Differentiation Between Balanitis and Carcinoma In Situ Using Reflectance Confocal Microscopy

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Importance: Zoon plasma cell balanitis is a benign inflammatory disease of genital skin. It may be difficult to clinically distinguish between balanitis and carcinoma in situ (CIS); thus, a biopsy may be needed to exclude malignant disease. Reflectance confocal microscopy (RCM) is an in vivo imaging method to get morphologic information about architecture and single cells in the skin.

Objective: To evaluate the ability of RCM to differentiate between balanitis and CIS compared with the gold standard histopathologic methods.

Design: Observer blinded study.

Setting: A referral center.

Participants: Fifteen patients with balanitis or CIS.

Intervention: Patients were assessed by clinical, histologic, and RCM findings. All lesions were imaged with the Vivascope 1500. In 5 cases of balanitis, the surrounding, noninvolved skin also was evaluated.

Main Outcome Measures: Local recurrence, nodal metastasis, disease-specific death, overall death.

Results: The clinical diagnoses showed 9 cases of balanitis and 6 cases of CIS. With histopathologic analysis, 12 cases of balanitis and cases of CIS were diagnosed, and RCM evaluation confirmed these diagnoses. The most relevant RCM criteria for CIS were atypical honeycomb pattern, disarranged epidermal pattern, and round nucleated cells. Balanitis showed a nucleated honeycomb pattern and vermicular vessels. Scattered small bright cells and round vessels were present in all lesions. The adjacent normal skin showed a typical honeycomb pattern and round papillary vessels.

Conclusions and Relevance: We were able to differentiate between balanitis and CIS. Reflectance confocal microscopy may help to avoid biopsies at this sensitive site.


Zoon plasma cell balanitis is a benign idiopathic inflammatory skin disease of the glans penis, the coronal sulcus, and the inner aspect of prepuce that typically occurs in middle-aged or elderly uncircumcised men. Clinically, sharply demarcated, occasionally eroded patches with a glossy surface and reddish-brown color owing to erythrocytic extravasation and accumulation of siderophages are observed. Lesions on the glans and opposing prepuce (kissing lesions) are common. Histopathologic findings include a dense, often bandlike plasma cell infiltrate as well as prominent blood vessels and extravasated erythrocytes. The overlying epidermis can be atrophic but may also be of normal thickness with spongiosis and parakeratosis. Local infections, caused by poor hygiene, sweating, and friction are causative factors.

In some cases, there is an overlap between Zoon balanitis and chronic nonspecific balanitis both clinically and histopathologically. To simplify matters, we will use the term “balanitis” for both entities. The clinical differentiation between balanitis and other penile dermatoses may be challenging. Erythroplasia of Queyrat (penile intraepithelial neoplasia grade 3; carcinoma in situ [CIS]), lichen sclerosus et atrophicus, lichen planus, psoriasis, and infectious diseases are all differential diagnostic considerations. In clinically equivocal cases, a biopsy is needed to exclude a diagnosis of malignant disease, especially since CIS may develop in balanitis, possibly as a consequence of the chronic inflammation.

Confocal laser scanning microscopy (reflectance confocal microscopy [RCM]) is an in vivo imaging method used to obtain morphologic information about both architecture and single cells in the epidermis and superficial dermis. The aim of this
study was to use RCM to identify distinct in vivo morphologic criteria for balanitis and carcinoma.

METHODS

Fifteen patients (mean age, 65 years [range, 48-80 years]) were evaluated by clinical, histologic, and RCM findings at the Department of Dermatology and Venereology, Medical University of Graz, Graz, Austria (Table 1). In this prospective study (October 10, 2009–March 15, 2010) we included patients with the histopathologically confirmed diagnosis of balanitis or carcinoma. Other diseases, such as lichen sclerosus et atrophicus, psoriasis, or infections, were excluded to obtain representative data for these 2 entities only. The patients presented with atrophic to hyperkeratotic, red to brown lesions on the glans and/or inner aspect of prepuce. Seven lesions were eroded, 7 were elevated and had an infiltrated granular surface, and 1 was a verrucous plaque. The lesions had been present for several weeks in 1 case up to 15 or more years in other cases (mean, about 6 years). As mentioned, all lesions were borderline cases in which a punch biopsy was essential to confirm the diagnosis. Results from serologic testing for syphilis were negative in about 6 years). As mentioned, all lesions were borderline cases in which a punch biopsy was essential to confirm the diagnosis. Results from serologic testing for syphilis were negative in all cases. We also studied normal skin at the periphery of 5 cases of balanitis. Clinically, these regions were sharply delineated from the inflamed areas. Normal skin was evaluated using the same RCM criteria of this study.

All patients gave written consent for the study, and institutional rules governing clinical investigation of human subjects were strictly followed. Approval of the local ethics committee was obtained (Figure 1).

CLINICAL IMAGING

Baseline clinical images were taken from all lesions with a Nikon D200 digital camera (10.2 megapixels; Nikon Corp). A dermatologist (P.K.) who specializes in genital skin lesions, evaluated the digital clinical images without further information on the patient’s medical history and treatment.

RCM IMAGING

All lesions were imaged with the Vivascope 1500 (Lucid Inc). After attaching the tissue ring, a dermoscopic image (“macroscopic image”) using the VivaCam was captured. This image is used for orientation during the RCM examination. The Vivascope 1500 provides thin horizontal virtual tissue sections with a field of view of up to 8 × 6 mm (Vivablock), composed of sequential single RCM images (500 × 500 μm). A standardized imaging procedure was performed in all cases. The Vivascope 1500 was centered on a representative lesion area on the glans and/or inner aspect of prepuce that was easily accessible to the adhesive tissue ring that couples the microscope to the skin. Depending on the location, the field of view varied from 4 to 7 mm². These mosaics were composed for different skin levels, ranging from stratum corneum (if present), granular and spinous layer to the dermoepidermal junction (DEJ) and then upper and optionally deeper dermal layers. In addition, we provided VivaStacks from the center of the lesions: a series of 30 images—500 μm in diameter—in 5 μm steps to a depth of 150 μm. Comparable with routine histopathologic testing, the evaluation of RCM images was first performed at low magni-

<table>
<thead>
<tr>
<th>Patient No./Age, y</th>
<th>Location</th>
<th>Duration of Illness</th>
<th>Clinical Diagnosis</th>
<th>Histopathologic Diagnosis</th>
<th>RCM Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/58 Glans/prep</td>
<td></td>
<td>10 y</td>
<td>Balanitis</td>
<td>Balanitis (Zoon)</td>
<td>Balanitis</td>
</tr>
<tr>
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<td>9 y</td>
<td>Balanitis</td>
<td>Balanitis</td>
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<td>Balanitis</td>
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</tr>
<tr>
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<td>Unknown</td>
<td>&gt;15 y</td>
<td>Balanitis</td>
<td>Balanitis</td>
<td>Balanitis</td>
</tr>
<tr>
<td>5/69 Glans/prep</td>
<td>Several weeks</td>
<td>&gt;15 y</td>
<td>Balanitis</td>
<td>Balanitis</td>
<td>Balanitis</td>
</tr>
<tr>
<td>7/56 Prepuce</td>
<td>Several months</td>
<td></td>
<td>CIS</td>
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<tr>
<td>8/67 Prepuce</td>
<td>1-2 y</td>
<td></td>
<td>CIS</td>
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<td>Balanitis</td>
</tr>
<tr>
<td>9/60 Glans/prep</td>
<td></td>
<td>&gt;2 y</td>
<td>Balanitis</td>
<td>Balanitis (Zoon)</td>
<td>Balanitis</td>
</tr>
<tr>
<td>10/76 Glans/prep</td>
<td></td>
<td>2 y</td>
<td>Balanitis</td>
<td>Balanitis (Zoon)</td>
<td>Balanitis</td>
</tr>
<tr>
<td>11/58 Glans/prep</td>
<td></td>
<td>&gt;2 y</td>
<td>Balanitis</td>
<td>Balanitis (Zoon)</td>
<td>Balanitis</td>
</tr>
<tr>
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<td>Balanitis</td>
<td>Balanitis</td>
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<tr>
<td>13/61 Glans</td>
<td>Several months</td>
<td></td>
<td>Balanitis</td>
<td>Balanitis</td>
<td>Balanitis</td>
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<tr>
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<td>Balanitis</td>
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<tr>
<td>15/62 Glans/prep</td>
<td></td>
<td>Several months</td>
<td>Balanitis</td>
<td>Balanitis (Zoon)</td>
<td>Balanitis</td>
</tr>
</tbody>
</table>

Abbreviations: CIS, carcinoma in situ; prep, prepuce; RCM, reflectance confocal microscopy.

Figure 1. CONSORT diagram. RCM indicates reflectance confocal microscopy.
fication to examine the overall lesion architecture and then at higher magnification by zooming to get cytomorphologic details.

The presence or absence of characteristic RCM criteria was assessed by 3 independent observers (V.A.-S., P.K., and R.H.-W.) without further information on the lesions. A criterion was counted as positive if at least 2 observers found it.

HISTOPATHOLOGIC EVALUATION

The histologic sections were stained with hematoxylin-eosin and periodic acid–Schiff stains. The lesions were evaluated with regard to the presence or absence of established histopathologic criteria.4-6,13

RESULTS

In histopathologic evaluation, 6 cases were diagnosed as Zoon balanitis, 6 as nonspecific balanitis, and 3 as CIS.

EVALUATION OF CLINICAL IMAGES

The isolated clinical examination led to the diagnosis of 6 carcinomas (3 of them confirmed histologically) (Figure 2), 7 cases of balanitis, and 2 lesions of uncertain malignant potential (Figure 3).

EVALUATION OF RCM IMAGES

The following criteria were used for evaluating the RCM images (Table 2).

Typical,14 atypical,15 and nucleated honeycomb pattern and disarranged epidermal pattern/focal loss15 are architectural features of the epidermis. The cytomorphologic features included round nucleated, dendritic, and scattered small bright cells16; the latter also were observed in dermal layers. The dermis was evaluated for 2 types of blood vessels. One type of vessel runs parallel to the surface and appears vermicular, the other runs per-
pendicular to the horizontal confocal imaging, producing a round appearance. All 3 cases of CIS were diagnosed correctly by RCM. The CIS showed an atypical honeycomb pattern as well as disarranged epidermal pattern/local loss in all cases; nucleated and typical honeycomb pattern were not observed. In addition, round nucleated cells were present in 2 cases of CIS, and 2 cases showed scattered small bright cells in epidermis and dermis; dendritic cells were absent. Vermicular vessels were observed in 1 case only, whereas all CIS showed round papillary vessels.

The main architectural feature of the epidermis in balanitis was nucleated honeycomb pattern, which was observed in 11 of 12 cases; in addition, 6 cases showed typical honeycomb pattern. Atypical honeycomb pattern was viewed in 1 case and disarranged epidermal pattern in 3. In contrast to CIS, dendritic cells were verified in 6 cases of balanitis. All balanitis showed scattered small bright cells in the upper dermis, 11 cases in the epidermis. Vermicular vessels and round papillary vessels were found in all cases of balanitis.

The uninvolved skin (Figure 4) showed a typical honeycomb pattern in all cases (n = 5). A partially nucleated

Figure 3. Zoon balanitis. A, Clinical features: atrophic to erosive red patches on the inner aspect of the prepuce, corresponding erythematous macula on the glans. B, Histopathologic features: the epidermis is atrophic. In the papillary dermis a bandlike infiltrate of predominantly plasma cells and dilated vessels is present (hematoxylin-eosin, original magnification ×40). C, A reflectance confocal microscopic (RCM) image, epidermis: 1 × 1 mm: nucleated honeycomb pattern. D, An RCM image, dermoepidermal junction, 1 × 1 mm: vermicular vessels (green arrows), round papillary vessels (pink arrows) and scattered small bright cells (white arrow). (Also see the video; http://www.jamaderm.com.)
Vivascope 3000 could compensate for this disadvantage in some cases because of its size, handling, and duration of imaging.

One drawback of this study is the small diagnostic spectrum of lesions in the enrolled patients; common diseases like psoriasis, lichen sclerosus et atrophicus, and infectious diseases are missing. Our intention, however, was to differentiate between balanitis and CIS.

Atypical honeycomb pattern, disarranged epidermal pattern, round papillary vessels, and round nucleated cells have been described as a common pattern in squamous cell carcinoma.14 In our study, all these findings were observed in CIS. Round nucleated cells, representing the atypical and dyskeratotic cells in histopathologic findings, were present in 2 of the 3 cases of CIS. Round papillary vessels are not helpful because they were present in all lesions and even in normal skin.

A second type of vessels with a distinct morphologic characteristic was detected (video; http://www.jamaderm.com). Because of the flattened DEJ, an increased number of vessels running parallel to the surface with a tortuous, vermicular appearance was observed. This special kind of blood vessels was present in all cases of balanitis. We also observed this feature in normal skin at the periphery of carcinomas but never inside the lesions. We interpreted this as peritumoral inflammation. Nucleated honeycomb pattern is a kind of regular honeycomb pattern with small, bright, reflecting intercellular connections of cells containing bright reflecting nuclei and hyporeflective cytoplasm which the honeycomb pattern is not longer visible.

Reflectance confocal microscopy imaging of the skin has been shown to be a useful tool for the evaluation of skin diseases (eg, neoplasms).14-28 In general, genital skin is suitable for confocal microscopy because of its thin or absent cornified layer. In most of our cases, we observed a thin epidermis and flattened DEJ. These are optimized conditions for good image quality even in deeper dermal layers. A limiting factor of RCM on genital skin area is that, depending on the location, some lesions cannot be imaged with Vivascope 1500 because of difficulties in fixing the tissue ring on the lesion. The handheld Vivascope 3000 could compensate for this disadvantage.
larly distributed in both balanitis and CIS; therefore, they too are not useful for differentiating between inflammatory and malignant lesions on genital skin.

In addition, in 5 cases of balanitis we evaluated normal skin that was sharply demarcated from the area of balanitis. The most noticeable difference in comparison of balanitis and CIS was the presence of typical honeycomb pattern in all cases. Whether the nucleated honeycomb pattern in 3 cases was caused by the nearby inflammation could not be determined. All other features showed a nonspecific distribution. In summary, these observations give an idea how normal genital skin site may appear in RCM.

In conclusion, we have shown some new aspects of the use of RCM on genital skin, as well as confirming previous findings. It is advantageous that uncomfortable and painful biopsies from the genital area may be avoided in some cases. At the moment, the applicability of this method in routine practice is restricted not only by the need for confirmatory studies but also because the Vivascope 1500 is available only at a few specialized centers.

In general, genital skin is suitable for RCM imaging because of its morphologic attributes. Previously described criteria for squamous cell carcinomas, such as atypical honeycomb pattern, disarranged epidermal pattern/focal loss, and round nucleated cells, were observed also in the carcinomas of the glans and prepuce. A nucleated honeycomb pattern is associated with inflamed genital skin. Vermicular vessels were an impressive new feature (video), correlating with inflammation and flattened DEJ. The concomitant presence of a nucleated honeycomb pattern and vermicular vessels and the absence of an atypical honeycomb pattern, disarranged epidermal pattern, and round nucleated cells is a clue for a benign inflammatory genital skin disease, whereas the presence of atypical honeycomb pattern, disarranged epidermal pattern and round, nucleated cells is a hint for malignant disease.

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Author Contributions: All authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Arzberger and Komerci. Acquisition of data: Arzberger, Komerci, Massone, Chubisov, and Hofmann-Wellenhof. Analysis and interpretation of data: Arzberger, Komerci, Ahlgriimm-Siess, and Massone. Drafting of the manuscript: Arzberger, Komerci, Ahlgriimm-Siess, and Hofmann-Wellenhof. Critical revision of the manuscript for important intellectual content: Komerci and Chubisov. Administrative, technical, and material support: Arzberger and Ahlgriimm-Siess. Study supervision: Komerci and Massone.

Conflict of Interest Disclosures: None reported.

Online-Only Material: The video is available online at http://www.jamaderm.com.