Application of In Vivo Reflectance Confocal Microscopy in the Diagnosis of Bowen's Disease

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Abstract

Bowen's disease (BD) is a relatively rare early-stage squamous cell carcinoma in situ, most commonly affecting the middle-aged and elderly, and occurring on the skin or mucous membranes of various parts of the body. Its onset is concealed, the course of the disease is chronic, and some patients are accompanied by malignant tumors outside the skin, so it is necessary to diagnose and evaluate the disease in its early stage. This study aims to investigate the application of reflectance confocal microscopy (RCM) in diagnosing Bowen's disease. We performed RCM imaging on the lesion site of 113 patients initially diagnosed with Bowen's disease in clinic, of which 92 patients underwent skin biopsy for histological diagnosis. A retrospective analysis of the RCM result as well as the histological examination revealed that after analyzing RCM images, 69 out of 113 patients were diagnosed with Bowen's disease; out of 92 biopsy lesions, 61 were Bowen's disease, of which 54 were consistent with RCM diagnosis. Among 59 cases diagnosed with Bowen's disease by RCM, 54 cases were consistent with histological diagnosis. Afterwards, we analyzed the RCM characteristics in patients with Bowen's disease verified by biopsy, and compared the RCM images of two different lesions, Classic Bowen's Disease (CBD) and Pigmented Bowen's Disease (PBD), and further summarized the key points of Bowen's disease under RCM. Finally, we focused on the differential characteristics between Bowen's disease and other skin diseases under RCM. It is found that RCM is of great value in diagnosing Bowen's disease.

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Patient consent statement: All patients obtained informed consent before undergoing the examination.

Research Highlights:

1. A retrospective study of reflectance confocal microscopy (RCM) and histological diagnosis in patients with clinical diagnosis of Bowen's disease (BD).

2. Analyze the RCM characteristics of skin lesions verified by biopsy.

3. RCM has great value in the diagnosis and differentiation of BD.

Abstract

Bowen's disease (BD) is a relatively rare early-stage squamous cell carcinoma in situ, most commonly affecting the middle-aged and elderly, and occurring on the skin or mucous membranes of various parts of the body. Its onset is concealed, the course of the disease is chronic, and some patients are accompanied by malignant tumors outside the skin, so it is necessary to diagnose and evaluate the disease in its early stage. This study aims to investigate the application of reflectance confocal microscopy (RCM) in diagnosing Bowen's disease. We performed RCM imaging on the lesion site of 113 patients initially diagnosed with Bowen's disease in clinic, of which 92 patients underwent skin biopsy for histological diagnosis. A retrospective analysis of the RCM result as well as the histological examination revealed that after analyzing RCM images, 69 out of 113 patients were diagnosed with Bowen's disease; out of 92 biopsy lesions, 61 were Bowen's disease, of which 54 were consistent with RCM diagnosis. Among 59 cases diagnosed with Bowen's disease by RCM, 54 cases were consistent with histological diagnosis. Afterwards, we analyzed the RCM characteristics in patients with Bowen's disease verified by biopsy, and compared the RCM images of two different lesions, Classic Bowen's Disease (CBD) and Pigmented Bowen's Disease (PBD), and further summarized the key points of Bowen's disease under RCM. Finally, we focused on the differential characteristics between Bowen's disease and other skin diseases under RCM. It is found that RCM is of great value in diagnosing Bowen's disease.

KEYWORDS

diagnosis; Bowen's disease; reflectance confocal microscopy; histopathology

1 | INTRODUCTION

Bowen's Disease (BD) is a comparatively rare early-stage non-melanocytic intraepithelial malignant tumor. It is estimated that approximately 3% -5% of BD cases in the general population transform into invasive squamous cell carcinoma(Morton et al., 2014). The typical manifestation is chronic, asymptomatic, and scaly plaques. Its incidence rate is as low as 76.8 per 100,000 annually(Jansen et al., 2019). In general, the rate is slightly higher for women. For instance, the incidence rate for males and females in the Netherlands

in 2017 was 68 and 72 per 100,000 annually respectively(Jansen et al., 2019). However, due to the concealed onset of Bowen's disease and the general lack of obvious symptoms, patients often delay seeking medical treatment. Skin lesions of Bowen's disease lack obvious specificity in appearance and may be similar to other benign skin diseases clinically including eczema, seborrheic keratosis(SK), and superficial mycosis, which are easily misdiagnosed by clinical physicians(Mohandas et al., 2020).

Currently, the gold standard for diagnosing BD is biopsy confirmed by histology. Typical histopathology includes hyperkeratosis and parakeratosis or accompanied by scabs, disordered arrangement of cells in various layers of the epidermis, atypical cell morphology and size, large and deeply stained nuclei, some dyskeratotic cells, elongated and widened skin processes, and intact basal cell layer(Palaniappan & Karthikeyan, 2022). Since biopsy is an invasive method and most lesions of patients with BD occur in such exposed areas as the face and the head, many patients are reluctant to undergo such examinations. Besides, its incidence rate is low, clinicians often do not like to consider this diagnosis first. Therefore, a non-invasive examination technique is quite needed.

At present, there are several non-invasive examination techniques used for skin diseases, including dermatoscopy, reflectance confocal microscopy (RCM) and optical coherence tomography, and so on(Pellacani & Argenziano, 2022). Among which dermatoscopy is widely used. Dermatoscopy can magnify the surface of the skin and observe its submicroscopic structure and pathological changes simply and conveniently(Mazzilli et al., 2020). Researchers have probed into the value of dermatoscopy in diagnosing Bowen's disease, but its diagnostic value is limited since many skin diseases, such as actinic keratosis(AK), psoriasis, melanoma, contact dermatitis, may be similar to Bowen's disease in clinical practice and under dermatoscopy(Namiki et al., 2017; Wozniak-Rito & Rudnicka, 2018).

Compared to dermatoscopy, RCM is based on the principle of optical confocal imaging, which utilizes a system to focus on the reflected light of a low energy laser light source at a specific skin level. RCM can achieve a scanning depth of 150-250micrometer from the epidermis to the superficial dermis to capture cell level resolution images, enabling in vitro, non-invasive, real-time, and dynamic imaging (Lboukili et al., 2022; Shahriari et al., 2021), which offers more proofs for diagnosis and differential diagnosis. Some case reports discovered that RCM can assist in the diagnosis of Bowen's disease without considering the differential diagnosis(Shahriari et al., 2018; Yamanaka-Takaichi et al., 2019). In addition, it has been reported that RCM is capable of diagnosing and differentiating between neoplastic and inflammatory skin diseases(Ianoși et al., 2019; Tang et al., 2020). Some studies have discovered that RCM can be applied to distinguish Bowen's disease from some other diseases, including basal cell carcinoma(BCC) (Chen et al., 2023), and actinic keratosis(AK)(Zhou & Mistry, 2017). However, due to the low incidence of Bowen's disease, there are only a few case reports and small studies. Thus, no specific guidelines were formulated due to limited cases and inadequate evidence.

The study aims to probe into the application of RCM in the diagnosis and differential diagnosis of Bowen's disease, and to explore the complete scheme of BD diagnosis by RCM on the basis of clinical experience.

2 | MATERIALS AND METHODS

2.1 | Patients

The study focused on 113 patients (39 males and 74 females) initially diagnosed with Bowen's disease in clinic by Department of Dermatology, the Third Xiangya Hospital of Central South University from March 2016 to August 2023, with an average age of 51.3 (25-87 years old) and a course of disease ranging from 3 months to more than 10 years. The skin lesions include the face, limbs, back, lower abdomen, vulva, buttocks, scalp, and other parts. All patients obtained informed consent before undergoing the examination. This study was carried out on the principle of the Helsinki Declaration and received ethical approval.

2.2 | RCM imaging

RCM imaging was carried out on all selected cases using the VivaScope 1500 device produced by Lucid. The laser of the light source has a wavelength of 830nm and a power of less than 35mW at the tissue level. The

obtained images measure 500micrometer*500micrometer. The best images were obtained by dermatologists at the junction of the epidermis and dermis, as well as at the dermal papilla, and dermatologists diagnosed the patients according to RCM images.

2.3 | Histopathological examination

After RCM examination, 92 patients (29 males and 63 females) underwent skin biopsy. The biopsy tissue was fixed in formaldehyde, embedded in paraffin, sliced, stained with hematoxylin -eosin for routine treatment, and finally stored at room temperature. Dermatopathologists reviewed the images and conducted histological diagnosis.

2.4 | Analysis

A comprehensive data and descriptive analysis was conducted on the results of RCM and histopathological examination.

3.RESULTS

3.1 | Diagnosis by RCM

Based on RCM image analysis, 69 out of 113 patients (61.1%) were diagnosed with BD. 11 patients were diagnosed with AK, four with BCC, two with squamous cell carcinoma(SCC), five with SK, one with superficial fungal infection, two with eczema, three with bowenoid papulosis(BP), and one with extramammary Paget's disease (EMPD). Out of 113 patients, 15 (13.3%) couldn't be diagnosed by RCM, and were considered as undiagnosed. (Table 1)

3.2 | RCM diagnosis compared with histopathological diagnosis

Out of 113 patients, 92 underwent biopsy. Among these 92 cases, based on histopathological standard, 54 out of 59 cases were diagnosed with BD by RCM, which was consistent with histological diagnosis. Of the other five cases, two were diagnosed with AK, one with SK, and two with SCC. Five cases of AK, three cases of BCC, two cases of SCC, three cases of SK, two cases of BP, and one case of EMPD were diagnosed by RCM, which were consistent with the histological diagnosis. Two cases of BD were misdiagnosed as AK by RCM, and one case was misdiagnosed as BCC. After histopathological diagnosis of the 12 undiagnosed cases, one were diagnosed with BD, three with AK, two with BCC, two with SK, and one with discoid lupus erythematosus (DLE) (Table 2).

As shown in Table 2, the sensitivity and specificity of RCM in the diagnosis of BD were calculated as 88.5% and 86.8%, respectively.

3.3 | RCM characteristics in 54 patients of BD

3.3.1 | Skin lesions of Classic Bowen's Disease (CBD)

52 skin lesions were featured by red or dark red patches or plaques, often with scales, scabs, or exudates on the surface. Removing the scales and scabs can reveal a dark red granular or granulation-like moist surface, with almost no bleeding (Fig. 1a). In the histopathological images, the red or dark red patches or plaques show hyperkeratosis and parakeratosis, atypia in epidermal cell accompanied by dyskeratotic cells, and more obvious inflammation in the dermis (Fig. 1b). RCM shows five microscopic characteristics, including (1) scale/parakeratosis (Fig. 1c), (2) disarranged cells manifested as irregular honeycomb pattern in the spinous layer, (Fig. 1d), (3) target cells in the spinous layer (Fig. 1e), (4) enlargement of interpapillary spaces in some cases, (5) vessels in tortuous morphology inside the dermal papilla and infiltration of inflammatory cells in the dermis (Fig. 1f).

3.3.2 | Skin lesions of Pigmented Bowen's Disease (PBD)

9 skin lesions were pigmentary, manifested as black or brown scale patches or plaques with clear boundaries (Fig. 2a). In histopathology, the lesions were characterized by atypia of keratinized cells in the entire layer of the epidermis with scattered abnormal keratinized cells and basilar hyperpigmentation(Fig. 2b). Under

RCM, the following were observed: (1) varying degrees of hyperkeratosis and parakeratosis, (2) irregular honeycomb pattern and target cells in the spinous layer (Fig. 2c), (3) spindle-shaped cells with dendritic processes at the spinous layer (Fig. 2d), (4) edged papillae (Fig. 2e) and enlargement of interpapillary spaces, (5) Vessels in tortuous morphology inside the dermal papilla, with visible melanophages and inflammatory cells (Fig. 2f).

In summary, target cells and irregular honeycomb patterns are important RCM features of BD, while the presence of dendritic cells was not stable in different categories of lesions. Abnormal keratinization of the stratum corneum, irregular hyperplasia of the dermal papillae, and infiltration of inflammatory cells might all be present, but edged papillae and melanophages in the dermis were typical manifestations of pigmented BD. (Table 3).

3.4 | Differential diagnosis of Bowen's disease from other skin diseases

In order to further improve the accuracy of RCM in diagnosing BD and better differentiate it from other skin diseases, we listed the main manifestations of BD, AK, BCC, SK, and SCC (Table. 4).

4 | DISCUSSION

BD, also known as squamous cell carcinoma in situ, is a rare skin tumor characterized by being asymptomatic and slow growth. It is prone to the chronically exposed skin of the elderly. BD usually has no pigmentation, but occasionally pigmentary changes can be seen, known as PBD. PBD is an uncommon variant of BD, accounting for no more than 2% of all BD cases (Zhou & Mistry, 2017). Due to the atypical clinical manifestations, CBD is difficult to be distinguished from such diseases as AK, SK,tinea and eczema while PBD is difficult to be distinguished from diseases like SK, BCC and melanoma(Zhou & Mistry, 2017). The current gold standard for diagnosing Bowen's disease is still biopsy (Palaniappan & Karthikeyan, 2022). However, since the skin lesions often occur at the exposed part, and the diagnostic method for it is invasive and time-consuming, compliance of patients is poor. As a result, there are certain difficulties in conducting tissue biopsy, which often delays diagnosing.

RCM is an optical microscope that utilizes various refractive indices of light in different tissues or cells to present images of varying brightness levels under the microscope, forming in vivo three-dimensional images (Lboukili et al., 2022). RCM can intuitively observe the morphology and arrangement of cells, which is consistent with histopathology. Therefore, RCM could be used to identify atypical cells in vivo to a certain extent. Currently, RCM has been used to assist in the diagnosis of various neoplastic dermatoses. In some case reports and small studies, RCM has been utilized to assist in the diagnosis of BCC, Paget's disease, and SCC(Chen et al., 2023; Ozdemir et al., 2017; Shahriari et al., 2018; Tan et al., 2022).

At present, in some case reports or small studies, RCM has been reported as a diagnostic aid for BD (Ianosi et al., 2019; Lacarrubba et al., 2021; Mazzilli et al., 2020; Namiki et al., 2017; Rstom et al., 2022; Shahriari et al., 2018). According to existing studies, the easily accepted diagnostic points of RCM for BD include irregular honeycomb patterns in the epidermis, atypical cellular structures in the spinous layer, vessels in tortuous morphology inside the dermal papilla, and infiltration of inflammatory cells. In the RCM observation of this study, BD cases exhibited the above findings. Researches have found that for atypical cells, some of them can be recognized as target cell and are considered as manifestations of dyskeratosis cells(Ianosi et al., 2019; Karaarslan et al., 2018; Lacarrubba et al., 2021). It was observed in our study that target cells often appeared in the spinous layer, and in some cases, they might involve the entire layer of the epidermis, manifesting as an increase in cell volume, irregular morphology, low nuclear refraction, large nucleus, enhanced peripheral cytoplasmic refraction, and target-like structures with dark nuclei and bright cytoplasm. These cells are considered as dyskeratosis cells. Target cell were observed by RCM in BD cases in this study with high specificity, indicating that the appearance of target cells was a typical manifestation of BD. We also found dendritic phenomenon in some spinous layer cells in PBD, and found high refractive index melanophages in the dermal papilla. Therefore, we speculated that this might be a typical manifestation of PBD. The study found that dendritic cells (43%) with spindle-shaped spines were confirmed as lccs by immunostaining (Rstom et al., 2022), which might be due to the observed high reflectivity under RCM

mirrors after uptaking of melanosomes by LCs. In addition, we found that the reduced dermal papilla contained a majority of highly refractive pigment structures, which might represent melanocytes, and might be another feature of PBD. The characteristics observed in the lesions of BD cases, such as the disappearance of normal honeycomb pattern in the epidermis, disarranged cells, vascular proliferation in the dermal papilla, and infiltration of inflammatory cells, might also occur in other skin tumors, which might be an important reason for misdiagnosis with diseases including highly malignant SCC, AK, and BCC.

Because of its low incidence, atypical clinical manifestations, and the need for invasive biopsy for diagnosis, physicians usually do not like to diagnose it as BD at the initial diagnosis. The application of non-invasive technology makes it easier for patients to accept the advice of physicians for examination, and also makes it easier for physicians to include BD in clinical diagnosis and differential diagnosis.

Both BD and AK clinically manifest as erythema accompanied by scales, which can cause bleeding after being scraped off. After analysis of their RCM characteristics, it was found that both BD and AK exhibited hyperkeratosis, irregular honeycomb pattern and target cells in the epidermis, which made the two diseases easily confused. The proliferation of dermal papilla and superficial blood vessels, as well as the degree of inflammatory cell infiltration, cannot effectively distinguish between the two. Therefore, based on histopathology, we reanalyzed their RCM characteristics and found some subtle differences. AK showed more hyperkeratosis and parakeratosis in the stratum corneum, while BD showed more obvious hyperkeratosis. Besides, epidermal cells in BD were disarranged, with target cells appearing in the spinous layer or even the entire epidermis. In comparison, target cells for AK were mostly in the basal layer, and there were normal honeycomb patterns in the spinous layer. This manifestation may be a feature of gradual progression from AK to BD. SCC is a tumor that occurs in epithelial cells and is more malignant than AK and BD, with a wide invasion range. An early manifestation of SCC is infiltrative erythema, which gradually develops into plaques or warty lesions, often accompanied by ulcers and scabs on the surface. In this study, two cases of SCC were diagnosed as SCC after RCM and pathological examination. After analyzing the RCM characteristics, it was found that SCC had more severe hyperkeratosis in the stratum corneum, with keratin rupture, disordered arrangement of epidermal cells, and target cells throughout the entire layer of the epidermis. The difference was that in the dermis, atypical proliferation and aggregation of keratinocytes were displayed, and the cell volume was larger than that of surrounding inflammatory cells. This is an important distinguishing feature for identifying SCC with AK and BD.

SK is the benign epidermal proliferative tumor most commonly affecting the elderly, with a slow course and early manifestation of brown flat papules or patches with clear boundaries. Due to the long course of the disease, the lesion may gradually change after exposure to sunlight and scratching. At present, the commonly recognized points for diagnosing SK by RCM include hyperkeratosis, papillary hyperplasia of the epidermis, increased pigmentation in basal layer, formation of keratin-filled cystic inclusions, and cerebriform architecture of the epidermis(Guo et al., 2018). In this study, one patient was diagnosed with BD through RCM, but ultimately diagnosed with SK through pathology. Analysis of RCM characteristics revealed that SK and BD couldn't be accurately distinguished by hyperkeratosis, epidermal hyperplasia and dendritic cells in the basal layer. The presence of melanophages in the dermis was a factor that made it difficult to distinguish between SK and PBD.

BCC is the most common skin tumor with diverse and complex clinical manifestations. Superficial BCC is characterized by erythema with or without scales, which is easily confused with non-pigmented BD. Skin lesions of pigmented BCC is often black or brown, making it easy to be misdiagnosed as PBD. Therefore, exploring the RCM characteristics of BD has profound significance for the diagnosis of BD and BCC. The RCM characteristics of BCC include: disarranged epidermal cells, loss of normal honeycomb patterns, proliferation of dermal cells, elongated tumor masses in the form of palisade or spindle-shaped cells, numerous high refractive pigmented masses in the middle, dendritic cell structures, and aggregation of melanophages and inflammatory cells(Niculet et al., 2022). Our study found that the spindle-shaped cell structure of BD was mistakenly believed to be a tumor mass of BCC extending into the dermis, accompanied by melanophages, and was misdiagnosed as pigmented BCC. The spindle-shaped structure within the epidermis was a typical

manifestation of PBD, and the position of the tumor nest at different skin layers might affect the diagnosis of disease. Moreover, the structure and cell morphology of the blood vessels within the dermal papilla also require detailed analysis and observation.

It was found in our study that brown patches or plaques that occurred in the external genitalia also made it difficult to distinguish BP and PBD in clinical practice. Therefore, we analyzed the RCM characteristics of BP: papillomatous proliferation in the epidermis, disarranged spinous cells, irregular cell structures with high refractive and large nuclei, dendritic cells in the basal layer, and a small amount of melanophages and inflammatory cells in the dermis. These highly refractive heteromorphic cells with large nuclei were easily considered as target cells. Whether there is a certain association between BD and BP may require further exploration.

Due to the fact that in clinical practice, BD is more likely to be overlooked, we have posted clinical experience using RCM to distinguish BD from other diseases. (1) AK, BD and SCC are tumors derived from epithelial cells, with clinical manifestations of erythema accompanied by scales, ulcers, and scabs. RCM is featured by target cells, and we can differentiate diagnosis based on the presence of atypical keratinocytes in different layers of the skin. (2) When the clinical manifestation is black or brown skin lesions, dermatologists cannot ignore the possibility of BD. PBD is more likely to be confused with SK and pigmented BCC. The pattern of epidermal hyperplasia can be used to determine BD and SK, and palisade-like tumor masses in the dermis are the typical manifestations of BCC. (3) It is difficult to distinguish between superficial BCC and BD with clinical manifestations of erythema. RCM can find target cells throughout the epidermis and there is no pigmentation, which can serve as a clue for CBD and BCC. (4) Bowenoid papulosis is usually associated with HPV infection, and RCM can detect large vacuolar-like cells (with large nuclei and low refraction) in the spinous layer, which may be a feature that could exclude BP.

In addition to diagnosing and distinguishing BD, RCM can also be used to evaluate and delineate the boundaries of BD surgery, monitor the treatment process of laser therapy, topical imiquimod, photodynamic therapy and 5-fluorouracil, and identify BD recurrence after treatment. (Combalia & Carrera, 2020)

Limitations still exist in our research. To begin with, the study was carried out in a retrospective way. In addition, the majority of patients undergoing histopathological examination were patients with atypical clinical manifestations and RCM characteristics that require further diagnosis, so the biopsy samples were not randomly selected. In addition, the number of patients in this study was limited.

In summary, RCM exhibits high sensitivity and specificity in diagnosing BD, indicating that RCM is of great value in the diagnosis and differential diagnosis of BD, though more researches are required to testify it. The goal of our further efforts is to perfect the diagnostic standards for RCM and improve the consistency between RCM and histology.

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AUTHOR CONTRIBUTIONS

Shu Ding and Zhen Tang designed the study. Shu Ding, Zhen Tang, Lingxue Hu, Yu Rao analyzed the data and wrote the manuscript. Shu Ding, Lingxue Hu, Yu Rao collected patients' clinical and examination data. Ruijian Ren, Xiaoliang Tong, Aiyuan Guo designed the graphics. Shu Ding,Zhen Tang, Lingxue Hu ,Aiyuan Guo, Jian Huang performed the examination. All authors critically reviewed the manuscript.

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