Urol Res (2002) 30: 295–300
DOI 10.1007/s00240-002-0255-4

ORIGINAL PAPER

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Autofluorescence and 5-aminolevulinic acid induced fluorescence diagnosis of penile carcinoma – new techniques to monitor Nd:YAG laser therapy

Received: 13 November 2001 / Accepted: 20 March 2002 / Published online: 18 June 2002

Abstract Nd:YAG laser coagulation is possible for superficial tumors of the penis. The value of photodynamic diagnosis (PDD) and autofluorescence imaging (AF) in detecting malignant lesions on the penis was evaluated. Twelve patients with biopsy-confirmed squamous cell cancer (SCC) of the penis were examined with PDD and AF. For the PDD and AF the penis was illuminated with the blue excitation light from a xenon arch lamp. Biopsies were taken from suspicious lesions detected by PDD or AF and then treated with Nd:YAG laser coagulation. Neoplastic lesions presented with a positive red fluorescence under PDD or a diminished appearance under AF. The HPV-analysis was positive in eight of the 12 lesions. Fluorescence diagnosis is used for the detection of neoplastic lesions. It assists the urologist in detecting neoplastic and preneoplastic lesions, ensuring a more reliable destruction of all tumor material in penile sparing surgery.

Keywords Cancer control · Flat lesions · Fluorescence diagnosis · Laser therapy · Penile sparing surgery

Introduction

Penile carcinoma is a devastating diagnosis for the patient due to the aggressive nature of the disease and the gold standard of partial or total penectomy. It is a rare disease in the United States and Europe [10] accounting for 0.4%–0.65% of all malignancies. In Brazil penile cancer is responsible for 2.1% of all malignancies and even reaches up to 17% in some regions due to the higher prevalence of human papilloma virus (HPV) and phimotic balanoposthitis [4]. Although it primarily affects men with a mean age of 57 years, up to 16% of patients are below the age of 40 [5, 6, 13]. Early diagnosis and treatment are crucial since the 5 year survival rate of 66% for patients with organ confined disease is halved once metastatic spread to the inguinal nodes has occurred [27]. Superficial tumor stages up to T1 can be safely treated with penile sparing surgery, avoiding a mutilating amputation. But the recurrence rates are higher in comparison with the amputation techniques. The main reason can be found in the wider resection margins as well as the elimination of accompanying occult neoplastic lesions with amputation. In order to optimize penile sparing techniques, it is necessary to develop more sensitive methods for the detection of carcinoma in situ (CIS), potential preneoplastic lesions such as penile intraepithelial lesions (PENIN) as well as subclinical high risk HPV lesions. PENIN describes cell atypia and atypical mitotic figures in the epithelial layer above the basal membrane. PENIN grade III is identical with CIS [14]. Additionally, the exact definition of tumor edges is mandatory to avoid local recurrence. The acetic acid test (AAT) is the standard test for the diagnosis and demarcation of subclinical HPV lesions of the outer genitalia [19]. Good results in identifying malignant and HPV associated lesions in the urethra and on the penile shaft by fluorescence diagnosis after application of 5-aminolevulinic acid (5-ALA) were described in a previous study [24]. Autofluorescence imaging (AF) is used for detecting neoplastic lesions in several medical disciplines including pulmonology [12] and ENT [3]. The value of photodynamic diagnosis (PDD) and AF in the detection of penile cancer was evaluated in a pilot study and the results of this new techniques were compared with those of the AAT.

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Material and methods

Twelve patients with biopsy-proven penile cancer were designated to undergo Nd:YAG laser therapy. Six of these patients were diagnosed with CIS. Of the other six patients, two were diagnosed with T1 G1 and the other four with T1 G2 tumors. Eight of the 12 patients were examined with AAT and PDD, four patients were additionally inspected with AF. All images were recorded on video tape for later comparison. All patients were Caucasians between 47 and 68 years old (mean age: 62 years) and had no signs of lymphatic or metastatic spread. Depending on tumor stage, patients underwent clinical examination of the inguinal nodes only in superficial stages up to T1 tumors and for stages T1 and higher evaluation with CT or MRI scan. A total of 28 biopsies were taken. At 24 h before the planned operation, all patients underwent acetic acid mapping of the penis after the application of 5% acetic acid for 20 min, for later comparison. On the day of surgery, a 1% solution of 5-ALA and lidocain jelly (Instillagel, Farco Pharma, Cologne, Germany) was applied to the glans and the shaft of the penis. A condom was placed to expose the entire epithelium of the penis to the 5-ALA allowing an even distribution of the agent, as previously described [24]. 5- ALA, a compound in the heme biosynthesis pathway, is intracellularly converted into fluorescent protoporphyrin IX (PPIX), an agent that selectively accumulates in tumors [25]. Following excitation with the blue light from a xenon-arch lamp (Fa. STORZ, Tuttlingen, Germany) with a wavelength between 375 and 440 nm, PPIX emits fluorescence in the red wavelength range (peaks at 635 and 705 nm) [26]. The red fluorescence can be visualized with the help of a yellow observation filter to cut out a large portion of the blue excitation light. The image can be seen either with the naked eye or a special RBG-camera (red-blue-green).

Autofluorescence imaging does not require the preoperative application of 5-ALA. Autofluorescence of the tissue can be visualized after the tissue is excited with the same xenon-arch lamp as used for the PDD. Instead of a yellow, a green observation filter is placed on the RBG-camera and the images can be visualized by the naked eye or through the camera on a video screen. Suspicious lesions are detected by their diminished autofluorescence appearance in the otherwise even green background of the normal mucosa (Fig. 1).

In the operation theater, the condom was removed and the penis inspected under regular white light. Afterwards, the room lights were dimmed and the penis was illuminated with the blue excitation light for the PDD. Suspicious penile lesions were detected by their bright red fluorescence. After changing to the green observation filter, the suspicious lesions were now visualized by their diminished autofluorescence. The AF and PDD images were then compared with the images obtained from the AAT performed the day before. Biopsies were taken from all suspicious lesions that showed a positive result under any of the described imaging techniques. One biopsy was taken from the tumor margin, being identified by the red fluorescence and diminished autofluorescence, respectively. It was send separately for histopathological examination. Additionally, one biopsy was taken from an area which was non-suspicious under AAT, AF and PDD. Afterwards the detected lesions were treated with Nd:YAG laser coagulation (Dornier, Germering, Germany) in continuous wave mode at 30–50 W console power in air ($\lambda = 1{,}064$ nm). The treatment energy was dependent on the size of the lesion with an irradiation time of 100 s (range: 60–150 s). After treating the lesion, a safety margin of 3 mm around the lesion was coagulated. The laser energy was delivered by a 600 µm bare fiber (Dornier, Germering, Germany) with no tissue contact and a fiber-tissue distance between 0.5 and 1 cm (NA 0.34). The focal spot depended on the fiber-tissue distance and followed a gaussian intensity profile. The laser treated areas were ablated with a scalpel and biopsies were taken from the tumor base. After the laser coagulation, the penis was again examined with fluorescence diagnosis to check for remaining fluorescence. Laser coagulated areas with residual positive fluorescence were biopsied and laser coagulated again. All biopsies, before and after laser treatment, and biopsies from the tumor base were sent for histopathological examination and HPV analysis. Most institutions treating penile carcinoma already have a Nd:YAG laser. For the PDD we used the same xenon-arch lamp as used for PDD of the bladder. While PDD also needs the application of 5-ALA, AF is done without 5-ALA. HPV-typing was performed by restriction fragment length polymorphism (RFLP) analysis of PCR products and subsequent hybridization with a generic oligonucleotide probe, as recently described by Meyer et al. [20].

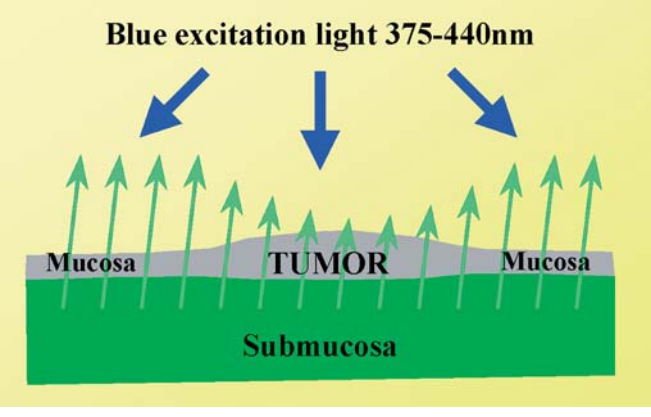

Fig. 1. Autofluorescence imaging. Diminished autofluorescence in areas of increased cell layers (tumor) after blue light excitation (375–440 nm) by a xenon arch lamp

Results

All of the lesions which were macroscopically visible under white light ($n = 6$) were detected with each of the described imaging techniques, revealing noninvasive SCC up to T1 (Table 1). The biopsies taken from the non-suspicious areas ($n = 12$) under AAT, PDD and AF all presented histopathologically as nonmalignant squamous cells. In the HPV analysis, the preneoplastic PENIN lesions as well as the neoplastic squamous cell cancers were associated with the high risk HPV types 6, 16 or 33 in six of the 12 patients (Table 1).

With the use of the AAT, all PENIN ($n = 2$), bowenoid papulosis (BP) ($n = 2$), and CIS ($n = 6$), as well as eight out of nine subclinical HPV lesions (Table 1) were identified by the typical whitish demarcation.

After being excited with the xenon-arch lamp all but one of the histopathologically proven neoplastic lesions showed a bright red fluorescence (Fig. 2). The HPV infected areas presented with a sharply demarcated positive red fluorescence as well. One hyperplasia and one dysplasia also presented as fluorescence positive. After being excited to emit autofluorescence, all histopathologically proven neoplastic lesions were detected by their diminished autofluorescence (Fig. 3). Although the HPV lesions were detected, the AF image missed the sharp demarcation in comparison with the PDD (Table 1). The separately taken biopsy of the PDD and AF positive margin around the lesion proved to be SCC.

Since all imaging techniques were recorded on video tape, an exact correlation of the three methods was

Table 1. Histopathological results and imaging techniques of the biopsies from12 patients. Number of biopsies, histopathological results, HPV types and the corresponding positive (pos) or negative (neg) results of the three different imaging techniques in comparison with the results under white light. PDD: photodynamic diagnosis, AF: autofluorescence diagnosis, HPV: human papilloma virus, BP: bowenoid papulosis, PENIN: penile intraepithelial neoplasia, CIS: carcinoma in situ, SCC: squamous cell carcinoma

	White light		Acetic-acid test		PDD		AF	
	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive
Normal epithelium	12		12		12		4	
Condylomata acuminata	1			1		1		1
Hyperplasia	1			1		1		1
Dysplasia	1		1			1		1
Ulcer		1	1		1			1
HPV 6	1			1		1		1
HPV 16	5		1	4		5		2
HPV 33	3			3		3		2
BP	2			2		2		
PENIN III	2			2		2		1
CIS	6			6	1	5		6
SCC	1	5		6		6		1

Fig. 2. Macroscopic squamous cell carcinoma detected with photodynamic diagnosis with clearly demarcated tumor edge

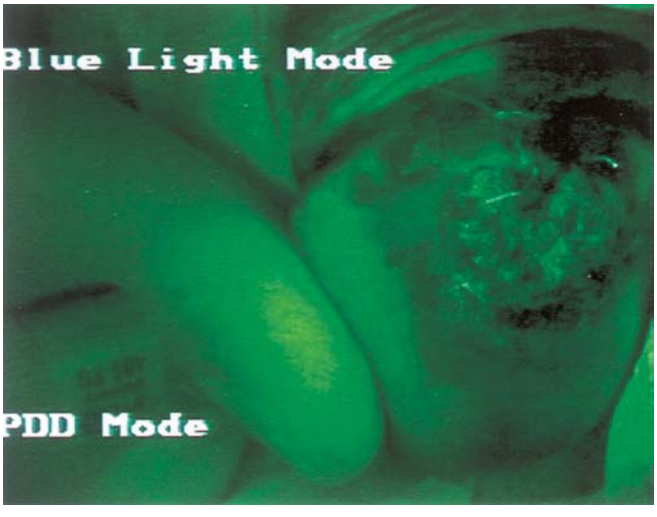

Fig. 3. Same lesion as in Fig. 2 under autofluorescence imaging, identifying the lesion in the same borders

possible. The results of the AF correlated with the PDD results in almost all of the cases. In all the cases in which the results of PDD and AF matched, the lesions were detected within the same borders. In two patients with large macroscopic lesions, residual fluorescence was detected after extensive laser coagulation and ablation of the whole area (Fig. 4). Those areas under white light which were not suspicious were biopsied again and proved to be positive for SCC in the histopathological examination (Fig. 5).

The red laser pilot of the Nd:YAG laser does not allow the laser coagulation of the lesions under PDD since the red pilot is camouflaged by the red fluorescence of the lesions under the excitation light. But the red pilot can easily be detected during the AF imaging and therefore an online-controlled coagulation of the suspicious lesions within their exact borders was performed. The whitish coagulated tissue after laser treatment cannot be differentiated from the whitish demarcation of the AAT.

No side effects from the AAT, the PDD or the AF imaging techniques were observed.

Discussion

The association of penile cancer with preexisting inflammatory lesions such as kerotic balanitis, leukoplakia and especially the high risk human papilloma viruses (HPV) types 16,18 and 33 are well established [18]. For benign lesions as well as for premalignant lesions, laser coagulation is an established form of treatment [22]. While PENIN-lesions, CIS, noninvasive verrucous carcinoma (Ta) and T1 tumors can be safely treated with penile sparing surgery, the recommended treatment for T2 and higher tumors remains partial or total penectomy [15]. Due to good tumor control and adequate cosmetic results, Nd:YAG laser coagulation has became the standard form of treatment for superficial penile tumors up to T1 [7, 23]. Although successful laser coagulation

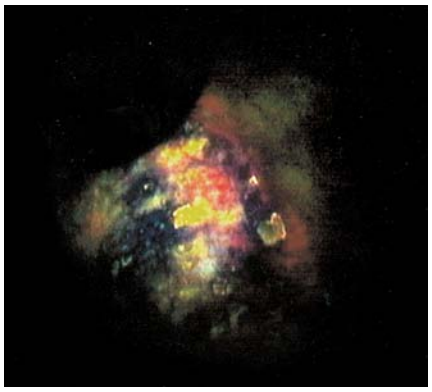

Fig. 4. Remaining squamous cell cancer after Nd:YAG laser coagulation and manual ablation, detected by the red fluorescence under control PDD

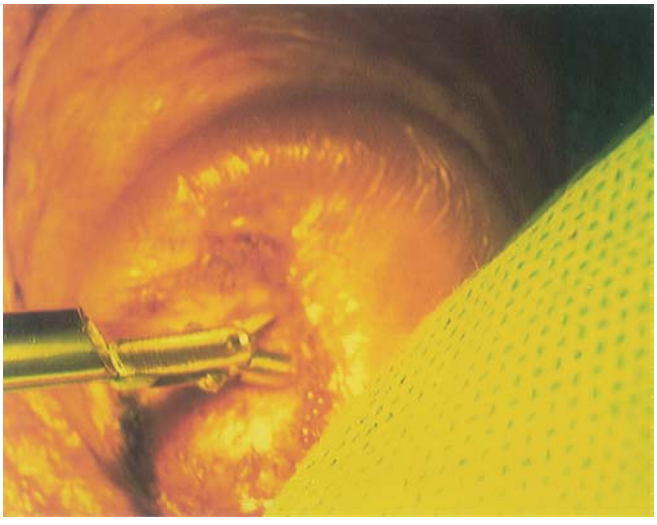

Fig. 5. Exact biopsy of the fluorescence positive lesion described in Fig. 4

has been reported for T2 tumors, the recurrence rates for invasive tumors suggest that partial or total penectomy with a safety margin of 2 cm is superior for invasive tumors [15].

Fluorescence diagnosis after application of 5-ALA is a sensitive method for the detection of subclinical HPV lesions on the penile skin. Additionally Schneede et al. [24] were able to detect flat condylomata acuminata in the urethra due to their red fluorescence during urethroscopy after the urethral instillation of a 0.1% solution of 5-aminolevulinic acid. As with bladder cancer, neoplastic lesions can be visualized by their bright red fluorescence after excitation with the blue light of a xenon-arch lamp.

In this report, autofluorescence imaging of the penis was performed in order to detect premalignant, malignant and HPV infected areas, the results were then compared with the PDD and the AAT. Since the results from the three diagnostic detection methods were recorded on video, a comparison and critical evaluation of the results from the histopathological specimens which were obtained was possible. Special consideration was given to their value in the assistance of Nd:YAG laser therapy.

With the use of 5% acetic acid, subclinical HPV associated and malignant lesions of the penis can be detected under magnification by a whitish demarcation from their non-suspicious surrounding [19]. While it is a sensitive test with detection rates of 95% for HPV associated lesions, the specificity only ranges around 42% [2]. Due to the unspecific whitening of inflammations or injuries of the penile epithelium, a discrimination from neoplastic areas is hardly possible. The sensitivity and specificity of the AAT for the identification of penile carcinoma has not been evaluated on larger numbers of patients and it is not certain if the whitish demarcation is due to the HPV infection or the neoplastic changes of the epithelium.

Autofluorescence imaging is already used in the early detection of lung and gastrointestinal SCC to enhance the detection of severe dysplasia and carcinoma in situ [1, 9]. Initial trials with laser induced autofluorescence (LIF) described a high sensitivity and specificity in the detection of epithelial lesions in the bladder [17]. An autofluorescence imaging system for the detection of malignant lesions in the bladder has recently been developed [8].

Autofluorescence is described as the tissues own fluorescence which can be perceived after the fluorochromes (e.g. flavin or porphyrin) in the submucosa of the corresponding tissues are excited by a suitable light source. The intensity of the perceptible AF is dependent upon the amount of cell-layer above the submucosa and the number of fluorochromes. The increased proliferation and decreased apoptotic activity of malignant cells leads to an increase of cell-layers above the submucosa resulting in reduced autofluorescence in the observed areas [8]. (Fig. 2). In our study, the macroscopically visible malignant lesions produced a clearly diminished AF (Fig. 3) and were correctly identified as neoplastic lesions. Additionally, the tumor edges as well as the biopsy-confirmed flat precancerous and malignant lesions were clearly demarcated by their diminished AF from the non-suspicious surrounding squamous epithelium of the penis. The images recorded from the PDD after application of 5-ALA and the images from the AF detected the suspicious lesions within the same borders. The whitish demarcated areas recorded from the AAT corresponded with the images generated by the fluorescence methods in location, but the borders of the lesions did not appear as clear-cut and were often smaller in diameter. The individual value of the three different imaging techniques can only be appreciated when applied to a larger number of patients. PDD has proven to be a highly sensitive method in detecting neoplastic tissues, but PPIX, although sensitive, has a higher false positive rate as it accumulates in benign conditions like condylomata acuminata and infections. While AF has a higher specificity than PDD, the combination of PDD

and AF achieved a better overall specificity in bladder cancer [8].

As pointed out by Malek [19], various degrees of PENIN, CIS and HPV associated lesions exist in the periphery of the visible macroscopic lesion. In penile sparing surgery these lesions, as well as tumor edges, may be left behind, building the grounds for recurrent disease. With penile amputation, the co-existing lesions are removed as well, keeping the recurrence rates as low as 20% [16]. Organ sparing surgery on the other hand has recurrence rates of up to 50%, however, the rate improves (10–30%) with laser coagulation [24]. Fluorescence diagnosis helps to guide laser therapy, therefore achieving a complete denaturation of all existing tumor material. Although the red laser pilot cannot be seen in the positive red fluorescence of the suspicious lesions, the light entities can be easily switched from blue to white using a foot switch [12] allowing the coagulation results to be checked either during or after the laser therapy. In our investigation, two patients had large macroscopic lesions of 2 cm in diameter which were extensively coagulated with the Nd:YAG laser at 30 W and afterwards ablated. Under white light, no residual tumor material was visible after the treatment. Under PDD on the other hand, a small fluorescence positive area on the edge of the already coagulated tissue was identified and proved to be residual SCC in the histological examination (Figs. 4, 5). Therefore the intraoperative performance of a control-PDD helped to detect leftover neoplastic tissue and allowed a more complete tumor destruction.

The AAT on the other hand detected suspicious lesions as a result of their whitish demarcation compared to the otherwise normal squamous epithelium. The thermal energy of the Nd:YAG laser denatures both malignant and benign tissue equally, leaving the successfully coagulated areas showing white. Therefore it is not possible with the AAT to distinguish intraoperatively between successfully laser-coagulated tissue and residual preneoplastic or malignant areas due to the similar whitish impression. Consequently, in order to detect recurrences, the AAT must be repeated once the wound is completely healed. Additionally AAT is not very specific and therefore many lesions showing a whitish demarcation are not malignant in the final histopathology, but are benign inflammations. Under AF, the red light from the red laser pilot becomes absorbed by the green filter and appears white under the green impression of the background. A constant monitoring of the laser coagulation process is therefore possible. The diminished AF of the suspicious lesions appears much lighter as the denaturation of the tissue is progressing, guiding the urologist towards the complete destruction of all existing neoplastic tissue. Following the laser coagulation, the AF images were controlled with PDD and no further red fluorescence was detected. In contrast to the other imaging techniques, AF imaging enables the urologist not only to detect and localize preneoplastic and neoplastic lesions but also permits the laser coagulation of the suspicious lesions under the direct impression of fluorescence. This exact localization of the tumor edge helps to determine the extent of the safety rim around the lesion which is usually 3 mm. The safety rim prevents recurrent disease and is largely responsible for the lower recurrence rates of laser treatment in comparison with excisional organ sparing techniques [28].

The additional costs for fluorescence diagnosis include the xenon arch lamp which provides the blue excitation light and the 1% 5-ALA dissolved in Lidocain jelly.

The combination of fluorescence detection and laser treatment of penile carcinoma is a promising technique for the performance of penile sparing surgery. It saves the patient a mutilating penile amputation but still enables the urologist to perform safe oncologic surgery with free tissue margins. Additionally, fluorescence diagnosis of penile cancer may lower the recurrence rate in organ sparing surgery by detecting co-existing malignant lesions on the periphery of the main tumor and by an earlier identification of premalignant lesions, respectively. Although recurrences have been described more than 10 years after the initial treatment, patients usually present within the first 18 months with new or recurrent lesions. Therefore the patients should be closely followed throughout this period [21]. The value of fluorescence diagnosis has to be evaluated on a larger number of patients in a randomized trial with longer follow up.

References

1. Abe S, Izuishi K, Tajiri H, Kinoshita T, Matsuoka T (2000) Correlation of in vitro autofluorescence endoscopy images with histopathological findings in stomach cancer. Endoscopy 32: 281
2. Barraso R, De Brux J, Croissant O, Orth G (1987) High prevalence of papilloma virus-associated penile intraepithelial neoplasia in sexual partners of women with cervical intraepithelial neoplasia. N Engl J Med 317: 916
3. Betz CS, Mehlmann M, Rick K (1999) Autofluorescence imaging and spectroscopy of normal and malignant mucosa in patients with head and neck cancer. Lasers Surg Med 25: 323
4. Brumini R (1982) In: Cancer in Brazil: histopathological data 1976–1980. Ministry of Health, Rio de Janeiro
5. Derakhshani P, Neubauer S, Braun M, Bargman H, Heidenreich A, Engelman U (1999) Results and 10-year follow-up in patients with squamous cell carcinoma of the penis. Urol Int 62: 238
6. Derrick FC, Lynch KM, Kretkowski RC, Yarbrough WJ (1973) Epidermoid carcinoma of the penis: computer analysis of 87 cases. J Urol 110: 303
7. Ficarra VD, Amico A, Cavalleri S, Zanon G, Mofferdin A, Schiavone D, Malossini G, Mobilio G (1999) Surgical treatment of penile carcinoma: our experience from 1976 to 1997. Urol Int 62: 234
8. Frimberger D, Zaak D, Stepp H, Schmeller N, Knüchel R, Hofstetter H (2001) Autofluorescence imaging to optimize the specificity of the 5-ALA induced fluorescence endoscopy. Urology 58: 372
9. George PJ (1999) Fluorescence bronchoscopy for the early detection of lung cancer. Thorax 54: 180

10. Gloeckler-Ries LA, Hankey BF, Edwards BK (eds) (1990) Cancer Statistics Review 1973–1987. National Cancer Institute, National Institutes of Health Publication No. 90. National Institutes of Health, Bethesda, p 2789
11. Hanash KA, Furlow WL, Utz DC, Harrison EGJr (1970) Carcinoma of the penis: a clinicopathologic study. J Urol 104: 291
12. Häussinger K, Stanzl F, Huber RM, Pichler J, Stepp H (1999) Autofluorescence detection of bronchial tumors with the D-Light/AF. Diagn Ther Endosc 5: 105
13. Hölzel D (1999) Tumorregister München. Jahresbericht 1999 des klinisch-epidemiologischen Krebsregisters am Tumorzentrum Muenchen. W. Zuckschwerdt, Munich, p 16
14. Horenblas S, van Tinteren H (1994) Squamous cell carcinoma of the penis. IV. Prognostic factors of survival: analysis of tumor, nodes and metastatic classification system. J Urol 151: 1239
15. Horenblas S, van Tinteren H, Delemarre JF, Boon TA, Moon LM, Lustig V (1992) Squamous cell carcinoma of the penis. II. Treatment of the primary tumor. J Urol 147: 1533
16. Izuishi K, Tajiri H, Fujii T, Boku N, Ohtsu A, Ohnishi T, Ryu M, Kinoshita T, Yoshida S (1999) The histological basis of detection of adenoma and cancer in the colon by autofluorescence endoscopic imaging. Endoscopy 31: 511
17. Koenig F, McGovern FJ, Enquist H, Larne R, Deutsch TF, Schomacker KT (1996) Laser induced autofluorescence diagnosis of bladder cancer. J Urol 156: 1597
18. Lynch DJr, Schellhammer PF (1988) Tumors of the penis. In: Walsh PC, Retik AB, Vaughan EDJr, Wein AJ (eds) Campell's urology. Saunders, Philadelphia, p 2453
19. Malek RS, Goellner JR, Smith TF, Espy MJ, Cupp MR (1993) Human paillomavirus and intraepithelial, in situ, and invasive carcinoma of the penis. Urology 42: 159
20. Meyer T, Arndt E, Stockfleth HT, Flammann H, Wolf U, Reischl U (1995) Strategy for typing Human Papillomaviruses by RFLP analysis of PCR products and subsequent hybridization with a generic probe. BioTechniques 19: 632
21. Montie JE (1994) Follow-up after penectomy for penile cancer. Urol Clin North Am 21: 725
22. Norouzi BB, Shanberg AM (1998) Laser treatment of large cavernous hemangiomas of the penis. J Urol 160: 60
23. Rothenberger KH (1986) Value of the neodymium-YAG laser in the therapy of penile carcinoma. Eur Urol 12 (Suppl 1): 34
24. Schneede P (1997) Condylomata accuminata and other viral skin lesions. In: Hofstetter A (ed) Lasers in urological surgery. Springer, Berlin Heidelberg, p 47
25. Steinbach P, Weingandt H, Knuechel R, Hofstetter H (1995) Cellular fluorescence of the endogenous photosensitizer protpporphyrin IX following exposure to 5-aminolevulinic acid. J Photochem Photobiol 62: 887
26. Stepp H, Baumgartner R, Hofstetter A (1991) Fluorescence imaging and spectroscopy of ALA-induced protoporphyrin IX preferentially accumulated in tumor tissue. Proc Int Soc Optical Eng (SPIE) 2627: 13
27. Sufrin G, Huben R (1987) Benign and malignant lesions of the penis. In: Gillenwater JY, Grayhack JT, Howards SS et al. (eds): Adult and pediatric urology. Medical Publishers, Chicago p 1462
28. Tietjen DN, Malek RS (1998) Laser therapy of squamous cell dysplasia and carcinoma of the penis. Urology 2: 559