹⁶ Chang et al¹⁷ found that a more concentrated ALA solution (10% Vs 1%) gave higher levels of PPIX in the urothelium, but less selectivity between layers of bladder wall. Kennedy et al¹⁴ observed the best fluorescence usually is produced by relatively low doses of photosensitiser. Similarly, D'Hallewin et al¹³ showed that a reduction of ALA instillation dose to 0.5 gm could decrease the incidence of false positive results. However, this effect was not evaluated in this study.

In order to improve its specificity, further studies are required to evaluate optimal method to improve tumour selectivity by enhancing the fluorescence differentials between normal and abnormal urothelium. To achieve a better understanding of the underlying metabolic differences between normal and tumour cells, quantitative analysis of fluorescence using, for example confocal microscopy is currently undertaken in the department. Hopefully this would help to evaluate the fluorescence kinetics of bladder tumour and normal urothelium.

Besides urgency symptoms in 2 patients, intravesical ALA instillation was well tolerated in all patients. None of the patients developed any clinically significant urinary tract infection during the postoperative period. All patients were followed up at 1 month and no phototoxicity or other complication was reported in any of the patients. Routine blood investigations such as liver function test and plasma porphyrin levels were not performed postoperatively because systemic absorption were not documented in either studies by both Kriegmair et al¹² and Datta et al.¹⁵ Moreover in the studies by Steinbach et al¹⁸ and Datta et al,¹⁵ fluorescence microscopy revealed ALA-induced fluorescence is mainly limited to the urothelium. Therefore one would not expect any long-term side effects on the bladder capacity and function after photodynamic diagnosis and therapy.

Conclusions

In conclusion, intravesical administration of ALA provides an efficacious and safe route for photodynamic diagnosis of bladder tumour with better sensitivity. In between 2 to 5 hours, the duration of ALA instillation did not seem to affect the accuracy of photodynamic diagnosis.

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REFERENCES

1. Kantor A F, Hartge P, Hoover R N, Fraumeni J F Jr. Epidemiological characteristic of squamous cell carcinoma and adenocarcinoma of the bladder. *Cancer Res* 1998; 48:3853-5.
2. Prout G R Jr Bladder carcinoma and a TNM system of classification. *J Urol* 1987; 117:583-90.
3. Althausen A F, Prout G R Jr, Daly J J. Non-invasive papillary carcinoma of the bladder associated with carcinoma in situ. *Urology* 1976; 116: 575-80.
4. Kelly J F, Snell M E. Hematoporphyrin derivative: A possible aid in the diagnosis and therapy of carcinoma of the bladder. *J Urol* 1976; 115: 150-1.
5. Kriegmair M, Baumgartner R, Lumper W, Waidelich R, Hofstetter A. Early clinical experience with 5-aminolevulinic acid and for the photodynamic therapy of superficial bladder cancer. *Br J Urol* 1996; 77:667-71.
6. Kennedy J C, Pottier R H. Endogenous protoporphyrin IX a clinically useful photosensitizer for photodynamic therapy. *J Photochem Photobiol B* 1992; 14:275-92.
7. Kennedy J C, Pottier R H, Pross D C. Photodynamic therapy with endogenous PPIX: basic principles and present clinical experience. *J Photochem Photobiol* 1990; 6:143-8.
8. Bedwell J, MacRobert A J, Phillips D, Bown S G. Fluorescence distribution and photodynamic effect of ALA induced PPIX in the DMH rat colonic tumour model. *Br J Cancer* 1992; 65:818-24.
9. Kriegmair M, Stepp H, Steinbach P, Lumper W, Ehsan A, Stepp H G, et al. Fluorescence cystoscopy following intravesical instillation of 5-ALA: A new procedure with a high sensitivity for detection of hardly visible urothelial neoplasia. *Urol Int* 1995; 55:190-6.
10. Kriegmair M, Baumgartner R, Knuchel R, Stepp H, Hofstadter F, Hofstetter A. Early clinical experience with 5-ALA for the photodynamic therapy of superficial bladder cancer. *Br J Urol* 1996; 77:667-71.
11. Flamm J, Dona S T. The significance of bladder quadrant in patients with primary superficial bladder carcinoma. *Eur Urol* 1989; 16:81-5.
12. Kriegmair M, Baumgartner R, Knuchel R, Stepp H, Hofstadter F, Hofstetter A. Detection of early bladder cancer by 5-ALA induced porphyrin fluorescence. *J Urol* 1996; 155:105-10.
13. D'Hallewin MA, Vanherzeele H, Baert L. Fluorescence detection of flat transitional cell carcinoma after intravesical instillation of aminolevulinic acid. *Am J Clin Oncol* 1998; 21:223-5.
14. Kennedy J C, Marcus S L, Pottier R H. Photodynamic therapy and photodiagnosis using endogenous photosensitization induced by 5-aminolevulinic acid: mechanisms and clinical results. *J Clin Laser Med Surg* 1996; 14:289-304.
15. Datta S N, Loh C S, MacRobert A J, Whatley S D, Matthews P N. Quantitative studies of the kinetics of 5-aminolaevulinic acid-induced fluorescence in bladder transitional cell carcinoma. *Br J Cancer* 1998; 78:1113-8.
16. Xiao Z, Miller G G, McCallum T J, Brown K M, Lown J W, Tulip J, et al. Biodistribution of PhotofrinII and 5-aminolevulinic acid-induced protoporphyrin IX in normal rat bladder and bladder tumor models: implications for photodynamic therapy. *Photochem Photobiol* 1998; 67:573-83.
17. Chang S C, MacRobert A J, Bown S G. Biodistribution of protoporphyrin IX in rat urinary bladder after intravesical instillation of 5-aminolevulinic acid. *J Urol* 1996; 155:1744-8.
18. Steinbach P, Kriegmair M, Baumgartner R, Hofstadter F, Knuchel R. Intravesical instillation of 5-aminolevulinic acid: the fluorescent metabolite is limited to urothelial cells. *Urology* 1994; 44:676-81.