DETECTION OF EARLY BLADDER CANCER BY 5-AMINOLEVULINIC ACID INDUCED PORPHYRIN FLUORESCENCE

MARTIN KREIGMAIR,* REINHOLD BAUMGARTNER, RUTH KNÜCHEL, HERBERT STEPP, FERDINAND HOFSTÄDTER AND ALFONS HOFSCHETTER

From the Department of Urology, University of Munich, Munich and Institute of Pathology, University of Regensburg, Regensburg, Germany

ABSTRACT

Purpose: We determined whether the sensitivity of detecting dysplasia or early bladder cancer can be improved by 5-aminolevulinic acid induced porphyrin fluorescence.

Materials and Methods: A 3% 5-aminolevulinic acid solution was instilled intravesically before cystoscopy in 104 patients. The 5-aminolevulinic acid induced porphyrin fluorescence was excited by violet light from a krypton ion laser (wavelength 406.7 nm.).

Results: The sensitivity of the fluorescence cystoscopy (96.9%) was significantly (p < 0.0001) greater than that of white light cystoscopy (72.7%). There was no impact on specificity.

Conclusions: Due to the high sensitivity of the procedure fluorescence guided biopsies are recommended instead of random biopsies.

KEY WORDS: bladder neoplasms, aminolevulinic acid, fluorescence, protoporphyrins

The fate of bladder tumor patients is closely correlated with dysplasias and early stages of urothelial tumors, which are concealed by nonspecific inflammatory lesions or normal-appearing bladder epithelium.1,2 For years methods of labeling flat urothelial neoplasms, such as dysplasias and carcinomas in situ as well as small papillary tumors in vivo, have been sought.

We developed a new method for fluorescence detection of urothelial neoplasms of the bladder based on intravesical application of 5-aminolevulinic acid, an initial substrate of heme biosynthesis. In animal experiments accumulation of fluorescent porphyrins in malignant tissues of epithelial origin could be demonstrated after exogenous administration of 5-aminolevulinic acid.3 We previously reported that the fluorescence of the urothelium induced by intravesical instillation of 5-aminolevulinic acid is correlated with dysplastic and malignant findings and, therefore, may be helpful in diagnosing bladder cancer.4 The specificity and sensitivity of the fluorescence detection of urothelial neoplasms after intravesical instillation of 5-aminolevulinic acid compared to conventional cystoscopy with white light were investigated.

MATERIALS AND METHODS

Patients. Cystoscopy was performed after intravesical instillation of 5-aminolevulinic acid in 106 patients with a suspicion of primary or recurrent bladder cancer. The 24 women and 80 men who could be evaluated were 41 to 85 years old (average age 68) and 77 had been treated previously for bladder cancer. The median number of early recurrences was 4 (range 1 to 35). The patients had a history of multiple transurethral operations and instillations, primarily of bacillus Calmette-Guérin (BCG) and mitomycin C.

Instillation of 5-aminolevulinic acid. Two to 3 hours before planned cystoscopy a 3% pH neutral solution containing 1.5 gm. 5-aminolevulinic acid hydrochloride dissolved in 50 ml. 8.4% sodium bicarbonate was instilled via a 14F catheter. The solutions were freshly prepared immediately before instillation and passed through a 0.2 μm. filter to eliminate pyrogens. Because of individual differences in bladder capacity and the routine schedule of daily operations there was an appreciable variability in the intravesical retention interval between voiding of the 5-aminolevulinic acid solution and the beginning of fluorescence cystoscopy (average 150 ± 80 minutes standard deviation, range 10 to 400). A total of 38 patients emptied the bladder at 15 to 420 minutes (average 140 ± 90) before fluorescence cystoscopy was begun.

Fluorescence cystoscopy. The violet light of a krypton ion laser was used for intravesical induction of fluorescence. The emitted wavelength of the krypton ion laser (406.7 nm.) is in the maximum of the fluorescence excitation spectrum of endogenous porphyrins. A 500 μm. plastic fiber with a biconical tip was used to conduct the laser light.5 By virtue of this modification a large area of illumination of the bladder wall is ensured with a rigid 5 or 30-degree cystoscope optical system. A rigid biopsy forceps with an additional channel to accommodate the plastic fiber for violet illumination was used to obtain the tissue specimens. A foot pedal was used to switch as desired between the violet laser light for induction of fluorescence and conventional white light illumination. The endoscopic findings were documented with a target integrating color video camera that provides for bright fluorescence images by integrating photons on the sensor chip. The integration time ranged from one-fifteenth to one-quarter of a second depending on the distance of observation.

Clinical investigation. The bladder was inspected carefully under white light. Particular attention was paid to the presence, number and location of papillary tumors. Biopsies were obtained from fluorescent and nonfluorescent areas of the bladder under excitation with violet light. The corresponding fluorescence findings (positive versus negative), macroscopic findings (hypervascularized, eroded or edematous), scars on the bladder wall, such as those observed after transurethral electro-resection, were considered normal findings. Hypervascularized, eroded or edematous areas of mucosa were considered nonspecifically inflamed.

Statistical analysis. The sensitivity and specificity were computed for the biopsies obtained under white light and compared to additional fluorescence detection on the basis of 2 x 2 frequency tables. The final histological status of the biopsies served as the confirmed condition in both tables.

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* Requests for reprints: Urologische Klinik, Klinikum Grosshadern, Marchioninistrasse 18, 81377 München, Germany.
white light was judged positive for papillary tumors or non-specific inflamed mucosa and negative for normal-appearing urothelium (table 1). For papillary tumors that were missed during the initial cystoscopy under white light but were detected subsequently because of the red protoporphyrin IX fluorescence using violet light the test result was judged positive for those within non-specific inflammatory mucosa and negative for those within normal-appearing areas of the bladder wall. The test result determined by fluorescence cystoscopy was considered positive when a lesion demonstrated fluorescence regardless of the macroscopic finding and when papillary but nonfluorescent tumors were present (table 2). The presence of nonfluorescent normal-appearing or non-specific inflammatory mucosa was considered a negative test result. The sensitivities and specificities were compared with chi-square analysis or Fisher's exact test, with p <0.050 considered statistically significant.

**RESULTS**

A total of 449 biopsies was obtained (average 4.4 per patient, range 1 to 11) and 433 excised specimens could be evaluated. Exclusively fluorescent lesions were biopsied in 28 patients and 21 had neoplastic lesions. The final tumor status is shown in table 3. Within this group 7 patients exclusively demonstrated false-positive findings. In 11 patients the entire urothelium was negative for fluorescence and all biopsies were true negative. Tissue samples were obtained from fluorescent and nonfluorescent areas of the bladder in 65 patients and none had exclusively false-positive biopsies, while 4 had false-negative findings. Calculated on a per patient basis, the sensitivity of the procedure was 94.2% and the specificity was 80.0%.

A total of 35 urothelial neoplasms (12 dysplasias, 2 carcinomas in situ, and 20 superficial and 1 muscle-infiltrating urothelial carcinomas) could not be detected under white light (table 1). Of these 35 tissue specimens 14 were from normal-appearing urothelium and 21 were from papillary tumors. Except for a low grade dysplasia, these macroscopically normal urothelial neoplasms could be identified by an intensive red fluorescence (table 2). In 1 case a muscle-infiltrating tumor was concealed under a fluorescent scar (table 1). The figure exemplifies such findings in 21 patients, in 11 of whom suspicion was raised due to repeatedly suspect urinary cytology results. Diagnosis was verified by fluorescence cystoscopy.

In the mucosa containing inflammatory lesions 15 of 58 fluorescent areas proved to be neoplastic, whereas a highly differentiated superficial urothelial carcinoma was encountered in only 1 of 27 nonfluorescent findings (table 2). Ten planar papillary lesions found on initial inspection of the bladder under white light were also evaluated as nonspecific inflammation (table 1). However, they were unequivocally identified as papillary on subsequent fluorescent cystoscopy.

For detection of dysplasia and urothelial cancer, the overall sensitivity of 5-aminolevulinic acid based fluorescence cystoscopy (96.9%) was significantly greater (p <0.0001) than that of white light cystoscopy (72.7%). The specificity of white light (68.5%) and fluorescence (66.8%) cystoscopy did not differ significantly. Dysplasias and carcinomas in situ were found with a sensitivity of 41.5% with white light, which increased to 95.8% on appraisal of the fluorescent findings (table 4). If the analysis concentrates on the intermediate dysplasias and carcinomas in situ, the gain in sensitivity remains significant. On exclusive evaluation of carcinomas in situ a significant difference could not be detected due to the few cases (6) evaluated. Papillary tumors are detected by fluorescence cystoscopy after intravesical instillation of 5-aminolevulinic acid with a sensitivity of 93.3%. Compared to conventional cystoscopy under white light, this increase is significant by 20%. No significant differences with regard to specificity between white light and fluorescent cystoscopy were noted in any case.

**Table 1. Numbers of bladder biopsies comparing the macroscopic findings under white light and histological results**

<table>
<thead>
<tr>
<th>Macroscopic Findings</th>
<th>Pos.</th>
<th>Neg.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dysplasia Grade 1</td>
<td>Dysplasia Grade 2</td>
</tr>
<tr>
<td>Normal</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Non-specific inflammatory mucosa</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Papillary tumor within non-specific inflammatory mucosa</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Papillary tumor</td>
<td>61</td>
<td>6</td>
</tr>
<tr>
<td>Non-specific inflammatory mucosa</td>
<td>21</td>
<td>6</td>
</tr>
</tbody>
</table>

* Papillary tumor confined to mucosa or invading the lamina propria.
† Tumor invading muscle at least superficially.

**Table 2. Number of bladder biopsies comparing macroscopic findings under white light cystoscopy in conjunction with fluorescence findings and histological results**

<table>
<thead>
<tr>
<th>Fluorescence Cystoscopy</th>
<th>Histology</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Dysplasia Grade 1</td>
</tr>
<tr>
<td>Pos.:</td>
<td>6</td>
</tr>
<tr>
<td>Normal</td>
<td>3</td>
</tr>
<tr>
<td>Non-specific inflammatory mucosa</td>
<td>1</td>
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<tr>
<td>Papillary</td>
<td>61</td>
</tr>
<tr>
<td>Non-specific inflammatory mucosa</td>
<td>21</td>
</tr>
</tbody>
</table>

* Papillary tumor confined to mucosa or invading the lamina propria.
† Tumor invading muscle at least superficially.
In normal bladder mucosa or bladder mucosa with nonspecific inflammation dysplasias or malignant neoplasms were detected by white light cystoscopy with a sensitivity of 53.3%. With excitation of the protoporphyrin fluorescence under violet laser light the sensitivity increased significantly to 93.3% (table 5). A significant increase in sensitivity without impairment of specificity could also be documented for the detection of dysplasias and malignant lesions from normal and nonspecifically inflamed urothelium in patients who received prior intravesical instillation of BCG or chemotherapeutic agents (table 4). It could not be established whether the duration of intravesical 5-aminolevulinic acid retention or the interval between voiding of the 5-aminolevulinic acid solution and the beginning of the endoscopic investigation affected the sensitivity and specificity. However, false-positive findings occurred more frequently in the region of the trigone, bladder neck and anterior bladder wall than in the remainder of the bladder, so that a significantly lower specificity was noted compared to that of the posterior and lateral walls of the bladder (table 6). A significantly increased incidence of biopsies with histological signs of chronic or acute inflammation from certain areas of the bladder could not be observed.

Despite positive fluorescence, 102 biopsies were considered benign. On precise analysis of these tissue specimens those with florid cystitis, hyperplastic urothelium or hyperemic submucosa were significantly more frequently false-positive than biopsies from normal, chronically inflamed or edematosely altered urothelium (p <0.0121).

No serious side effects were observed in any of the 104 patients investigated during or after intravesical 5-aminolevulinic acid instillation. After instillation of this agent 7 patients complained of supra-symphysial pain, alginuresis or symptoms of urgency. Consequently, 3 patients voided the 5-aminolevulinic acid solution within 30 minutes and 6 were only able to retain the solution for 30 to 60 minutes. However, all 9 patients with retention of 5-aminolevulinic acid for less than 60 minutes had a restricted bladder capacity of less than 250 ml. due to numerous prior transurethral operations, and they already suffered from pollakiuria and nocturia. After fluorescence cystoscopy, more severe alginuresis symptoms and pollakiuria were detected in 4 patients. Significant gram-negative bacteriuria was detected in 3 patients but the symptoms improved rapidly with appropriate antibiotics and spasmylytic agents.

Phototoxic skin reactions, such as those observed regularly with systemic administration of synthetic porphyrin mixtures, were not detected in any of the 106 patients. 5-Aminolevulinic acid and various products of porphyrin metabolism (porphobilinogen, uroporphyrin, coproporphyrin, protoporphyrin) were found in the urine, feces and erythrocytes from 14 patients in whom the solution was retained intravesically for at least 1 hour to detect a possible systemic absorption of
FLUORESCENCE DETECTION OF BLADDER CANCER WITH 5-AMINOLEVULINIC ACID

**TABLE 4. Sensitivity and specificity of white light versus fluorescence cystoscopy**

<table>
<thead>
<tr>
<th>Dysplasia Grade</th>
<th>White Light Cystoscopy</th>
<th>Fluorescence Cystoscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 + Ca In Situ</td>
<td>2 + Ca In Situ</td>
</tr>
<tr>
<td>No. true pos.</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>No. false-pos.</td>
<td>96</td>
<td>99</td>
</tr>
<tr>
<td>No. false-neg.</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>No. true neg.</td>
<td>209</td>
<td>217</td>
</tr>
<tr>
<td>% Sensitivity</td>
<td>41.5%</td>
<td>53.3%</td>
</tr>
<tr>
<td>% Specificity</td>
<td>68.6%</td>
<td>37.7%</td>
</tr>
<tr>
<td>% Prevalence</td>
<td>7.3%</td>
<td>4.0%</td>
</tr>
</tbody>
</table>

* Papillary tumor confined to mucosa or invading lamina propria.
† p < 0.0005 (chi-square analysis).
‡ p = 0.0774 (Fisher’s exact test).
§ p = 0.0001 (chi-square analysis).

**DISCUSSION**

Dysplasias, carcinomas in situ as well as small papillary tumors may be concealed in normal-appearing mucosa or by nonspecific lesions in bladder tumor patients. Particularly, high grade dysplasias and carcinomas in situ have a crucial effect on the rates of progression and recurrence.1-5 Histological diagnosis is made on the basis of random biopsies from various areas of the bladder.6 In a prospective analysis of regression, the detection of dysplasias or carcinomas in situ in the random biopsy proved to be an important prognostic factor with regard to tumor progression.10 However, even 40% of patients with a small solitary papillary tumor (stage pTa) and no other lesions had recurrences at 3 years. Therefore, it is logical to suspect that numerous early stage and preneoplastic lesions escape diagnosis by random biopsy.

Attempts have been made to develop various methods for in vivo labeling of early stage urothelial neoplasms of the bladder. Methods based on detection of the fluorescence of systemically administered tetracyclines or synthetic porphyrin derivatives have only been tested in a few patients and later abandoned.11 Intravesical instillation of methylene blue has also proved unsuitable, since 70% of carcinomas in situ and 84% of dysplastic lesions could not be stained.

We used 5-aminolevulinic acid to label urothelial neoplasms. After intravesical application 5-aminolevulinic acid gives rise to an accumulation of endogenous porphyrins in the urothelium. The typical red fluorescence of the porphyrin storing urothelial lesions can be induced with violet light. Compared to all other methods of fluorescence detection, fluorescence diagnosis with 5-aminolevulinic acid has the crucial advantage that the fluorescence can be discerned with the naked eye, so that elaborate image processing techniques are unnecessary.

In contrast to conventional cystoscopy with white light, a significant increase in sensitivity for diagnosis of planar urothelial lesions, such as dysplasias and carcinomas in situ, as well as for papillary tumors can be detected with additional evaluation of porphyrin fluorescence. Due to the few false-negative findings, the sensitivity of the procedure is outstanding. The method proved to be advantageous particularly for detection of urothelial neoplasms in areas of the bladder wall with nonspecific inflammation. Such changes are frequently observed in patients with recurrent bladder carcinomas and after intravesical instillation of BCG or chemotherapeutic agents. For these patients the sensitivity of cystoscopy can be significantly increased by 29% with fluorescent labeling. However, 35 dysplastic or malignant lesions were also discovered in the bladder wall, which appeared normal under white light.

The specificity of cystoscopy (approximately 70%) is not impaired by appraisal of the bladder wall with violet excitation light. Of note, the specificity in the region of the trigone, bladder neck and anterior bladder wall was significantly poorer than that in the posterior and lateral walls of the bladder, possibly due to a higher proportion of inflammatory changes in these regions but this suspicion could not be verified in our biopsies.

The technical requirements for this method merely comprise a violet light source in the region of the maximum fluorescence excitation spectrum of protoporphyrin IX. In the meantime, we replaced the krypton ion laser as a light source by a special xenon arc lamp with a corresponding band pass filter (375 to 440 nm.). The decisive advantage of this light source is that the violet excitation light is directly coupled into the optical system of the endoscopes. Therefore, all conventional available endoscopic instruments can be used in combination with fluorescence diagnosis.

Orientation in the bladder under violet excitation light is excellent. Due to the high contrast obtained the red fluorescence can be easily seen. Therefore, it also is desirable to perform transurethral electroresection of bladder tumors under fluorescent control, since the entire extent of the tumor cannot always be appraised with certainty under white light.
After transurethral resection of superficial bladder cancer, tumor remnants were found in 43.5% of the cases at repeat resection 1 or 2 weeks later. It is possible that the detection of the fluorescence induced by 5-aminolevulinic acid would enable increased radical excision during transurethral electroresection.

Fluorescence detection after intravesical instillation of 5-aminolevulinic acid has also proved to be helpful with cytologically doubtful findings. Diagnosis of a malignant urothelial neoplasm was confirmed histologically in 11 of 21 patients. Cytology and fluorescence cystoscopy should complement each other. Side effects that would contraindicate widespread clinical use of the method were not detected. In particular, there was no evidence of systemic absorption or cutaneous photosensitization.

CONCLUSIONS

Appraisal of 5-aminolevulinic acid induced fluorescence of the urothelium in addition to the macroscopic findings noted under white light has revealed a significant improvement in the sensitivity of the diagnostic process. Photodynamic diagnosis of urothelial neoplasms. In case of suspicious urine cytological findings, fluorescence cystoscopy might be useful to detect the precise site of the malignancy. A decrease in recurrence rates is expected for transurethral resection of bladder cancer performed under violet light following intravesical 5-aminolevulinic acid instillation. However, this result must be demonstrated in prospective randomized trials.

REFERENCES


REPLY BY AUTHORS

We welcome the editorial comment since it points out the controversy about the necessity of diagnosing neoplastic alterations of the entire urothelium. Some months ago Zimmern et al reported the first staining test with methylene blue in the diagnosis of premalignant and tumor lesions of the bladder. Eur. Urol., 13: 15, 1987.
clinical experience with a new approach for detection of bladder cancer based on intravenously administered fluorescein. The usefulness of this procedure was evaluated in 10 patients with bladder cancer and was underlined by an editorial comment.

The indifference noted in the comment is due to the relatively small percent of relevant neoplasms. However, 2 carcinoma in situ tumors were detected as fluorescing lesions in areas of normal appearing urothelium in patients with primary Ta grade 1 disease. Kiemeny et al found flat lesions like dysplasia and carcinoma in situ in 11% of patients with solitary Ta grade 1 tumors in select biopsies from normal appearing mucosa. Dysplasia and carcinoma in situ were revealed by 5-aminolevulinic acid induced fluorescence in 33% of our patients (5 of 15) with solitary Ta grade 1 lesions from normal appearing mucosa. Kiemeny et al did not report biopsies from non-specific inflamed areas and, therefore, it is questionable whether all carcinoma in situ lesions had been obtained from areas of normal appearing urothelium (reference 10 in article). Fluorescing lesions from nonspecific inflamed areas demonstrated 7 important tumors (4 carcinoma in situ and 3 Ta). Important lesions from nonfluorescing and nonspecific inflamed areas were not observed. We expect that obtaining biopsies from fluorescing normal or nonspecific inflamed areas will increase the detection of important lesions.

We detected 20 small Ta low grade tumors in the normal appearing mucosa and, in addition, 13 Ta lesions in the nonspecific inflamed areas due to their positive fluorescence signal. In contrast, only 1 of 27 biopsies from nonfluorescing but nonspecific inflamed lesions was false-negative, containing a Ta grade 1 neoplasm. Why should urologists refrain from resecting these tumors by an easy procedure even if there is little risk of progression? The small papillary tumor in part B of the figure exemplifies the tumor specificity of 5-aminolevulinic acid in a lesion that is known not to be highly proliferating, and indicates that the technique will be helpful in resecting tumorous areas more clearly and will most certainly detect small tumors of higher initial grades of malignancy as well. Patients presenting with recurrent multifocal papillary tumors every 3 to 6 months may benefit from a longer disease-free period.

A retrospective analysis can draw attention but cannot prove whether biopsies from normal mucosa are of prognostic impact. In 1993 Kiemeney et al demonstrated that the result of biopsies from normal appearing urothelium (normal versus dysplasia/carcinoma in situ) was statistically significant in determining disease progression (reference 10 in article). In 1994 the retrospective review by these authors described a statistically significant difference in progression between patients without random biopsies and those with dysplasia/carcinoma in situ in random biopsies (Cox multivariate regression analysis, relative risk 1.57, 95% confidential interval 1.03 to 2.39). No difference was noted regardless of whether random biopsies were performed or dysplastic changes were found. That situation is what we expect since finding flat neoplastic lesions in normal appearing mucosa is like finding the proverbial pin in a haystack. Due to the high sensitivity of the procedure select biopsies from fluorescence-negative areas of the bladder seem to be unnecessary, and in patients with repeatedly suspicious cytology findings the procedure will help to clarify the situation.

Meanwhile the procedure has been further simplified. The krypton ion laser for fluorescence excitation was replaced by a xenon arc lamp with a violet filter system. All conventional endoscopic instruments can be combined and transurethral resection can be performed under fluorescence guidance. Switching between violet and white light is easily controlled by a foot pedal. The procedure can be done in addition to all of the technical points specified by the editorial comment. The procedure meets all requirements for wide clinical testing and should be proved in prospective randomized trials.
