Title Confocal microscopy and lentigo maligna: An in vivo pilot study for the assessment of response to imiquimod therapy

Running Head Lentigo maligna patients follow up with in-vivo confocal microscopy

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Key Message In-vivo RCM seems to be an applicable method for diagnosis and follow-up of LM.

Keywords Imiquimod therapy; Lentigo maligna; Non-invasive follow-up; Reflectance confocal microscopy

Abstract

Background Reflectance confocal microscopy (RCM) is a noninvasive technique that provides real-time in vivo images of the epidermal layer. Imiquimod has been recommended as an alternative treatment in lentigo maligna (LM) when surgical excision is not the treatment of choice. In the present study we compare the results of in vivo RCM to the histopathological examination before and after treatment of LM with topical imiquimod.

Methods Thirty-four patients with confirmed LM were included. Imiquimod 5% was applied until a weeping erosion appeared in the LM affected skin. Evaluation was performed by clinical examination, dermatoscopy, histopathology and RCM.

Results During the follow-up, twenty-seven of 34 patients (79.42%) demonstrated a total tumor clearance by imiquimod treatment. In the treated area, a significant decrease of atypical cell using RCM ($P < 0.0001$) was detected. Furthermore, a significant positive correlation in detected atypical cells using confocal microscopy and histology (p-value = 0.0001, $r = 0.7335$, respectively) was shown.

Conclusion In patients not suitable for surgical intervention imiquimod treatment is an appropriate treatment alternative. Thereby, in vivo RCM was demonstrated to be an excellent examine device, which not only allows diagnosis of LM, but also therapy and follow-up examinations. An important benefit of RCM, in contrast to conventional histopathology, is the simple handling with in vivo examination of epidermal skin without any pain for the patient.

Introduction

Lentigo maligna (LM) is the most common melanoma in situ, which occurs in chronic sun-exposed skin. [1-2] Between 5-15% of all LM transform to an invasive lentigo maligna melanoma over the years. [3]

Although the histological examination is still the gold standard to confirm the diagnosis of LM, this method is time consuming, invasive and painful especially at cosmetic critical locations in the face or at the head. [4-6] Histologic features associated to chronic sun damaged skin of LM patients are the epidermal atrophy, the increased pigmentation in basal keratinocytes, and a prominent solar elastosis. [7] The limitations of histological examination have led to the development of new non-invasive diagnostic methods that offer real-time in vivo results without scarring. [5-6] Among these techniques, in vivo reflectance confocal microscopy (RCM) which has been actively developing in recent years, with a sensitivity of 85% and specificity of 76% for the diagnosis of LM allows real-time visualization of the skin up to a depth of 200 μm with the best imaging-histologic correlation. [6, 8]

Once LM is diagnosed, dermatologists have several management options for a patient including surgery, radiotherapy [9] and cryotherapy. [10] Although surgical excision with an appropriate safety margin is still the gold standard and the treatment of choice, [11] in critical cosmetic localization of the lesions for example in the face, the head or the neck alternative treatments might be considered for LM in some patients.

In recent studies, imiquimod (Aldara 5% cream), an active agent from the group of immune-response modifier, has been shown as an appropriate non-surgical alternative to treat LM. [12-13] Furthermore, imiquimod has been recommended for adjuvant therapy to the surgical intervention. [14] Therapy monitoring and regular surveillance are important issues in LM, which might be performed by RCM repeatedly over time, enabling a noninvasive analysis of treatment effects. Hence, in the current study we investigated in-vivo confocal microscopy efficacy in the assessment

of Imiquimod treatment in patients with LM by architecture comparison of epidermis before and after treatment using RCM pictures by evaluation of the loss of cellular atypia after the treatment.

Materials and Methods

For further details, see the supplementary materials. (Figure 1).

Results

Patients and tumor characteristics are demonstrated in Table 2. Thirty-four patients (21 women, 13 men) with LM were considered for the analysis in the current study (November 2011 - July 2017). All patients were European with Fitzpatrick skin types I-III and a median age of 69 (range, 38 to 87 years). Thirty-two of thirty-four LMs were located in the face or in other parts of the head. Average duration of treatment was 41 days (range, 8 to 108 days). Only one patient was treated longer, i.e. 225 days, due to delayed skin reaction.

Therapy-induced local inflammation of the skin was generally well tolerated in all patients. Therapy only failed in one patient. He showed no macroscopic, histopathological and confocal microscopic improvement of the LM in the course of imiquimod therapy.

During the follow-up period, imiquimod treatment showed a cure-rate of 79.42% in all treated patients. Only seven patients revealed a recurrence of LM. (Table 3) The average time elapsing until recurrence of LM was 3.07 years. Five of these patients then had a surgically removal of the lesion, the other two were treated again with imiquimod.

The primary evaluation of confocal microscopy images showed higher numbers of atypical cells in the patients with disease recurrence. Thus, 30 atypical cells per mm^2 more were detected in the group of recurrence. However, this difference between the two patient groups was not statistically significant (P=0.615).

In addition, the LM lesion before and after the therapy with Imiquimod were compared with RCM (Figure 3), where the mean number of detected atypical cell using confocal microscopy decreased

significantly from 121.04 / mm² before to 7.25/ mm² after the therapy (P<0.0001). Only one patient showed no clinical response and LM persisted. Although, the atypical cells in RCM decreased from 146 to 105 cells. Furthermore, RCM images of patients without recurrence were compared with those of patients with recurrence. This analysis after the therapy revealed that in the patients without relapse, there was a decrease of 107.77 atypical cells and in patients with recurrence a decrease of 141.47 atypical cells/ mm².

Moreover, Spearman's rank correlation declared a significant positive correlation between detected atypical cells using confocal microscopy and histology (p- value = 0.0001, r= 0.7335, respectively). (Figure 4)

Discussion

In recent years, in vivo confocal microscopy has gained increasing significance as a new method of painless and non-invasive examination of cutaneous tumors at high magnification. [6, 17] The current study evaluated RCM as an alternative to histology in the diagnosis of LM and efficacy assessment of therapy with Imiquimod for LM. Thereby we were able to show that RCM can be another useful investigational tool for treatment surveillance in LM, i. e. providing reliable information on transformation of epidermal lesions, without false positive and false negative findings of clinical examination and dermatoscopy.

LM is the most common melanoma in elderly patients with sun-damaged skin. [2] Although the progression rate of LM into invasive melanoma is below 5% overall, [18] it is important to treat LM and prevent progression of disease. In patients with LM, surgical excision is still the gold standard and treatment of choice. Thus, Mohs micrographic surgery was shown to have a recurrence rate of only 3% in a follow up period of 5 years. [19] Nevertheless, surgical treatment cannot be performed in all cases, either because of comorbidities or because of the risk of poor cosmetic results. Thereby, surgical treatment is mostly limited by the size and the location of LM.

In recent years, topical imiquimod has been shown to be a good therapeutic alternative to surgical excision in patients with comorbidities or at all risks of cosmetic disfigurement. [20]
Imiquimod works as a topical immune response modifier, which operates through binding TLR-7/8 in neutrophils, macrophages and dendritic cells and regulates different genes involved in the immune response, oncogenesis and apoptosis. [21] In the treatment of LM, it is supposed that Imiquimod induces a cytotoxic T-cell-mediated immune response, which elicits the destruction of malignant melanocytes. [22] According to the study of Naylor et al. [23] imiquimod treatment shows in more than 80% of cases no relapse in the first year after treatment. In a study of Gautschi at al. [15] even a study group of 82% with a relapse-free interval over 5 years was shown. In the present study 79.42% of the treated patients complained no recurrence of LM during the follow-up period. However, the patients treated with imiquimod represent a special study population, because of their treatment of choice (Aldara 5% cream).
In LM lesions, the main limitation of conventional histopathological analysis is the impossibility to examine the entire lesion accordingly including the risk to miss the correct diagnosis. In recent years, RCM has proven to be a new examination method, which allows noninvasive and high-resolution imaging for in vivo examination of pigmented cutaneous lesions. Thereby, RCM possessing the power to analyze large skin areas makes this technology appropriate to choose the correct skin biopsy site for LM diagnosis and probably also to evaluate non-invasive treatment responses. In the present study, we performed quantitative assessment of cellular changes of LM after imiquimod treatment in in vivo RCM images using a new RCM atypia scoring system (counted atypical cells per mm^2, shown in the LM-score of Guitera et al. [8]).
The number of detected atypical cell using RCM decreased significantly after imiquimod therapy ($P<0.0001$). In addition, the Spearman correlation coefficient showed significant positive correlation between histopathological and RCM atypical cell counting (p-value = 0.0001, r=0.7335, respectively).

The demonstrated decrease of atypical cells clearly indicates the therapeutic effect of Imiquimod well corresponded to the clinical outcome. Moreover, our results demonstrated that RCM is suitable for a follow-up examination and therapy-control of LM patients.

Gautschi et al. [15] showed that in relapsing patients, already in the initial biopsy number of the total melanocytes was significantly increased compared to the relapse-free patients. Thus, these high cell numbers might be an indicator for the risk of recurrence. In our study, we could see an analogous phenomenon. Both RCM and histopathology at the initial examination showed in later on relapsing patients an increased number of total melanocytes, however compared to Gautschi et al. our results showed no statistical significance (P=0.615). [15] Nevertheless, we believe high initial atypical cell numbers might be a prognostic marker for recurrence of LM.

In conclusion, in patients not suitable for surgical intervention due to comorbidities, tumor size or cosmetic reasons, imiquimod treatment is an appropriate treatment alternative. It offers compared to surgery a cheap and painless therapy with low morbidity demonstrating a reasonable efficacy. Although the risk of recurrence is about 20%, with RCM, there is an excellent examine device, which not only allows diagnosis of LM, but also treatment surveillance and follow-up examinations. An important benefit of RCM is the simple handling with noninvasive, in vivo examination of epidermal skin, even in cases of recurrent and/or previously treated lesions. However, the operators should be aware of possibility of false negative and false positive results on confocal microscopy examination. Therefore, confocal and dermoscopic examination, should be considered along with patient-related information and clinical history, to define an optimal patient management. [24-26]

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None.

Declaration of interests

The authors declare that they have no competing interests.

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Figures

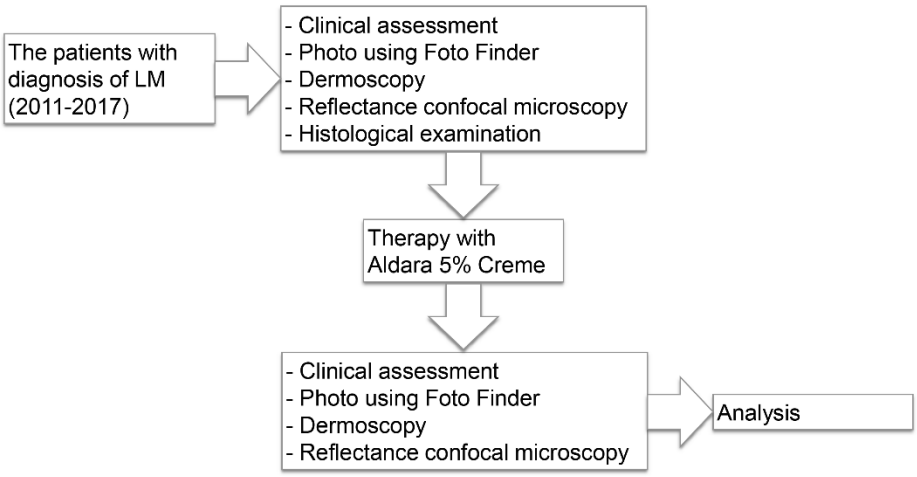

Fig. 1- Summary of the Materials and Methods

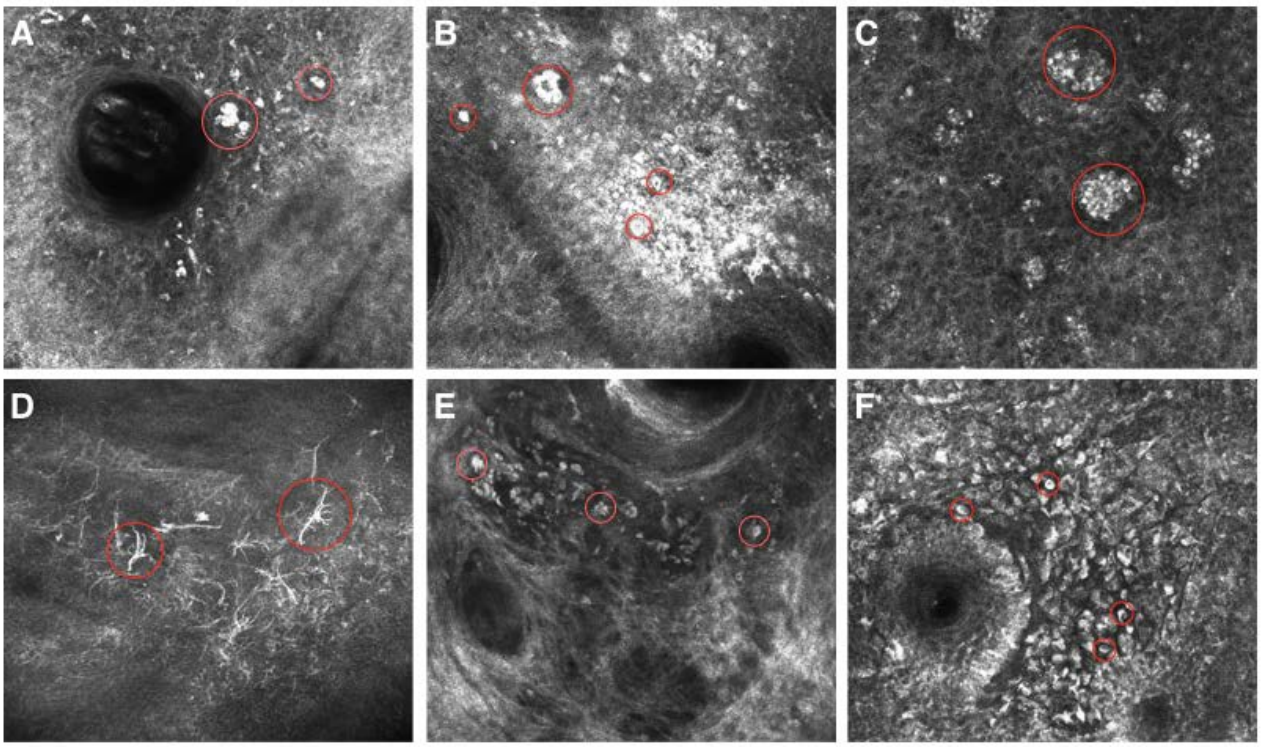

Fig. 2-Pictures of lentigo maligna under the RCM: (A) large round pagetoid cells in the epidermal layer (encircled). (B) large number of pagetoid and atypical cells(encircled). (C) cluster of cells (encircled). (D) atypical dendritic melanocytes within the epidermis (encircled). (E) non-edged papillae with atypical cells (encircled) in the area of the dermoepidermal junction. (F) follicular localization of atypical and nucleated cells (encircled).

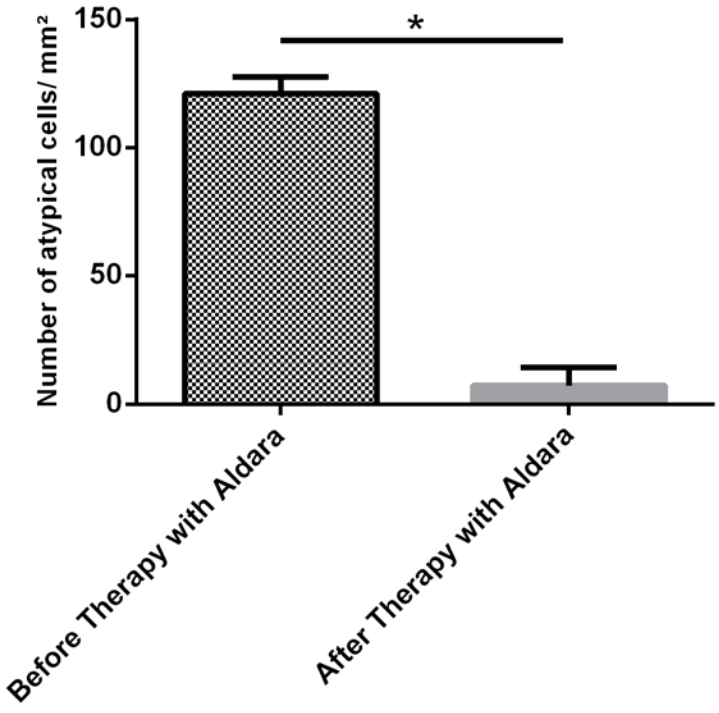

Fig. 3- Confocal microscopy parameters of all patients: Atypical cells per mm² before and after therapy with Aldara (*P< 0.0001)

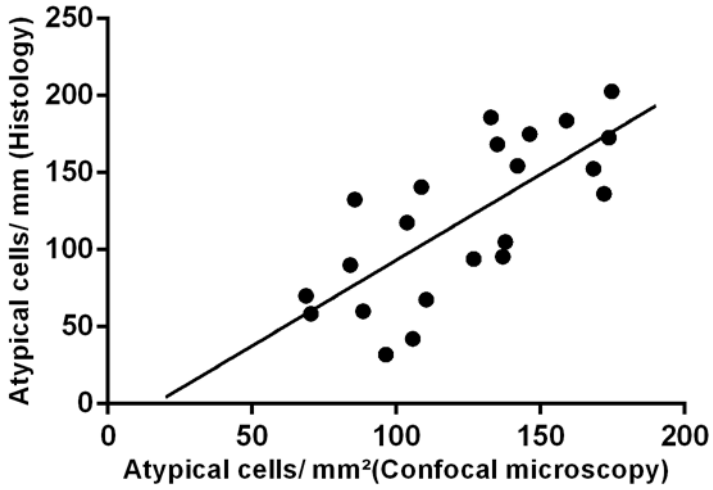

Fig. 4- Correlation of detected atypical cells using confocal microscopy and histology (p- value = 0.0001, r=, 0.7335).

Tables

Table 1- LM score, Guitera et al. [8]

Major features	Nonedged papillae	+2
	Large(>20μm), round pagetoid cells	+2
Minor features	Three or more atypical cells localized at the dermoepidermal junction in five 0.5 X 0.5 mm^2 images	+1
	Follicular localization of atypical and/or pagetoid cells	+1
	Nucleated cells in a dermal papilla	+1
	Broadened honeycomb pattern of the epidermis	-1

Table 2-Patients and tumor characteristics

Number of analyzed patients	34
Gender	21 women and 13 men
Age in years (range)	69 (38-87)
Localization of the lesion	32 lesions at the head, 2 at other localization
Recurrence rate	7 of 34 (20.58%)

Table 3- Time from end of the therapy to occurrence of disease recurrence (days)

Patient Number	Time from end of the therapy to occurrence of disease recurrence (days)
Patient 1	1353
Patient 2	683
Patient 3	831

Patient 4	3102
Patient 5	512
Patient 6	1111
Patient 7	256