Original Article

Optical coherence tomography accurately identifies patients with penile (pre) malignant lesions: A single center prospective study

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Abstract

Introduction: Currently, (multiple) biopsies are taken to obtain histopathological diagnosis of suspicious lesions of the penile skin. Optical coherence tomography (OCT) provides noninvasive *in vivo* images from which epidermal layer thickness and attenuation coefficient (μ_{oct}) can be quantified. We hypothesize that qualitative (image assessment) and quantitative (epidermal layer thickness and attenuation coefficient, μ_{oct}) analysis of penile skin with OCT is possible and may differentiate benign penile tissue from (pre) malignant penile tissue.

Materials and Methods: Optical coherence tomography-imaging was performed prior to punch biopsy in 18 consecutive patients with a suspicious lesion at the outpatient clinic of the NKI-AVL. Qualitative analysis consisted of visual assessment of clear layers and a visible lower border of the lesions, quantitative analysis comprised of determination of the epidermal layer thickness and μ_{oct}. Results were grouped according to histopathology reports.

Results: Qualitative analysis showed a statistically significant difference ($P = 0.047$) between benign and (pre) malignant lesions. Quantitative analysis showed that epidermal layer thickness and attenuation coefficient was significantly different between benign and (pre) malignant tissue, respectively, $P = 0.001$ and $P < 0.001$.

Conclusion: In this preliminary study, qualitative and quantitative analysis of OCT-images of suspicious penile lesions shows differences between benign lesions and (pre) malignant lesions. These results encourage further research in a larger study population.

Key Words: Carcinoma *in situ*, imaging, optical coherence tomography, penile carcinoma, penile intraepithelial neoplasia

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INTRODUCTION

Penile cancer is rare in the Western world. In some African, Asian and South American countries its incidence rate can be up to 10% of malignant disease in men. In Paraguay and Uganda incidence rates are 4.2 and 4.4, respectively, per 100,000 men.[1,2] These numbers are in contrast to Western Europe and the United States where age-standardized incidence rates range from 0.3 to 1.0 per 100,000 men. In these countries, penile cancer accounts for 0.4–0.6% of all malignancies.[3,4] More than 95% of penile tumors are squamous cell carcinomas (SCC).[5] SCC can be preceded by penile intraepithelial lesions (PIN).[6] The term PIN covers different clinical presentations of dysplastic lesions. The morphology of PIN lesions can vary greatly: From pigmented to leukoplastic papules, erythroplasic macules

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and keratinized condylomas. Histopathological features of PIN consist of disorganized basal and parabasal layers combined with cellular atypia, exhibiting atypical mitosis.[7] The goal of treatment of PIN is to eradicate the disease while limiting penile mutilation.[8] PIN can be treated with topical therapy like podophyllotoxin, imiquimod, and/or with surgical methods as laser ablation and excision. No randomized clinical trials are known, comparing various treatment modalities. The choice of treatment is generally based on preferences and skills of the urologist.[9] In our urology department, lesions with suspicion of PIN are first biopsied. Histologically proven PIN lesions are usually managed with CO_2 laser therapy.

Optical coherence tomography (OCT) is a new imaging technique equivalent to ultrasound.[10] Though, it uses back-scattered light to produce cross-sectional images instead of back-reflected sound waves. The high resolution images have a maximal depth of 2.0 mm due to loss of signal by scattering and absorption of light within the tissue. Nevertheless, OCT allows a qualitative assessment of tissue pathology by assessing the presence of clearly delineated tissue layers[11] or visibility of the lower boundary of the lesion. Quantitative measures can also be derived, such as epidermal layer thickness and the OCT signal decay. The decay of OCT signal in depth is directly related to the optical properties of the tissue[12] and is parameterized by the attenuation coefficient (μ_{oct}).[13] Recent studies show that quantitative measurement of μ_{oct} using OCT is sensitive for changes of optical properties in tissue due to changes in tissue (cellular) morphology.[13,14] A noninvasive tool like OCT, which can directly show the epithelial tissue layers and their organization in great detail, may help the urologist in differentiating between benign lesions and (pre) malignant lesions. Based on this, OCT may help in accurately selecting biopsy sites and limit the amount of biopsies taken, or even assist in choice of treatment, serve as a tool to follow-up the effects of noninvasive treatments, or be of added value in defining margins of resection during surgery.

We hypothesize that (a) the thickness of the epidermal layer of benign penile lesions as measured with OCT will differ from the thickness of (pre) malignant penile lesions and (b) that the μ_{oct} measurement in the epidermal layer of benign penile lesions will differ from the μ_{oct} of (pre) malignant penile lesions. OCT analysis will thus enable the urologist to investigate suspicious lesions of the penis *in vivo* and may be of added value in clinical decision making at the outpatient clinic during diagnosis as well as during follow up or may be used at the operation theatre to determine lesions margins.

METHODS

Data collection

From August 2010 to October 2012 we performed a prospective pilot study including 18 consecutive patients in the outpatient clinic of our institute (a tertiary cancer center), who underwent a biopsy because of a clinical suspicious lesion of the penis. These lesions were analyzed with OCT imaging followed by a punch biopsy for standard histopathological examination. Approval for the study was obtained from the Medical Ethical Committee of our institute, and the study was conducted according to the declaration of Helsinki principles.

Optical coherence tomography analysis

Before punch biopsies were taken, five two-dimensional OCT images and one three-dimensional OCT image of each lesion were made by two investigators (DMdB and RW). All OCT images were acquired during one imaging session from the same location. Images were made with a commercially available 50 kHz swept source OCT system (Santec Inner Vision 2000; 10 μm axial resolution, 40 μm lateral resolution, with light with a wavelength around 1300 nm). All images were stored to be analyzed by one investigator (RW) at a later date. Qualitative analysis was performed by determining the visibility of clear tissue layers and a visible lower border of the lesion in each OCT image. Quantitative analysis in each OCT image, that is, to determine epidermal layer thickness and the decrease of light intensity per millimeter (attenuation coefficient, μ_{oct} mm^{-1}) across the epidermal layer, was performed after careful calibration of the OCT system as described before.[11,13,14] When performing the analysis, the investigator was blinded for the pathology report.

Statistical analysis and grouping

The standard histopathology report was considered the gold standard for comparison and was categorized as benign, premalignant or malignant. From the OCT data of the suspicious lesions, five values of epidermal layer thickness were available in 15 patients. In three patients less than five values (two, three and four, respectively) were available because in these patients, very thin epidermal layers did not always allow accurate measurement of thickness in certain images. Similarly, five values of epidermal μ_{oct} were available in 16 patients. In one patient only three and in another patient only four values were available, due to thin layer thickness and therefore too little pixels to obtain reliable μ_{oct}. The mean epidermal layer thickness and mean epidermal μ_{oct} for each imaged suspicious lesion were subsequently calculated by averaging the values obtained from the available images; these averages were grouped according to the histopathology report. Next to benign lesions, all premalignant lesions and malignant lesions were grouped to form the "(pre) malignant" group. All data were collected and

analyzed in R version 2.12 (The R Foundation for Statistical Computing, Vienna, Austria). The difference in the presence of the qualitatively observed features in the images of benign and (pre) malignant tissue was tested using Fisher's exact test. The difference in mean epithelial layer thickness and mean attenuation coefficient μ_{oct} between benign and (pre) malignant tissue was tested using a mixed effects regression analysis. To analyze any possible redundancy in the measured quantitative parameters, Pearson's correlation was used to test for correlation between layer thickness and attenuation coefficient. Differences were considered statistically significant if the two-sided $P < 0.05$.

RESULTS

In total, 18 lesions out of 18 patients were available for analysis. The average age of the patients was 61 years (range: 42–76). Fourteen patients had a history of penile carcinoma and were treated by local excision in the past. Table 1 shows the patient characteristics. All lesions had a red appearance; in seven lesions hyperkeratosis was seen. In total, histopathology showed five benign lesions (two reactive tissue, three inflammation) and thirteen (pre) malignant lesions (two dysplasia/PIN, eight carcinoma *in situ* (cis) and three penile SCC). Typical examples of inflammation, a cis and a penile SCC are shown in Figures 1-3.

In four out of 5 (80%) benign lesions clear layers and a visible lower border of the lesions were seen [Figure 1]. In one benign lesion (20%) none of the above features were clearly visible. In ten of the thirteen (pre) malignant lesions (77%) no clear layers and no lower border of the lesions were seen [Figure 3], though in three (23%) (pre) malignant lesions these features were visible. There was a statistically significant difference in appearance of these features in benign lesions and (pre) malignant lesions ($P = 0.047$) [Table 2].

In quantitative assessment, the mean epidermal layer thickness of the benign lesions was 0.18 mm (SE 0.07 mm). In (pre) malignant lesions the mean epidermal layer thickness was 0.53 mm (SE 0.04 mm) ($P = 0.001$), Figure 4. No receiver-operating characteristic (ROC)-curve is shown, as the epidermal layer thickness is completely different between benign and (pre) malignant lesions.

The mean attenuation coefficient of the benign and (pre) malignant lesions was 2.5 mm^{-1} (SE 0.5 mm^{-1}) and 5.2 mm^{-1} (SE 0.3 mm) respectively ($P < 0.001$), [Figure 5]. The ROC-curve for the average μ_{oct} values per lesion is shown in Figure 5 as well. The area under the curve = 0.96 (0.88–1.00). When using the Youden index, the threshold is 3.1 mm^{-1} with a sensitivity of 1.00 and a specificity of 0.80.

Table 1: Patient characteristics (*n*=18)

Age	History of penile cancer		Colour of the lesions	
	Yes	No	Red	White
61 years (range: 42-76)	14	4	18	None

Table 2: Qualitative assessment of the lesions

Clear layers and visible border of the lesions (*P*=0.047) (%)			
Benign lesions (*n*=5)		Premalignant lesions (*n*=13)	
Yes	No	Yes	No
4/5 (80)	1/5 (20)	3/13 (23)	10/13 (77)

Within the benign and (pre) malignant groups, we assessed the correlation between layer thickness and attenuation coefficient. A Pearson's correlation of $R = 0.29$ (confidence interval [CI] 0.80–0.93, $P = 0.64$) was found for the benign lesions and $R = 0.28$ (CI: 0.32–0.72, $P = 0.36$) for the (pre) malignant lesions [Figure 6], indicating that epidermal layer thickness and attenuation coefficient are not correlated.

DISCUSSION

Since its development, most research in the field of OCT imaging has been performed in ophthalmology, due to the transparent nature of the corpus vitreum. Today, clinical commercially available OCT systems are being widely used in the ophthalmology clinic.[15] In other fields of medicine, such as dermatology, OCT has been studied thoroughly as well.[16] In urology, OCT has proved to differentiate between normal renal tissue and renal tumours.[17,18] In addition, it has been shown that OCT can be used to visualize upper urinary tract tumours *in vivo*.[11] In penile suspicious lesions, a noninvasive tool like OCT may be of assistance in accurately selecting biopsy sites and limit the amount of biopsies taken, or even help in choice of treatment, serve as a tool to follow-up the effects of noninvasive treatments, or be of added value in defining margins of resection during surgery.

To the best of our knowledge, this is the first prospective study that images suspicious penile lesions *in vivo* using OCT and quantifies image features related to morphological changes occurring during carcinogenesis (e.g. epidermal layer thickness and attenuation coefficient). Due to the low incidence of PIN and penile SCC,[3] patients available for research are limited and studies about (pre) malignant penile lesions are rare. There is one published case report, wherein the authors performed OCT imaging of an Erythroplasia of Queyrat of the penis (cis). They studied the images qualitatively and did not estimate epidermal layer thickness or attenuation coefficients, nor did they take a biopsy to histopathologically confirm the diagnosis. Nevertheless, they did show the potential of OCT as

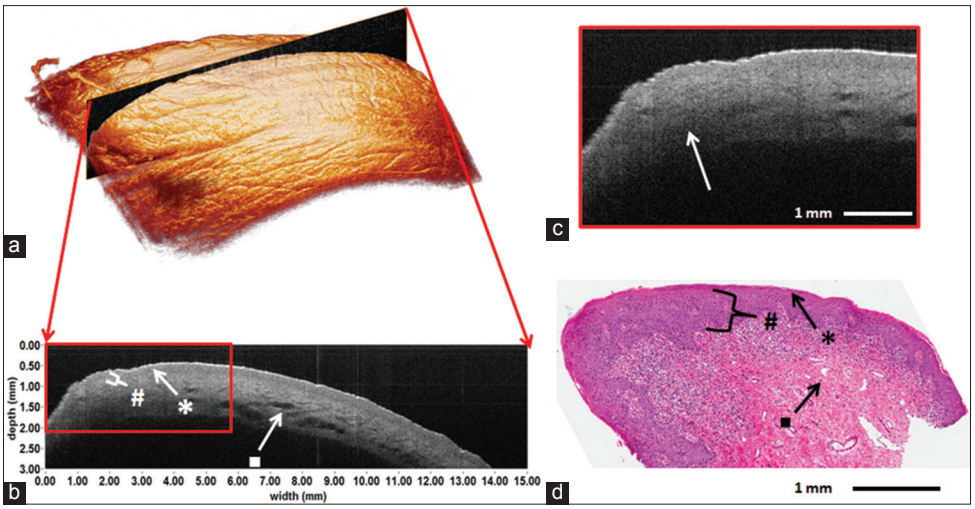

Figure 1: Three-dimensional optical coherence tomography (OCT) image (a) and two-dimensional OCT image (b) of a benign lesion (inflammation) of the glans penis with detailed image of the lesion (c) and corresponding histology (H and E) (d). In Figure 1C, an arrow shows the lower border of the lesion (*) The horny layer of the skin, (#) shows the epidermal layer and (•) shows a blood vessel

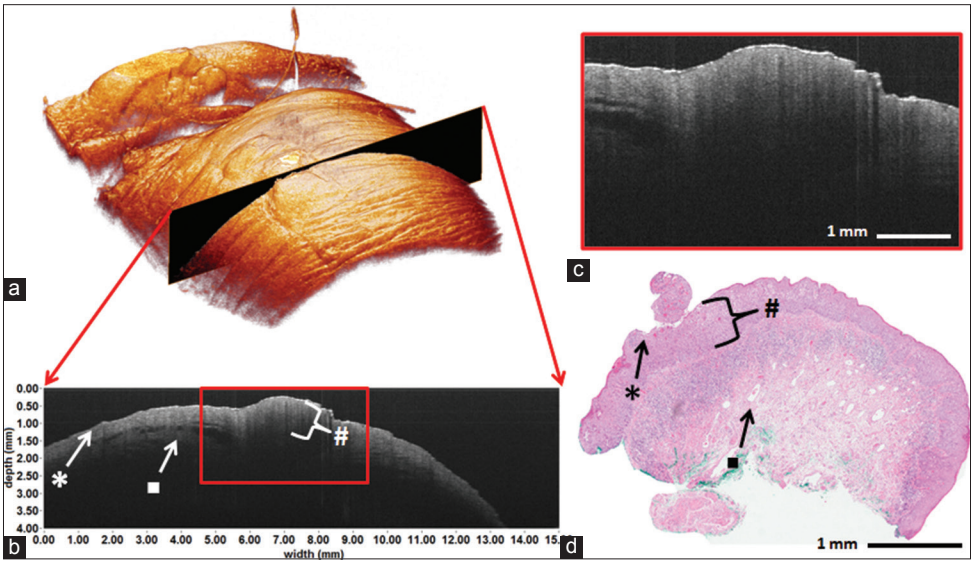

Figure 2: Three-dimensional optical coherence tomography (OCT) image (a) and 2-dimensional OCT image (b) of carcinoma *in situ* of the glans penis with detailed image of the lesion (c) and corresponding histology (H and E) (d) (*) The horny layer of the skin, (#) The (thickened) epidermal layer and (•) shows a blood vessel

a noninvasive tool to study lesions of the penis and investigate the effectiveness of noninvasive treatment.[19] Our sample size was too small to differentiate between premalignant and malignant lesions. Given the similarity in clinical management of premalignant and malignant lesions, we created two groups (benign and (pre) malignant) for analysis in this work.

In the qualitative and quantitative analysis of the OCT images, there were statistically significant differences between benign and (pre) malignant lesions, with regard to epidermal layer thicknesses and attenuation coefficients. In this study epidermal layer thickness and attenuation coefficients were not correlated. Therefore, quantification of layer thickness and attenuation coefficient could potentially serve as complementary parameters for discrimination between benign and (pre) malignant tissue. However, these results should be interpreted with some considerations. First of all, the study group was biased towards the (pre) malignant lesions, as there were 13 (pre) malignant lesions and only 5 benign lesions. Furthermore, 14 out of 18 patients had a history of penile cancer. Previous treatment might have influenced the epidermal layer thickness and attenuation coefficient. A larger study population is needed to further investigate these preliminary results.

Little data are available when it comes to penile carcinoma, as it is rare in the developed world. Vulvar carcinoma has many

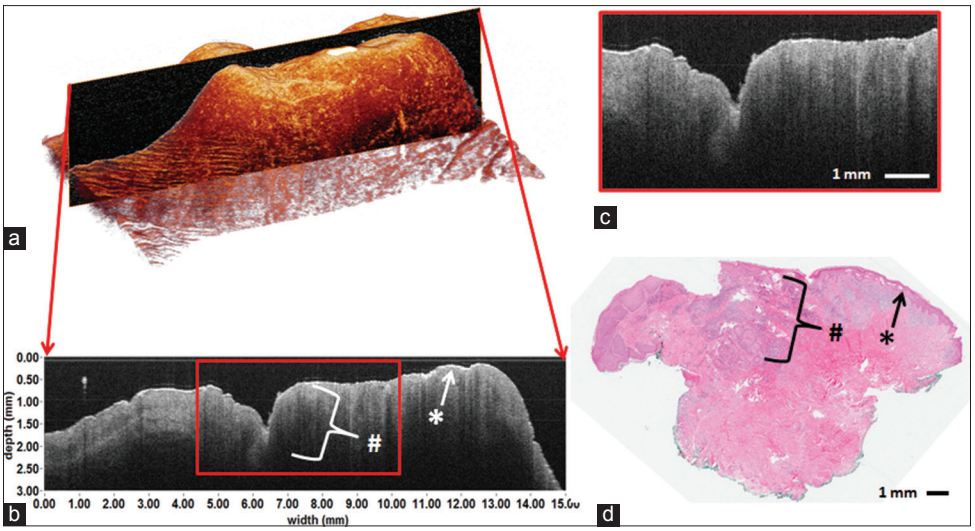

Figure 3: Three-dimensional optical coherence tomography (OCT) image (a) and two-dimensional OCT image (b) of penile squamous cell carcinoma of the glans penis with detailed image of the lesion (c) and corresponding histology (H and E) (d). The lower border of the lesion is not visible (c and d) (*) The horny layer of the skin, (#) The (thickened and disorganized) epidermal layer

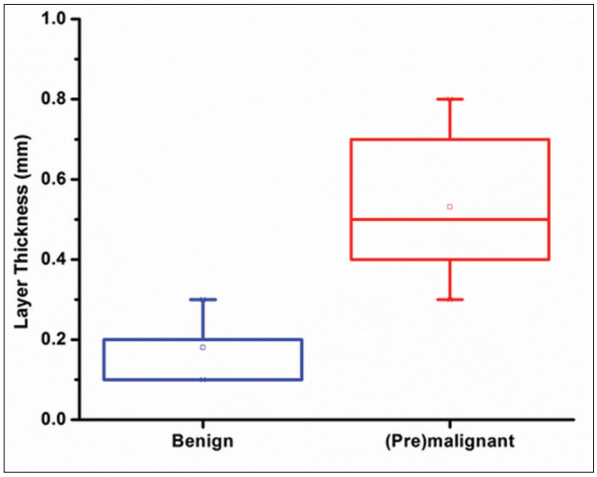

Figure 4: Boxplots of the average epidermal thickness values (mm) per lesion. Mean epidermal layer thickness of benign lesions was 0.18 mm (SE 0.07 mm), of (pre)malignant lesions epidermal layer thickness was 0.53 mm (SE 0.04 mm), (P = 0.001)

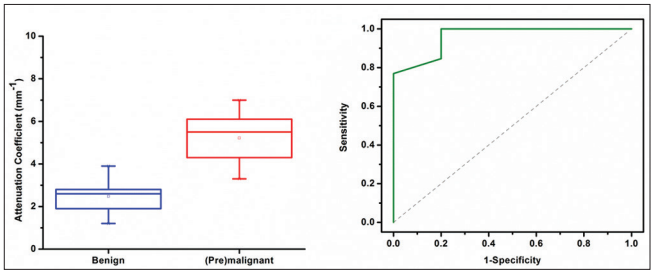

Figure 5: Boxplots of epidermal μoct values (mm^{-1}) of the average epidermal μoct values (mm^{-1})/lesion. Mean μoct of benign lesions was 2.5 mm^{-1} (SE 0.5 mm^{-1}); mean μoct of (pre)malignant lesions was 5.2 mm^{-1} (SE 0.3 mm), (P < 0.001). Receiver-operating characteristic curve for the average μoct values per lesion. The area under the curve is 0.96 (0.88–1.00). The Youden index has a threshold of 3.1 mm^{-1} with a sensitivity of 1.00 and a specificity of 0.80

parallels to penile carcinoma and is a much better studied disease.[20] Benedet *et al*.[21] performed epidermal thickness measurements in patients with vulvar intraepithelial neoplasia. The involved vulvar epithelium varied from 0.10 mm to 1.4 mm in thickness, with a mean of 0.46. Noninvolved epithelium varied in thickness from 0.10 mm to 0.70 mm, with a mean of 0.28. Vulvar carcinoma in women has many similarities to penile carcinoma in men. Therefore, it might be that the penile tissue thickens when (pre) malignant penile lesions develop, similar to vulvar intraepithelial neoplasia.

Not only the epidermal layer thickens increases, the morphology of the tissue changes as well. Light scattering measurements are sensitive to variations in tissue morphology (density) at sub-wavelength scale.[22] Our OCT measurements are sensitive to variations on length scales of around $\lambda/2 \approx 650$ nm, e.g., on the scale of organelles and cells.[23] Which processes and changes during carcinogenesis are responsible for the measured differences as shown in this study remains to be resolved. For example, cancers have a high amount of dividing cells[24] during which cells increase their DNA. The refractive index of the nucleus, governing light scattering properties, increases during the cell cycle when cells increase their DNA[25] leading to changes in scattering properties. In dysplastic cells, like the cells in (pre) malignant epithelial lesions such as PIN or SCC, DNA replication takes place as well,[26] therefore changes in scattering properties may be anticipated. Our findings confirm these differences between benign and (pre) malignant lesions of the penis.

In our study, quantification of layer thickness and attenuation coefficient was sometimes hampered by the very small size of

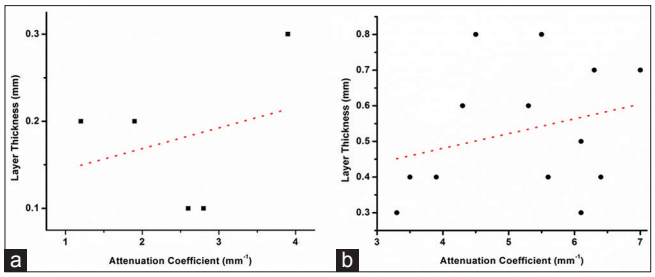

Figure 6: Scatter plot of the (average per lesion) attenuation coefficient against the layer thickness for the benign lesions (a) and the (pre) malignant lesions (b). No significant correlation between layer thickness and attenuation coefficient is observed in benign ($P = 0.64$) and (pre) malignant ($P = 0.36$) lesions

the epidermal layer. For layer thickness measurements, OCT systems with smaller axial resolution may, therefore, prove beneficial. Smaller axial resolution can be achieved using lower wavelengths, or light sources with larger optical bandwidths. In the case of a thin epidermal layer, determination of attenuation coefficient may remain challenging with our current approach. Nevertheless, recent advances show promise for obtaining attenuation coefficients of these thin layers[27] as well.

Until now, patients with a suspicious lesion of the penis undergo a biopsy for histological diagnosis. Unlike histopathology, OCT imaging can be performed noninvasively, *in vivo* and in real time. It takes only 2 s to generate a three-dimensional image and even <1 s to create a two-dimensional image. Like in histopathology, our present OCT analysis relies on postimaging processing of the data by the investigator. In the near future, automated region selection and μ_{oct}–determination might be possible. Until then, postimaging processing of the data are a process that has to be performed by the investigator.

CONCLUSION

Our results show that, qualitative and quantitative analysis of the OCT images is a potential promising tool to identify benign tissue from (pre) malignant tissue in suspicious penile lesions. In this way, OCT may accurately select biopsy sites and therefore limit the amount of biopsies taken. In the future, OCT may even help in defining lesions margins during surgery as well as during follow up of patients with a noninvasive treated penile lesion. A larger study population is needed to further research these preliminary results and the potential clinical applications.

REFERENCES

1. Rubin MA, Kleter B, Zhou M, Ayala G, Cubilla AL, Quint WG, *et al.* Detection and typing of human papillomavirus DNA in penile carcinoma: Evidence for multiple independent pathways of penile carcinogenesis. Am J Pathol 2001;159:1211-8.
2. Wabinga HR, Parkin DM, Wabwire-Mangen F, Nambooze S. Trends in cancer incidence in Kyadondo County, Uganda, 1960-1997. Br J Cancer 2000;82:1585-92.
3. Siegel R, Naishadham D, Jemal A. Cancer statistics for Hispanics/Latinos, 2012. CA Cancer J Clin 2012;62:283-98.
4. Parkin DM, Muir CS. Cancer incidence in five continents. Comparability and quality of data. IARC Sci Publ 1992;120:45-173.
5. Maiche AG. Epidemiological aspects of cancer of the penis in Finland. Eur J Cancer Prev 1992;1:153-8.
6. Cubilla AL, Meijer CJ, Young RH. Morphological features of epithelial abnormalities and precancerous lesions of the penis. Scand J Urol Nephrol Suppl 2000;205:215-9.
7. Wikstrom A, Hedblad MA, Syrjanen S. Penile intraepithelial neoplasia: Histopathological evaluation, HPV typing, clinical presentation and treatment. J Eur Acad Dermatol Venereol 2012;26:325-30.
8. Ficarra V, Maffei N, Piacentini I, Al Rabi N, Cerruto MA, Artibani W. Local treatment of penile squamous cell carcinoma. Urol Int 2002;69:169-73.
9. von Krogh G, Horenblas S. The management and prevention of premalignant penile lesions. Scand J Urol Nephrol Suppl 2000;205:220-9.
10. Cauberg EC, de Bruin DM, Faber DJ, van Leeuwen TG, de la Rosette JJ, de Reijke TM. A new generation of optical diagnostics for bladder cancer: Technology, diagnostic accuracy, and future applications. Eur Urol 2009;56:287-96.
11. Bus MT, Muller BG, de Bruin DM, Faber DJ, Kamphuis GM, van Leeuwen TG, *et al.* Volumetric *in vivo* visualization of upper urinary tract tumors using optical coherence tomography: A pilot study. J Urol 2013;190:2236-42.
12. de Bruin DM, Bremmer RH, Kodach VM, de Kinkelder R, van Marle J, van Leeuwen TG, *et al.* Optical phantoms of varying geometry based on thin building blocks with controlled optical properties. J Biomed Opt 2010;15:025001.
13. Faber D, van der Meer F, Aalders M, van Leeuwen T. Quantitative measurement of attenuation coefficients of weakly scattering media using optical coherence tomography. Opt Express 2004;12:4353-65.
14. Wessels R, de Bruin DM, Faber DJ, van Boven HH, Vincent AD, van Leeuwen TG, *et al.* Optical coherence tomography in vulvar intraepithelial neoplasia. J Biomed Opt 2012;17:116022.
15. Drexler W, Fujimoto JG. State-of-the-art retinal optical coherence tomography. Prog Retin Eye Res 2008;27:45-88.
16. Sattler E, Kästle R, Welzel J. Optical coherence tomography in dermatology. J Biomed Opt 2013;18:061224.
17. Barwari K, de Bruin DM, Cauberg EC, Faber DJ, van Leeuwen TG, Wijkstra H, *et al.* Advanced diagnostics in renal mass using optical coherence tomography: A preliminary report. J Endourol 2011;25:311-5.
18. Barwari K, de Bruin DM, Faber DJ, van Leeuwen TG, de la Rosette JJ, Laguna MP. Differentiation between normal renal tissue and renal tumours using functional optical coherence tomography: A phase I *in vivo* human study. BJU Int 2012;110:E415-20.
19. Schmitz L, Bierhoff E, Dirschka T. Optical coherence tomography imaging of Erythroplasia of Queyrat and treatment with imiquimod 5% cream: A case report. Dermatology 2014;228:24-6.
20. Longpre MJ, Lange PH, Kwon JS, Black PC. Penile carcinoma: Lessons learned from vulvar carcinoma. J Urol 2013;189:17-24.
21. Benedet JL, Wilson PS, Matisic JP. Epidermal thickness measurements in vaginal intraepithelial neoplasia. A basis for optimal CO2 laser vaporization. J Reprod Med 1992;37:809-12.
22. Muller MG, Valdez TA, Georgakoudi I, Backman V, Fuentes C, Kabani S, *et al*. Spectroscopic detection and evaluation of morphologic and biochemical changes in early human oral carcinoma. Cancer 2003;97:1681-92.
23. van der Meer FJ, Faber DJ, Aalders MC, Poot AA, Vermes I, van Leeuwen TG. Apoptosis- and necrosis-induced changes in light

attenuation measured by optical coherence tomography. Lasers Med Sci 2010;25:259-67.
24. Williams GH, Stoeber K. The cell cycle and cancer. J Pathol 2012;226:352-64.
25. Bista RK, Uttam S, Wang P, Staton K, Choi S, Bakkenist CJ, *et al.* Quantification of nanoscale nuclear refractive index changes during the cell cycle. J Biomed Opt 2011;16:070503.
26. Freeman A, Morris LS, Mills AD, Stoeber K, Laskey RA, Williams GH, *et al.* Minichromosome maintenance proteins as biological markers of dysplasia and malignancy. Clin Cancer Res 1999;5:2121-32.
27. Vermeer KA, Mo J, Weda JJ, Lemij HG, de Boer JF. Depth-resolved model-based reconstruction of attenuation coefficients in optical coherence tomography. Biomed Opt Express 2013;5:322-37.

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