
EXPLORING THE
MORPHOLOGY
OF THE SKIN
USING ADVANCED IMAGING TECHNIQUES
Kim Nguyen

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General introduction and outline of this thesis

1.1 Introduction

The skin is the largest organ of the human body. One of its main functions is to protect against pathogens and ultraviolet light (UV). The following layers can be identified in the skin: the epidermis, dermis, and underlying subcutis (Figure 1). In the epidermis, several layers can be distinguished. From the outside to the inside: the stratum corneum (SC), stratum granulosum (SG), stratum spinosum (SG) and stratum basale (SB). Keratinocytes represent the major cell type in the epidermis. They develop in the basal layer of the epidermis and differentiate while migrating upwards. They are designed to constantly renew the skin, which is important in skin wounding. Other cells in the epidermis include melanocytes and Langerhans cells.[1,2] The dermis consists of connective tissue protecting the epidermis and the vascular and nervous plexuses running through it. Furthermore, it contains few inflammatory cells that regulate inflammatory responses. The subcutis is the fat layer below the dermis and mainly consist of adipocytes. This layer plays an important role in thermoregulation and also functions as a shock absorber.[2]

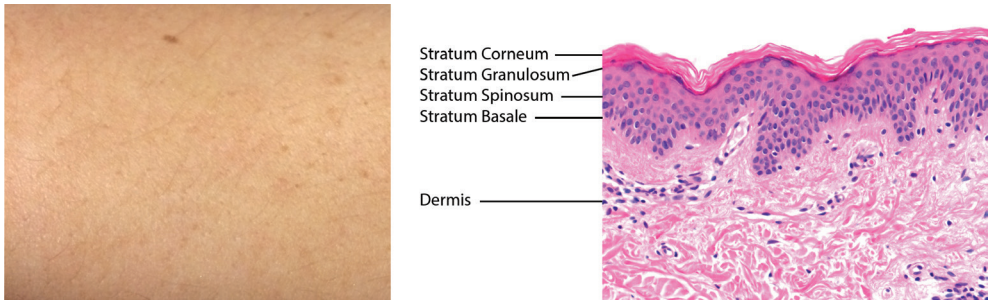

Figure 1. Clinical image and histological hematoxylin-eosin stained tissue section of normal skin. *(Reprinted from the thesis of M. Peppelman).*[3]

Due to its visibility and accessibility, the skin is easy to examine. Therefore, in dermatology, morphological characterisation of the skin is important in diagnosing and monitoring of treatment effects. Dermoscopy is a non-invasive method that allows evaluation of skin lesions using a tenfold magnification. Dermoscopy has been implemented in clinical dermatology, mainly in the field of dermato-oncology, using standardised pattern algorithms.[4,5] Despite its advantage, histological confirmation is often necessary to secure the diagnosis. However, histological confirmation, by obtaining a punch biopsy, is an invasive method. In addition, only part of the lesion can be examined and therefore sampling error may occur. In recent years, various advanced imaging techniques have been developed that reveal more detailed morphological features of the skin non-invasively. These techniques may be used as diagnostic and monitoring tools and evade the need for a skin biopsy. Reflectance confocal microscopy (RCM) and videodermoscopy (VD) are examples of these techniques.

For RCM, there is increasing evidence for its applicability in melanocytic lesions.[6-10] However, less is known about the role of RCM in non-melanoma skin cancer (NMSC), especially in squamous cell carcinoma (SCC) and its precursor lesions.[11-18] Also, (video)dermoscopy is mainly used in neoplastic skin lesions.[4,5,19-27] However, less is known about its value in inflammatory skin diseases (ISD).[28-31] Moreover, there is scarce knowledge on the possible additional value of VD, compared to dermoscopy, in this area.[32-34] Therefore, this thesis will focus on the diagnostic and monitoring values of RCM in NMSC and (video)dermoscopy in ISD in order to optimise the current diagnostic techniques, such as histological examination.

1.2 Non-melanoma skin cancer

In the Caucasian population, skin cancer is the most common type of cancer with rising incidence rates worldwide.[35,36] Skin malignancies can be divided into two main categories that encompasses melanoma and NMSC, that represent approximately 10% and 90% of the skin malignancies, respectively.[37,38] The term NMSC is mostly used to define basal cell carcinoma (BCC) and squamous cell carcinoma (SCC).[39] The incidence ratio between SCC and BCC is approximately 1:4.[35,40] Risk factors for developing BCC and SCC include individuals with fair skin, actinic damaged skin, exposure to ultraviolet radiation, chronic use of immunosuppressive medication, exposure to carcinogens (e.g. psoralens, arsenic, ionizing radiation), presence of chronic ulcers or burn scars, or genetic syndromes.[39] The majority of patients with a skin cancer are prone to develop skin cancer again.[41] About one-third of all patients who had a first BCC will develop at least a second BCC and in some cases a SCC.[42] These elevated risks vary geographically and reflect the underlying incidence rates.[42]

1.2.1 Basal cell carcinoma

Though, there is no consensus yet about the precise aetiology, BCCs are most likely to arrive from stem cells within hair follicles and interfollicular epidermis.[43,44] There are different histological subtypes (superficial, nodular, micronodular and infiltrative).[45] Each subtype has its own biological behaviour that affects treatment options and likelihood of tumour recurrence.[46] Most BCCs occur in the head and neck area, followed by trunk and extremities.[47,48]

Clinical features

Nodular BCC (nBCC) is the most common subtype, accounting for 50-79% of all BCCs.[47,49] These lesions are characterised by nodules with a pearly shine and small arborizing teleangiectasias. A crust may appear over a central depression and the lesion may ulcerate over time. Micronodular BCCs manifests as plaque-like indurated lesions with a poorly demarcated contour and have a higher incidence of local recurrence.[50] Superficial BCC (sBCC) is the second most common subtype, accounting for up to 15% of all BCCs.[47] They typically appear as well-described, scaly, pink-to-red maculae, thin papules or plaques. Infiltrative BCC (iBCC) occurs less often,

accounting for 5-10% of all BCCs.[47] Lesions present as pink-to-ivory, shiny, smooth, scar-like, indurated plaques or depressions with ill-defined borders.

Histopathology

Basal cell carcinoma can be divided into two main groups: non-aggressive growth subtypes (superficial and nodular) and aggressive growth subtypes (micronodular and infiltrative). Aggressive subtypes are more likely to recur and tend to cause extensive local destruction. When a BCC consist of more than one histological subtype, they are called a mixed type BCC. The incidence of mixed type BCCs ranges from 19% to 74%.[51-54] Basal cell carcinomas are histologically characterised by aggregations of basaloid keratinocytes with, at times, peripheral palisiding of nuclei (Figure 2). Often, the basaloid aggregations are surrounded by stromal tissue and regularly there is a microscopically visible cleft present between the tumour nests and peritumoural stroma. Nodular BCC is characterised by large, round to oval, nests of basaloid cells in the papillary or reticular dermis with peripheral palisiding and peritumoural clefts.[45] Ulceration may be present and cystic spaces within larger tumour islands due to necrosis. Like nBCC, micronodular BCC (mnBCC) show round or oval tumour nests but are smaller. They are approximately the size of hair bulbs and are more widely dispersed, and extending deeper into the dermis.[55] In some cases, these nests can penetrate the subcutis. Superficial BCCs are characterised by nests of basaloid cells residing high in the dermis, usually in a multifocal pattern.[45] Infiltrative BCCs consist of irregular, non-rounded strands or nests of basaloid cells surrounded by peritumoural stroma.[45] Sometimes the basaloid nests even penetrate the subcutis. Peritumoural clefting is uncommon and there is no peripheral palisiding.

Treatment

Basal cell carcinoma has a very low potential to metastasize. However, there might be considerable functional and cosmetic morbidity due to localised tissue invasion and therefore adequate treatment is necessary.[35,39,56] Various treatment options for BCC are available and depend on the histological BCC subtype. The treatment option of mixed type BCCs depends on the most aggressive BCC subtype. Surgical excision can be used to treat all BCC subtypes and is generally considered to have the lowest failure rate.[57] According to the Dutch guidelines, the advised excision margin for low risk BCCs (nBCC and sBCC) $\leq$ 10 mm in diameter is 3 mm. For high-risk BCCs (iBCC, mnBCC or recurrences) and/or tumours larger than 10 mm, the advised margin is 5 mm.[58] Non-surgical treatments include photodynamic therapy (PDT), 5-Fluorouracil (5-FU) cream and Imiquimod cream, and are mainly used to treat sBCCs. These treatment options are non-invasive and usually result in better cosmetic outcomes compared to surgical excision.[59,60] Furthermore, in some cases radiotherapy can be performed, mainly for high-risk tumours in patients who are unwilling or unable to tolerate surgery.[61]

1.2.2 Squamous cell carcinoma and its precursors

Squamous cell carcinoma can arise from precursor lesions such as actinic keratosis (AK), actinic cheilitis (AC), and squamous cell carcinoma *in situ* (Bowen's disease and erythroplasia of Queyrat).[39] While AK develops in the skin, AC arises on the lips.[62] Bowen's disease (BD) is an *in situ* SCC of the skin, while in erythroplasia of Queyrat (EoQ) the penile glans or prepuce are commonly involved.[63] Keratoacanthoma (KA) is another cutaneous neoplasm that clinically and histologically resembles a SCC. There is controversy about whether KA is a separate entity or a subtype of SCC (low-grade SCC).[64] According to various studies the estimated rate of progression of AK to SCC is up to 20%.[65,66] However, it was also concluded that reliable estimates of malignant transformation of AK could not be derived from existing data, especially due to the lack of reliable means of monitoring AKs over time.

Clinical features

Actinic keratosis usually appear as scaly, skin coloured, pink or brown superficial papules or plaques, often with an erythematous base (Figure 2).[38] Actinic cheilitis manifests as an atrophic white papule or plaque that usually becomes fissured, eroded, or ulcerated.[38] Bowen's disease usually presents as an erythematous, well-demarcated, scaly plaque.[38] Erythroplasia of Queyrat appears as a well-demarcated, flat, non-infiltrated eythematous plaque with no tendency of spontaneous regression.[67] Well-differentiated SCCs are classically recognised by thick, scaly papules and plaques, whereas poorly differentiated SCCs are often soft, non-scaly, ulcerated or hemorrhagic.[38] Keratoacanthoma presents as a dome-or bud-shaped, well-demarcated, umbilicated nodule with a hyperkeratotic plug in the centre. It evolves in 3 clinical stages: proliferative, mature, and resolving.[68]

Histopathology

Actinic keratosis is characterised by keratinocyte dysplasia with aggregates of atypical, pleomorphic keratinocytes with nuclear atypia, dyskeratosis, and loss of polarity. It can be classified according to the Keratinocyte Intraepithelial Neoplasia (KIN) system, with subdivision into three histological grades.[69-71] In AK KIN I, the keratinocytic atypia involves only the lower third of the epidermis. In AK KIN II, the atypical cells affects the lower two-thirds of the epidermis. In AK KIN III, including BD, the full epidermis is affected without infiltration of atypical cells into the dermis. Furthermore, hyperkeratosis, parakeratosis and solar elastosis in the dermis are often seen.[71] Squamous cell carcinoma has similar histological features as AK, but can be distinguished from AK due to the presence of tumour cells passing through the basement membrane into the dermis. Furthermore, nests of atypical tumour cells are formed in the dermis. Usually, there is full thickness epidermal atypia and a dermal inflammatory infiltrate.[71] Cellular characteristics of KA are similar to those of SCC.[68]

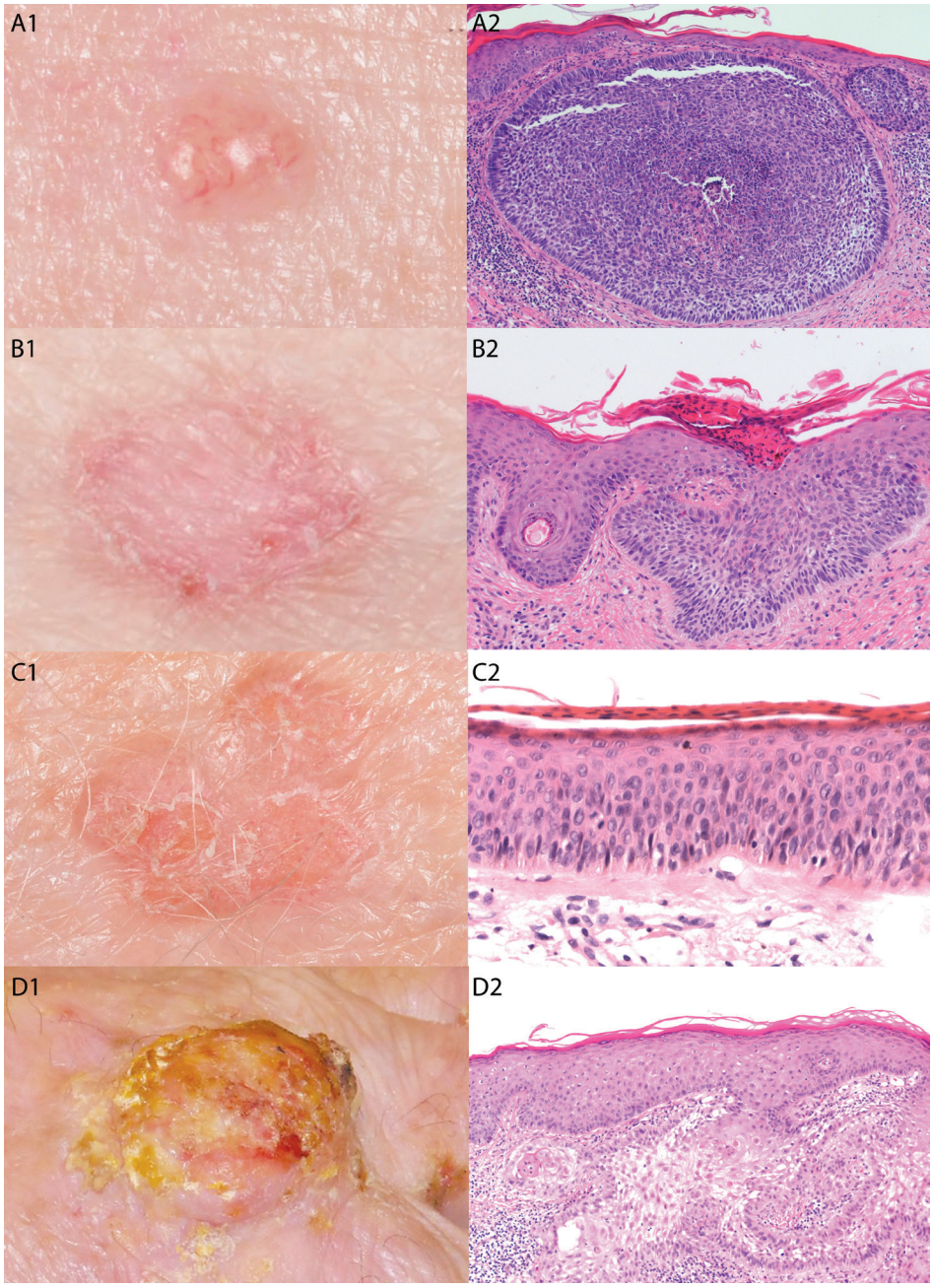

Figure 2. Clinical images of (pre)malignant skin lesions (1) with their corresponding hematoxylin-eosin stained tissue sections (2). A) Nodular basal cell carcinoma. B) Superficial basal cell carcinoma. C) Actinic keratosis. D) Squamous cell carcinoma.
(Reprinted from the thesis of M. Peppelman).[3]

Treatment

In a small number of cases, SCC might be life-threatening as it has the ability to metastasize. Therefore, adequate treatment is important. Surgical excision or radiation therapy are the preferred treatment for SCC.[38] Furthermore, treatment of the precursor lesions is recommended, as it is not possible to predict which of the precancerous lesions will progress into a SCC. The most common applied treatment for AK is liquid nitrogen cryotherapie. More extensive or multiple AK can be treated with 5-FU cream, Imiquimod cream and PDT. Other therapies for AK include Ingenolmebutaat or Diclofenac 3% gel.[72,73] Most of these treatments can also used to treat AC.[62] Treatment options for BD and EoQ include e.g. liquid nitrogen cryotherapie, PDT, 5-FU, Imiquimod cream and surgical excision.[74] For KA, surgical excision is the first choice of treatment.[68]

1.3 Inflammatory skin diseases

The term ISD encompasses a broad range of diseases in which inflammatory processes play an important role. Inflammation can be defined as a complex defence mechanism of the body to dangerous endogenous or exogenous stimuli. An overview of various ISD is described in the textbook of Billings *et al*.[75] The clinical classical signs of inflammation are rubor (redness), calor (heat), dolor (pain), tumour (swelling), and function laesa (disturbed function). Treatment options may vary from topical to systemic therapy. Since many cell types and mechanisms are involved in ISD, it is challenging to unravel the pathogenesis. Therefore, in histopathology, morphologic criteria are used for classification.

Histopathology

In general, most inflammatory dermatoses can be divided into two categories: epidermal and dermal patterns.[76] In the epidermal patterns, there are 3 primary patterns (spongiotic, psoriasiform, and interface). The spongiotic pattern is characterised by intra-epidermal accumulation of oedema. Psoriasiform patterns are identified by epidermal hyperplasie, whereas the interface pattern is recognised by damage to the basal layer of the epidermis by an inflammatory infiltrate. The dermal patterns lack significant epidermal changes. Broadly, the dermal patterns can be divided into 4 patterns (perivascular, nodular and diffuse, palisiding granulomatous and sclerosing). The perivasculair pattern shows an inflammatory infiltrate mainly around dermal blood vessels in a superficial, or superficial and deep distribution. In the nodular and diffuse pattern, the infiltrate is less concentrated around the blood vessels. The palisiding granulomatous pattern is characterised by infiltrates surrounding zones of altered collagen. In sclerosing dermatoses, fibrosis of the dermis is present, usually with relatively little inflammation. Even though most ISD can be sorted into one of the major categories, there are inevitable dermatitides that defy classification or show overlapping features.[76] In addition, a particular dermatitis may have a completely different appearance early in the course of the

disease compared to late in the disease process. Therefore, some inflammatory lesions may be sampled either too early or too late in their evolution to be diagnostic. This dynamic process of inflammation results in lesions that are heterogeneous. Therefore, it is critical to sample the appropriate site with knowledge of the point in time when the lesion was sampled, to detect morphological changes that are optimal for microscopic interpretation and diagnosis.[77]

1.4 Limitations of histopathological evaluation

In dermatology, the majority of skin lesions are diagnosed clinically after visual inspection of the skin. Sometimes a dermoscopy is used to aid in the clinical diagnosis. In case of suspicion of NMSC or clinical difficult to diagnose ISD, a diagnostic punch biopsy may be obtained. However, punch biopsies are invasive diagnostic methods which may result in scarring, inflammation, hindering the ability to monitor the dynamic processes over time and the number of biopsies is limited due to practical and cosmetic reasons. Furthermore, it may be a challenge to select the appropriate biopsy site to sample, particularly in heterogeneous lesions. There is a risk of sampling error, in which the biopsy was not taken from the most aggressive or active part of the lesion and therefore the diagnosis is missed.[52,53,78-80] As a result, e.g. the aggressive BCC subtype or the presence of a SCC in a large AK lesion may be missed. In addition, due to the heterogeneity of an inflammatory lesion in ISD, it can be challenging to choose an appropriate site for sampling. This can lead to non-specific and descriptive diagnoses, making it difficult to select an adequate treatment. Moreover, when a punch biopsy is obtained, only a part of the punch biopsy will be histopathologically evaluated. Theoretically, the most aggressive or active part of the punch biopsy may be missed resulting in under- or misdiagnosis. This may lead to patient discomfort and high treatment costs due to subsequent treatments. Therefore, it is interesting to evaluate the sampling error within a punch biopsy. In addition, the usage of advanced imaging techniques may play a role in reducing the risk of sampling errors.

1.5 Advanced non-invasive imaging techniques

1.5.1 Reflectance confocal microscopy

In 1957, Marvin Minsky invented the confocal microscope.[81,82] During that time, the microscope was only suitable for imaging of *ex vivo* tissue samples. Thereafter, changes were made enabling *in vivo* imaging of human skin. Currently, the most frequently used *in vivo* RCM devices in the field of dermatology are the Vivascope 1500 and 3000 (handheld device) by Caliber Imaging & Diagnostics Inc. (Rochester, New York, USA) (Figure 3). For *ex vivo* purposes, the Vivascope 2500 is available.

Figure 3. Vivascope 1500 device.
A metal ring with an adhesive window is placed onto the skin. A drop of fluid is applied between the window and the skin. The VivaCam is used to obtain a clinical dermatoscopic image. Afterwards, ultrasound gel is placed onto the window and the objective lens housing is attached to the metal ring. After starting the laser, images in black and white will appear on the computer screen.
(Reprinted from the thesis of M. Peppelman).[3]

Technical principle

The reflectance confocal microscopy uses a point source of light, derived from a near-infrared 830 nm laser[83], which penetrates into the skin and illuminates a small point inside the tissue (Figure 4). The laser power lies between 5 and 10 mW and does not cause any damage to the skin. Structures within the skin reflect the laser light. Subsequently, a detector collects the reflected light through a small pinhole and out of focus light from another tissue point will be blocked from detection. Therefore, only reflected light from the focal region is detected. The lateral resolution of RCM is less than 1.25 μm and the axial resolution is less than 5 μm. This technique produces images of the skin parallel to the skin surface (en face) and can examine the skin up to a depth of approximately 250 μm. The contrast in RCM images relies on the differences in the reflectivity of tissues, which is depending on their chemical and molecular structures. Structures with a high refractive index, like melanin and melanosomes, appear bright in RCM (Figure 5). The reflectivity of white blood cells, chromatin, collagen and elastin is lower.[84-88] In normal skin, RCM can visualize the epidermis, papillary dermis and superficial part of the reticular dermis. Each layer has its own set of distinct RCM features (Figure 5). A digital camera (VivaCam) is part of the Vivascope set up. Prior to RCM imaging, the VivaCam is used

to capture a clinical dermatoscopic image of the lesion. Afterwards, this clinical image can serve as a navigation map during RCM imaging.

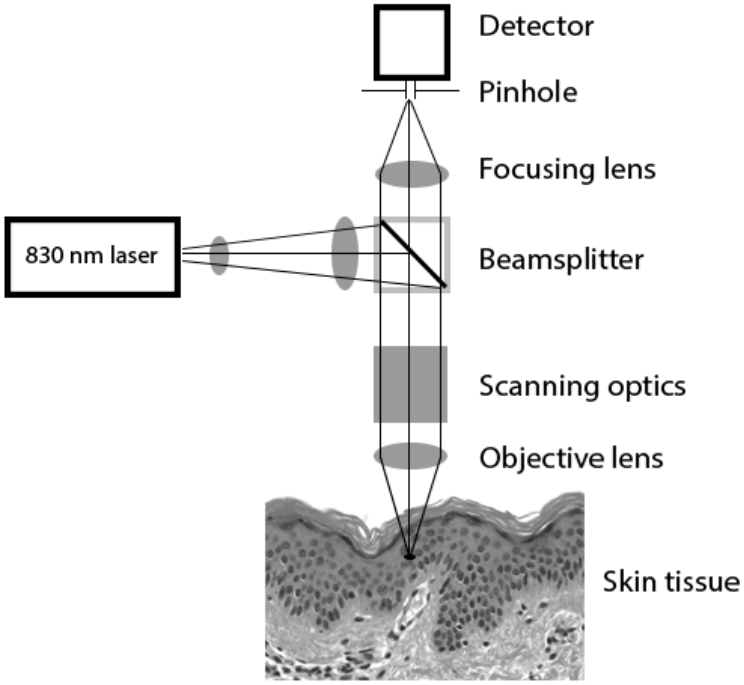

Figure 4. Schematic illustration of the technical principle of the reflectance confocal microscopy. *(Reprinted from the thesis of M. Peppelman).*[3]

Practical aspects

In order to obtain RCM images of a lesion, a metal ring with a polymer window is attached to the skin. This ring is magnetically connected to the objective lens housing to stabilize the imaging location. To reduce the amount of backscattering of light and to optimise image quality, a drop of water or oil is applied between the window and skin. In addition, ultrasound gel is placed between the window and objective lens housing. The standard acquired RCM image depicts an area of 500 x 500 μm of the skin (confocal image) (Figure 6). The separate horizontal confocal images can be assembled into a large mosaic image of maximum 8 x 8 mm (VivaBlock). Moreover, the objective lens can move vertically to capture images in depth (VivaStack), while maintaining the same field of view. This VivaStack has an automatic section depth of 4.5 μm, but can be adapted to other preferable depths. In addition, videos at 9 frames per second can be captured to document dynamic processes (e.g. blood flow, and migration of leukocytes).

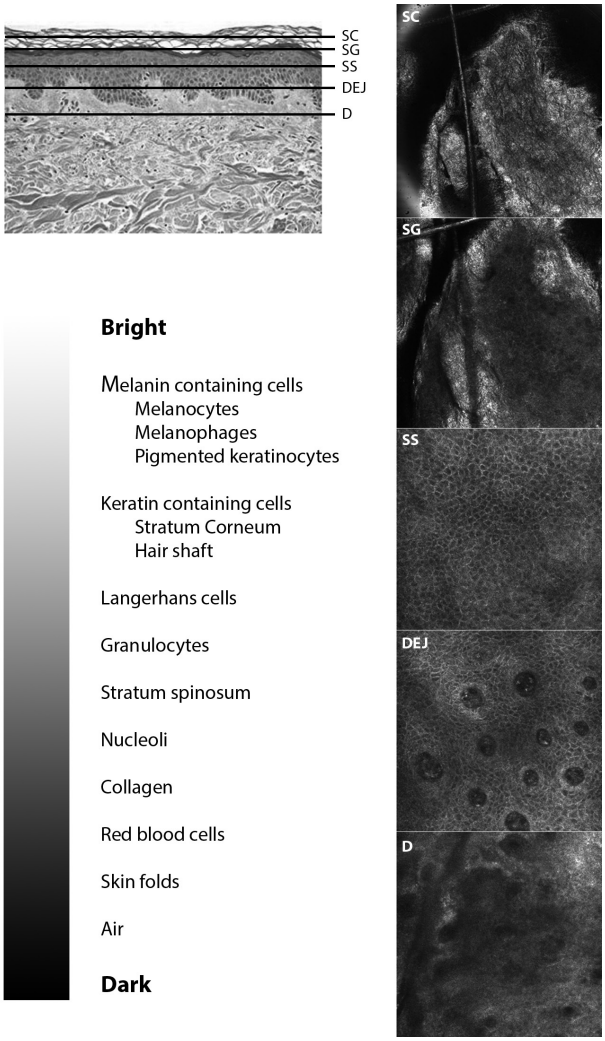

Figure 5. Histological tissue section of the normal skin (in vertical plane) with lines at the level of stratum corneum (SC), stratum granulosum (SG), stratum spinosum (SS), dermo-epidermal junction (DEJ) and dermis (D). At these levels, reflectance confocal microscopic (RCM) images are obtained (in horizontal plane). Each level has its own distinct RCM features. SC: grey, with no nuclei or cellular outlines. SS and SG) dark round nuclei with surrounding bright cytoplasm in a regular honeycomb pattern. DEJ) dermal papillae visible as dark circles. D) dark grey with reflective reticular fibers. Illustration depicts the reflectivity of cellular and subcellular structures of the skin decreasing in brightness.

(Reprinted from the thesis of M. Peppelman).[3]

Relevance in dermatology

RCM allows in-depth imaging of the skin in a comfortable, non-invasive, manner without causing pain or scarring. The skin tissue is not altered by tissue processing (e.g. fixation, mounting) or staining. Real-time data collection is faster than routine histology and a whole lesion can be investigated. This might reduce the risk of sampling error and decrease delay in diagnosis and adequate treatment. Furthermore, the same location of the skin can be repeatedly imaged over time. This allows monitoring of a lesion to evaluate processes such as lesion progression or response to therapy.

Melanin and melanosomes are key histological features of melanocytic lesions and are strong contrast sources for RCM imaging. Therefore, most research was focussed on melanocytic skin lesion at the time RCM was first introduced in the field of dermatology.[6-10] Thereafter, studies on the application of RCM in NMSC appeared, mainly focussing on BCC and its subtypes.[12,14,18,89-95] Only a small number of research was performed on other types of (pre)malignant skin cancers in NMSC, namely SCC and its precursor lesions.[13,80,89,96] In addition, the role of RCM in ISD has been studied.[97-101]

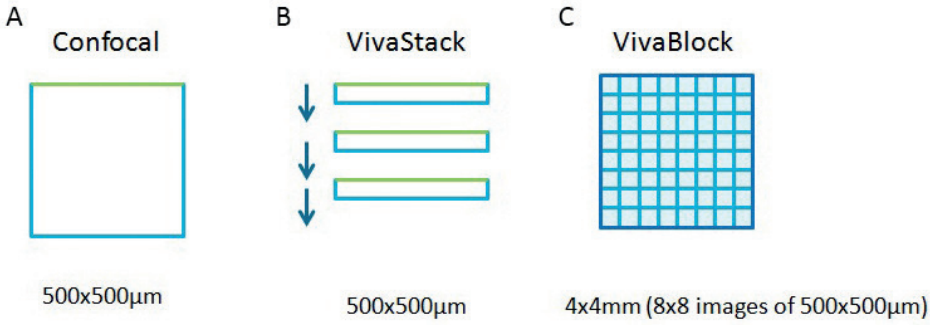

Figure 6. Reflectance confocal microscopic images.
A) Confocal image: basic image of 500 μm x 500 μm. B) VivaStack: a sequence of confocal images (500 μm x 500 μm) captured at the same horizontal position but at different depths. C) VivaBlock: a mosaic of 8 x 8 confocal images (4 mm x 4 mm) providing a larger field of view.
(Reprinted from the thesis of L. Hoogedoorn).[102]

1.5.2 (Video)dermoscopy

In 1663, skin surface microscopy was first introduced by J.C. Kolhaus, who applied the method originally to inspect the nail fold capillaries.[103] The term 'dermatoscopy' was first used in 1921 by J. Saphier.[104] The technique was further developed by Goldman in the 1950s and called 'dermoscopy'. He was the first dermatologist to use this new technique for the evaluation of pigmented skin lesions.[105] Since then, many studies have been published on the use of

dermoscopy in pigmented skin lesion.[106-111] Today, dermoscopy has become a routine practice in Europe and gaining acceptance in other countries.

Technical principle

Dermoscopy (also known as surface microscopy or epiluminescent microscopy) is a technique that allows a rapid and magnified *in vivo* observation of the skin surface. By definition, it is performed with handheld devices (dermatoscopes), allowing 10x magnification (Figure 7). Conventional dermoscopy uses non-polarised light sources to illuminate the skin. It requires a liquid interface and direct contact between the scope and the skin. In this way, the amount of light reflected, refracted and diffracted at the skin surface is reduced, allowing the observer to visualize structures below the stratum corneum.[112] Currently, also polarised dermatoscopes are available. Unlike non-polarised light dermoscopy (NPD), polarised light dermoscopy (PD), allows visualization of deep skin structures without the necessity of a liquid interface.[113]

Figure 7. Dermatoscope.

Between 1980 and 2000, there were many improvements in digital camera and computer technology which have led to the development of the digital dermatoscopy (videodermatoscope).[114] Videodermoscopy (VD) is performed by a video camera equipped with optical fibers and lenses that allow high-resolution imaging at magnifications up to 1000x (Figure 8). The images obtained are visualised on a monitor and can directly be stored digitally, to identify and compare changes over time.[115] To aid the dermatologist, some of the systems offer the possibility of computer-assisted diagnosis for malignant melanoma or for consulting an expert through telemedicine.

Relevance in dermatology

Both dermoscopy and videodermoscopy are mainly used for the evaluation of pigmented skin tumours. Multiple algorithms have been developed to diagnose melanoma (e.g. ABCD rule, 7-points checklist and Menzies method).[83,111,116-119] On the other hand, they are also used in the area of ISD, in order to assist the clinical diagnosis and reduce the need for invasive skin biopsies.[120-122] As videodermoscopy allows higher magnification, it may offer visualization of more specific features compared to dermoscopy. Therefore, it would be interesting to evaluate the current and future role of (video)dermoscopy in ISD and the possible benefit of VD over dermoscopy in this area.

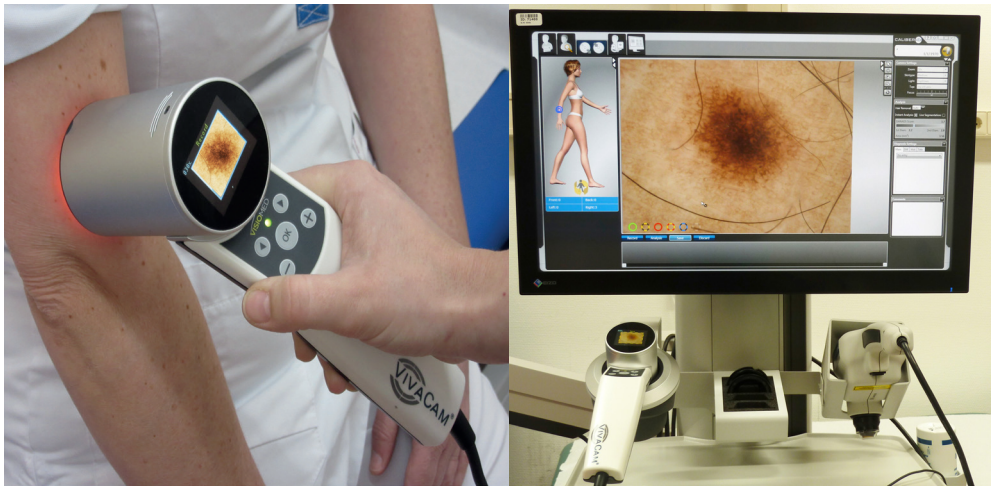

Figure 8. Videodermatoscope.
The naevus on the arm is visualised on the computer screen of the videodermatoscope with a magnification of 80x.

1.6 Aims and outline

At present, skin biopsies are the gold standard for diagnosing cutaneous malignancies and clinical difficult to diagnose ISD. However, a punch biopsy is prone to sampling errors. This may occur due to sampling an inappropriate site within a heterogeneous lesion or perhaps due to missing the most aggressive or active part within a punch biopsy during histopathological examination. Consequently, this can lead to under-, mis- or non-specific diagnoses and inadequate treatment.

The overall objective of this thesis is to explore the morphology of the skin using advanced imaging techniques in order to optimise the current diagnostic process. The thesis primarily focuses on 3 aims:

1. To investigate the sampling error in sBCC and its clinical effect
2. To investigate the applicability of *in vivo* RCM in NMSC
3. To investigate the applicability of (video)dermoscopy in ISD

Chapter 2 focuses on sampling errors and its clinical effects in lesions diagnosed as sBCCs using punch biopsies. **Chapter 2.1** evaluates whether more aggressive BCC subtypes can be detected with additional step sectioning in the histopathological examination procedure in punch biopsies, that were initially diagnosed as sBCCs. In **chapter 2.2** the clinical efficacy of two different MAL-PDT protocols for the treatment of sBCC is studied. Interestingly, it evaluates whether treatment failures and recurrences are related to sampling error of the primary punch biopsies.

The use of advanced imaging techniques, such as the RCM or (video)dermoscopy, might be able to diminish the risk of sampling error. Therefore, these techniques are evaluated in **chapter 3 and 4**. Using RCM, an in-depth exploration of the skin can be performed to detect the most affected area within a lesion. It may be used as a diagnostic tool and evade the need for a skin biopsy. However, prior to the use of RCM as a diagnostic tool, explorative studies have to be conducted to evaluate diagnostic RCM features for specific skin conditions. Therefore, in **chapter 3.1** specific RCM features for skin entities within the continuum of AK and SCC are studied. Furthermore, **chapter 3.2** addresses the study where specific RCM features, to differentiate between SCC and AK, are explored. In addition to AK and SCC, multiple explorative studies have already determined diagnostic RCM features for the diagnosis of BCC, including its subtypes, and features to differentiate it from other skin entities. Therefore, the regular use of RCM as a diagnostic tool for the diagnosis of BCC in daily clinical practice is getting closer. Nonetheless, a large RCT is necessary to investigate the cost-effectiveness and whether RCM is (non-) inferior to histopathological evaluation of punch biopsies, in diagnosing BCC and its subtype, prior to implementation in dermatological practice (**chapter 3.3**).

The use of dermoscopy and VD offer clinicians the possibility to examine a greater area under a high magnification. Both techniques are mainly used within the pigmented dermato-oncology. However, they can also be useful in ISD, as these entities can occur in the facial region, where invasive skin biopsies are not cosmetically favoured. (Video)dermatoscopy may improve the diagnostic accuracy of a clinical diagnosis without the need for invasive skin biopsies. Though, prior to the use of (video)dermoscopy as a diagnostic tool, specific (video)dermoscopic features for different ISDs have to be determined (**chapter 4.1**).

In **chapter 5** the results presented in this thesis are summarised and discussed.

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2.1

Standard step sectioning of skin biopsies diagnosed as superficial basal cell carcinoma frequently yields deeper and aggressive tumour subtypes

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Abstract

Background: Correct diagnosis of superficial basal cell carcinoma (sBCC) is essential due to the increase of non-surgical treatments for this subtype. Histological confirmation by punch biopsy for the diagnosis of BCC and its subtype is recommended. However, a standardised histological method for sectioning punch biopsies is currently missing.

Objective: To compare the accuracy of histological examination of only one level in sBCC punch biopsies (current method) with a more extensive step-section method. Additionally, to investigate whether tumour thickness, ulceration and adnexal extension are determinants of treatment failure or recurrence in initial diagnosed sBCCs.

Methods and methods: 116 sBCC punch biopsies, obtained between 2014-2015 at the Department of Dermatology (Radboud university medical center, Nijmegen, the Netherlands), were cut in 4 additional levels and thereafter histopathologically evaluated.

Results: In 22.4% a more aggressive BCC subtype was missed with the current examination process. In 25% of the treatment failure and recurrence group, sBCCs were thicker than 0.4 mm. Ulceration and adnexal extension did not occur in this group.

Conclusion: This study shows that histological examination of only one level in a punch biopsy leads to underdiagnosis of more aggressive BCC subtypes in biopsies diagnosed as sBCC. Recommendations for a revised examination method are proposed to reduce the risk of missing more aggressive BCC subtypes and to prevent undertreatment.

Introduction

Basal cell carcinoma (BCC) is the most common malignancy in the Caucasian population with a rise in incidence worldwide, emphasizing the importance of BCC diagnosis and managment.[1] European guidelines recommend a punch biopsy of clinically suspected BCC prior to treatment to confirm diagnosis and identify the histological subtype.[2] There are different histological BCC subtypes, each with its own biological behaviour that affects the treatment options and likelihood of tumour recurrence.[3] Aggressive BCC subtypes include infiltrative (iBCC) and micronodular (mnBCC) types that have a high risk of incomplete excision and recurrence. Nodular (nBCC) and superficial BCC (sBCC) are considered non-aggressive BCC subtypes.[4] While nBCC is the most common histological subtype, a significant higher increase in the proportion of sBCC is noticed.[5] The dramatic increase in sBCCs leads to a greater applicability of non-surgical treatment options such as methylaminolaevulinate photodynamic therapy (MAL-PDT) and topical application of Imiquimod and 5-Fluorouracil (5-FU).[6,7] Mixed type BCCs consist of multiple subtypes. The treatment option of mixed type BCCs is dependent on the most aggressive BCC subtype. However, misdiagnosis of the subtypes within mixed type BCCs can occur due to a sampling error within the lesion. Multiple studies have evaluated the reliability of a punch biopsy in accurately subtyping BCCs by comparing the initial punch or shave biopsy with the excision specimen. The discordance ranged between 18.0% and 39.1%.[8-11] However, to the best of our knowledge, no studies have analysed the sampling error within a single punch biopsy. Failure to detect aggressive subtypes by histopathological evaluation of a punch biopsy may result in undertreatment and eventually lead to more patient discomfort and higher healthcare costs.

According to the Dutch guideline, the histopathological examination report of a BCC punch biopsy must include at least the lesion's location, histological growth pattern and whether it is a recurrent BCC.[12] However, the histological examination process of punch biopsies performed in lesions suspicious for BCC is not standardised. Therefore, there is a variation across institutions with respect to histopathological evaluation methods. At the Department of Pathology at the Radboud university medical center (Radboudumc), Nijmegen, the Netherlands, the current protocol for the histological examination of a 3 mm punch biopsy suspicious for BCC consists of evaluation of two to three, 4 μm thick, hematoxylin and eosin (H&E) stained tissue sections. These sections are obtained from one level, in which the contour of the punch biopsy is completely visible (at approximately 1000 μm). In case a BCC histological subtype is detected in the initial H&E sections obtained from the first level, no additional levels will be cut and the histopathological diagnosis will be based on the detected BCC subtype. When no BCC is identified in the initial H&E sections, the punch biopsy is cut at four more levels with an interval of approximately 200 μm. One to two H&E sections per level are analysed. In case a mixed subtype is found, the histopathological diagnosis will either be based upon all the detected BCC subtypes or only the most aggressive subtype that is of most relevance for treatment selection.

There is a debate whether factors other than the histological subtype can guide treatment of BCC. Multiple studies have evaluated clinical ulceration, tumour thickness and adnexal extension in sBCCs as determinants of treatment failure.[13-16] However, the association between these parameters and non-invasive treatment outcome is still uncertain.

This retrospective study sought to analyse the sampling error within a 3 mm punch biopsy suspicious for BCC by evaluating the current histological examination method using a revised examination process. Because the therapeutic impact is the highest for mixed type BCC in which a sBCC is found and a more aggressive BCC subtype (nBCC, mnBCC, iBCC) is missed, the focus will be on sBCC. The secondary objective was to aid in developing a standard sectioning protocol for BCC. Furthermore, we investigated whether tumour thickness, ulceration and adnexal extension are determinants of treatment failure or recurrence in initial diagnosed sBCCs treated with MAL-PDT, Imiquimod or 5-FU.

Materials and methods

This retrospective study was approved by the institutional review board of the Radboudumc, Nijmegen, the Netherlands.

Population

The McNemar test was used for the power calculation with the following variables: $\alpha = 0.05$, power = 80% and probability = 1%. The prevalence of finding a sBCC subtype in histopathological diagnosed mixed type BCCs in excision specimens was at least 16%. This was deducted from the study of Wolberink *et al.*[8] The prevalence of a superficial subtype in histopathological diagnosed mixed type BCCs in punch biopsies was estimated to be around 10%. Using this information, a sample size of 116 punch biopsies was determined. One hundred and sixteen sBCCs in the years 2014-2015 were included. A chart-review was performed of punch biopsy proven primary sBCCs. Appropriate charts were selected from Radboudumc, using the Dutch pathologic anatomic national computerised database (PALGA). This database contains histopathological reports and diagnosis of all tissues evaluated by pathologists in the Netherlands. Superficial BCCs with the following criteria were included: (i) derived from 3 mm punch biopsies (ii) histopathological diagnosis of sBCC without any other BCC subtypes (iii) histological analysis involved sectioning in one level only (at approximately 1000 μm) after formalin fixation and paraffin embedding. Exclusion criteria were punch biopsies from patients with (i) genetic skin disorders (ii) using immunosuppressive medications (iii) previous radiotherapy (iv) previous documented BCC at the same location, which could represent a recurrent BCC.

Data collection

The included sBCC punch biopsies, embedded in paraffin blocks after tissue processing, were requested from the Department of Pathology, Radboudumc. The punch biopsies were cut at four levels at an interval of 200 μm. After every 200 μm, ten sections of 4 μm each were cut of which the middle two sections were stained with H&E and evaluated. A total of 5 levels per punch biopsy were available for histopathological evaluation (Fig. 1). BCC subtype classification was based on the Dutch guidelines.[12] Superficial BCC was defined as nests of basaloid cells residing high in the dermis (above the level of the vascular plexus), usually in a multifocal pattern. Nodular BCC was defined by the presence of large, rounded tumour nests. Micronodular BCC resembled nBCC but with smaller nests. Infiltrative BCC consisted of irregular, non-rounded strands or nests of basaloid cells surrounded by desmoplastic stroma. Presence of adnexal extension of sBCC was defined as tumour cells along hair follicles or sebaceous gland ducts growing deeper than the deepest located tumour nest. Micronodular BCCs and iBCCs have the smallest BCC nests. The size of a mnBCC nest is comparable to the bulbus of a hair follicle which is approximately 270 μm.[12,17] The choice of cutting step sections at 200 μm intervals was aimed to identify even small BCC nests. The sectioning, staining and evaluation of the H&E stained sections was done by a pathologist-in-training (GJK) at the Radboudumc. In case of doubt and/or in case another BCC subtype was observed, evaluation was also performed by a certified dermatopathologist (WAMB). Additionally, H&E stained sections from one out of every ten punch biopsies were also assessed by the dermatopathologist. The dermatopathologist was blinded for the diagnosis made by the pathologist-in-training. Tumour thickness was evaluated by measuring the basaloid nest from the stratum granulosum up until the deepest point of invasion using a 0.1 mm precise ocular micrometer.

For all patients age and gender were recorded. For all included sBCCs the following data was assessed: location, size (<10 and >10 mm), histopathological diagnosis after examination of the additional sections, presence of ulceration and adnexal extension, tumour thickness, initial therapy given with corresponding treatment effect, histopathological diagnosis of the excision (if applicable) and follow up time (until 29-1-2016). In case of treatment failure, the histopathological diagnosis of the repeated punch biopsy and secondary therapy with corresponding treatment effect were also determined. All included patients were treated with Imiquimod cream, 5-FU, excision or MAL-PDT.

Statistical analysis

Patient and tumour characteristics were presented as numbers and percentages in case of categorical variables or as mean and standard deviations. The difference in sBCC tumour thickness in the initial H&E and additional H&E sections was analysed using the independent T-test. Follow up time was calculated from date of treatment to date of treatment failure, recurrence or last follow up. The analyses were performed using SPSS Statistics 22 (SPSS Inc., Chicago, IL).

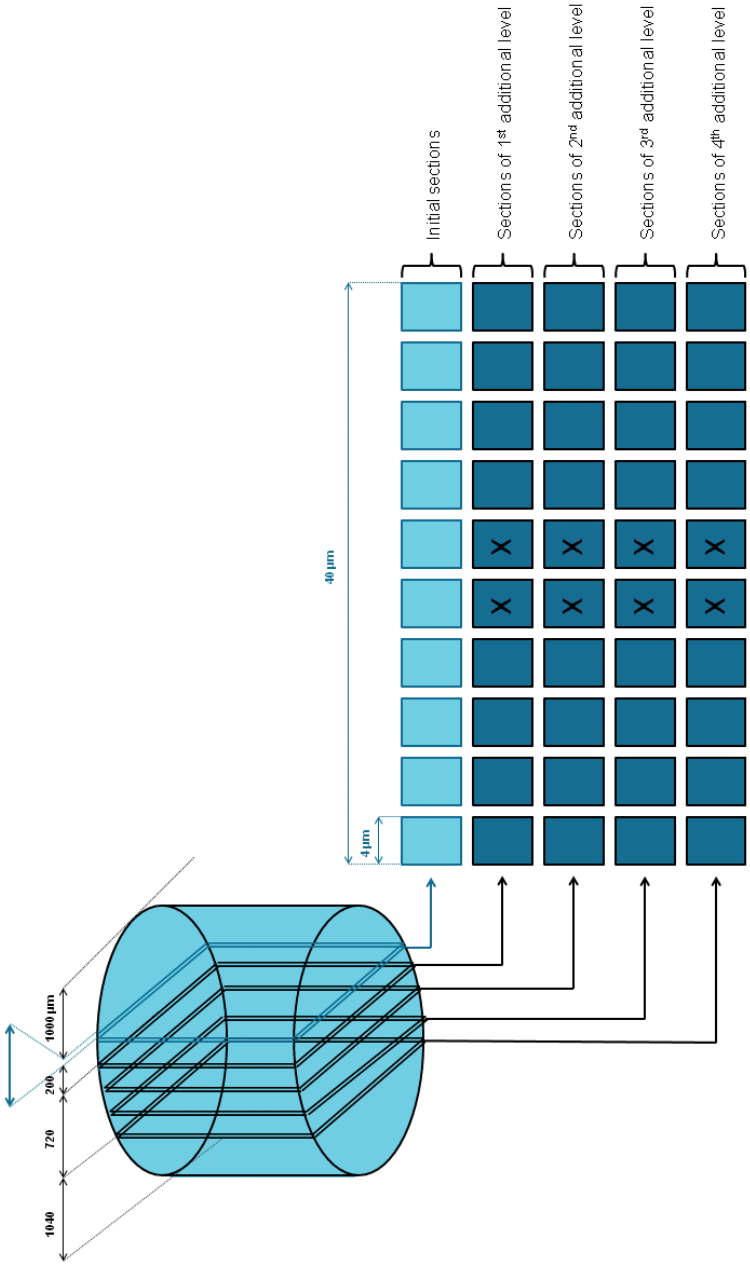

Figure 1. Histopathological examination method of a punch biopsy suspicious for BCC.
Schematic overview of the histopathological examination process. The dimensions of the punch biopsy might be smaller than depicted in this figure due to shrinkage following formalin fixation and histological processing, embedding and mounting.
X: These sections are stained with hematoxylin and eosin and used for histopathologial examination. In the initial sections it is unknown which slices are stained and examined.

Table 1. Demographics

Patient characteristics	**N (%)**	**Mean ± SD**
Gender		
- Male	43 (48.3)	
Age (years)		66.0 ± 12.4
Tumour characteristics	**N (%)**	**Mean ± SD**
Location		
- Head and neck	15 (12.9)	
- Upper extremities	11 (9.5)	
- Trunk	64 (55.2)	
- Lower extremities	26 (22.4)	
Size		
- <10 mm	88 (75.9)	
- >10 mm	28 (24.1)	
Presence of clinical ulceration		
- Yes	1 (0.1)	
Presence of adnexal extension		
- Yes	13 (11.2)	
Tumour thickness (mm)		
- Initial H&E stained sections		0.32 ± 0.19
- Additional H&E sections		0.41[1] ± 0.23
Treatment		
- Imiquimod	23 (19.8)	
- 5-FU	19 (16.4)	
- MAL-PDT	24 (20.7)	
- Excision	50 (43.1)	
Follow up time (days)[2]		312 ± 194

[1] *p*-value when compared to the initial H&E stained sections was 0.001.

[2] Until 1-2-2016.

Table 2. Histopathological diagnosis

	Diagnosis of punch biopsy n (%)[1]							
Primary punch biopsy diagnosis[2]	**sBCC**	**nBCC**	**iBCC**	**mnBCC**	**n/ mnBCC**	**n/iBCC**	**n/mn/i BCC**	**Total**
sBCC	90 (77.6)	16 (13.8)	1 (0.9)	1 (0.9)	4 (3.4)	1 (0.9)	3 (2.6)	116 (100.1[3])
More aggressive subtype missed n (%)	26 (22.4)							

[1] Based on histopathological evaluation of 5 levels per punch biopsy.

[2] Based on histopathological evaluation of 1 level per punch biopsy.

[3] Numbers do not add up to 100 due to percentages round off.

Table 3. Histopathological evaluation: presence of additional BCC subtypes

	H&E stained sections in which other BCC subtypes are present[1]				
Punch biopsy	**Initial H&E**	**1st additional level**	**2nd additional level**	**3rd additional level**	**4th additional level**
1	No	No	No	No	Yes
2	No	Yes	Yes	Yes	Yes
3[3,4]	No	Yes	No	No	No
4	No	No	No	Yes	Yes
5	No	No	Yes	Yes	Yes
6	No	No	Yes	No	Yes
7	No	Yes	Yes	Yes	Yes
8	No	No	Yes	Yes	Yes
9	No	No	No	Yes	Yes
10	No	Yes	Yes	Yes	Yes
11	No	Yes	Yes	Yes	Yes
12	No	No	Yes	Yes	Yes
13	No	No	No	Yes	Yes
14	No	Yes	Yes	Yes	No
15	No	Yes	Yes	Yes	Yes
16	No	No	No	No	Yes
17[2,3]	No	No	No	Yes	No
18	No	Yes	No	No	Yes
19	No	No	No	Yes	Yes
20	No	Yes	Yes	Yes	Yes
21	No	Yes	Yes	Yes	Yes
22	No	Yes	No	No	Yes
23	No	No	Yes	Yes	Yes
24	No	No	Yes	Yes	Yes
25	No	No	No	No	Yes
26	No	No	No	Yes	Yes
Overall presence of additional BCC subtypes (%)	0	11 (42.3)	14 (53.8)	19 (73.1)	23 (88.5)

[1] Other than superficial basal cell carcinoma subtype.

[2] Other BCC subtype(s) missed when evaluating only 1st and 4th additional level.

[3] Other BCC subtype(s) missed when evaluating only 2nd and 4th additional level.

[4] Other BCC subtype(s) missed when evaluating only 3rd and 4th additional level.

Results

In total, 116 punch biopsies of 89 patients (48.3% male; mean ± SD age at analysis 66.0 ± 12.4 years, Table 1) were evaluated. The majority of sBCCs were located on the trunk (55.2%), smaller than 10 mm, did not show clinical ulceration or adnexal extension and were treated by excision. The mean sBCC tumour thickness in the additional H&E sections was larger than in the initial H&E (Table 1). This difference was statistically significant. Follow up was available for all lesions with a mean of 312 ± SD 194 days. In 22.4% of the lesions (n= 26) other BCC subtypes (nBCC, mnBCC, iBCC) were found in the additional H&E sections (Table 2). The BCC diagnosis, after evaluating 5 levels of the punch biopsy, is shown in Table 2. The presence of additional BCC subtypes per sectioning level is depicted in Table 3 (Fig. 2). Clinical recurrences were present in two cases (1.7%) which were also confirmed by a second punch biopsy (Table 4). In two lesions that were treated with PDT, one showed no response and one showed partial response. In 25% (n=1) of the treatment failure and recurrence group the sBCC was thicker than 0.4 mm. Ulceration and adnexal extension did not occur in this group.

Discussion

BCC management is mainly based on the expected biological behaviour that correlates highly to the histological BCC subtype. Treatment modalities have expanded and the usage of non-surgical treatments have increased. However, these treatments are only suitable for sBCCs. Therefore, correct diagnosis of sBCC is of great importance. Currently, the punch biopsy is the gold standard for detection of BCC subtypes. However, a standardised method for histopathological examination of punch biopsies in lesions suspicious for BCC is missing. For this reason, the aim of this paper was to evaluate the accuracy of the current histological examination method in sBCC punch biopsies using a more extensive step section method. Furthermore, we sought to investigate whether tumour thickness, ulceration and adnexal extension are determinants of treatment failure or recurrence in sBCC treated with MAL-PDT, Imiquimod or 5-FU. The overall accuracy of sBCC punch biopsies that are histopathologically evaluated in only one level was 77.6%. Thus, in almost one out of every four sBCC a more aggressive BCC subtype was missed.

Hoogedoorn *et al.* analysed the treatment failure and recurrence of sBCC following MAL-PDT. They discovered that more than half of the treatment failures was due to underdiagnosis of the primary punch biopsy.[18] In those tumours, a more aggressive subtype was detected that was missed in the primary punch biopsy. In approximately 50% of the recurrences a mixed type BCC was present. These numbers were based on histological re-examination of repeated

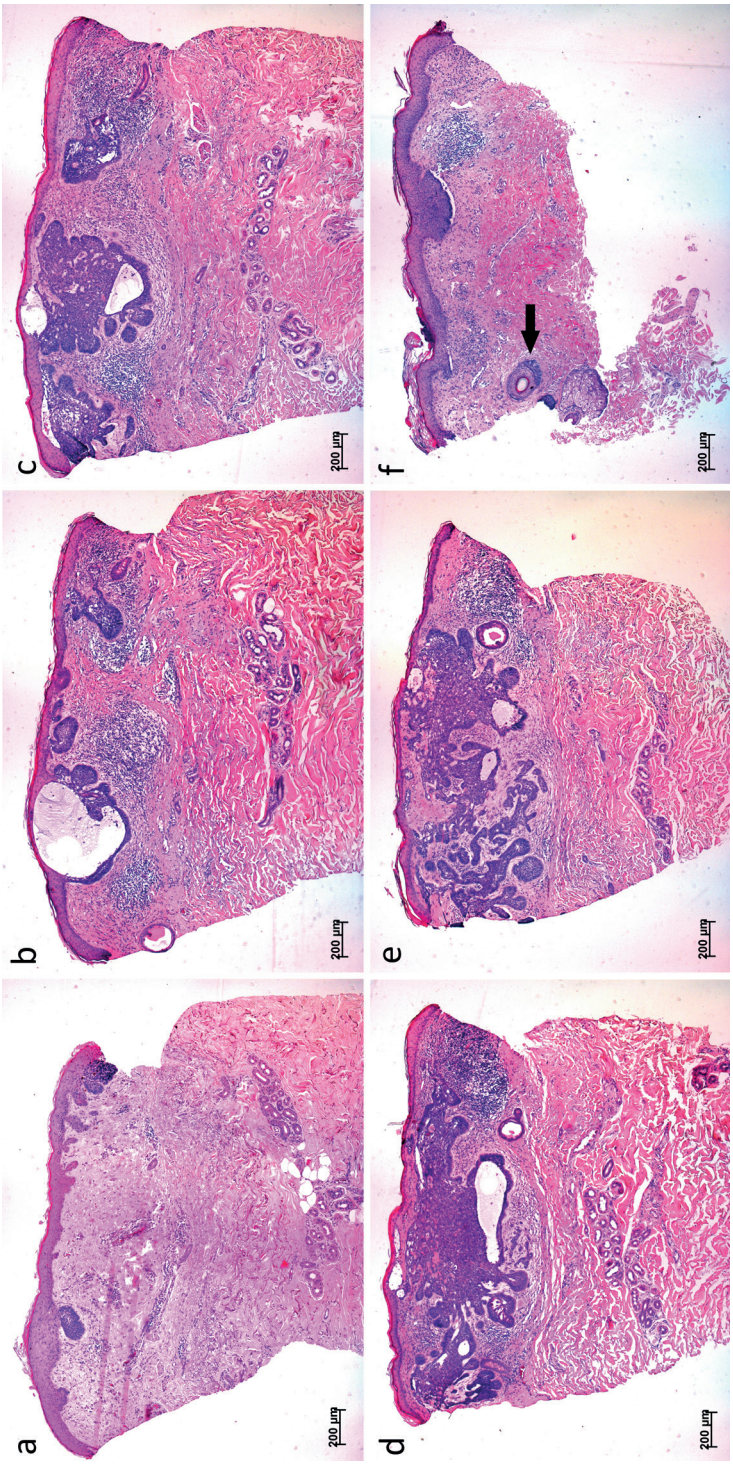

Figure 2. Detection of various BCC subtypes and adnexal extension in H&E stained sections.
a) In the initial section of punch biopsy 24 (Table 3) a superficial component is seen. b-e) In the additional sections of the same punch biopsy (micro-) nodular and infiltrative subtypes are detected. f) Some lesions showed an adnexal extension (arrow). 5x original magnification.

Table 4. Clinical treatment failure or recurrence

Patient	Treatment failure[1] or recurrence[2]	Location	Lesion size (<10, >10 mm)	Presence of clinical ulceration	Presence of adnexal extension	sBCC thickness (mm)[3]	Initial treatment	Histo-pathological diagnosis of 2nd punch biopsy[4]	Histo-pathological diagnosis of excision specimen	Histo-pathological diagnosis of punch biopsy[5]
1	Recurrence	Lower extremities	>10 mm	No	No	0.32	5-FU	sBCC	sBCC	sBCC
2	Recurrence	Lower extremities	>10 mm	No	No	0.20	MAL-PDT	sBCC	sBCC	sBCC
3	No response	Trunk	>10 mm	No	No	0.33	MAL-PDT	Not performed	sBCC	sBCC
4	Partial response	Head	>10 mm	No	No	0.74	MAL-PDT	mn/nBCC	mn/nBCC	sBCC

[1] Clinical treatment failure: partial or no response after initial treatment.

[2] Recurrence: presence of tumour tissue detected in follow up after previous tumour clearance.

[3] Largest measurement taken from either the initial or additional H&E sections.

[4] Extra punch biopsy taken after treatment failure or recurrence for diagnosis and determination of excision margin for treatment.

[5] Based on histopathological evaluation of 5 levels per punch biopsy.

biopsies and excision specimen. Because the punch biopsy represents only a small part of the entire tumour, sampling error of the location of the punch biopsy can also play a role in the underdiagnosis. This sampling error might be the reason why in one of our patients, who had a partial response to MAL-PDT, the histopathological diagnosis of the punch biopsy did not match the diagnosis of the excision specimen (Table 4). These authors also reported a higher number of clinical treatment failures (6.0%) and recurrences (9.2%) compared to our study.[18] Their median follow up period was two years. The short follow up period in our study might be the reason that no association was found between presence of a more aggressive BCC subtype in the punch biopsy and treatment failure or recurrence.

Studies have shown that sBCCs with tumour thickness > 0.4 mm are significantly more likely to recur after treatment with Imiquimod and that ulceration and tumour thickness were associated with lower response to MAL-PDT.[14,15] The follow up period in these studies ranged between a mean of 23.5–34 months, with a median between 20–27.3 months. In our treatment failure and recurrence group, however, ulceration and adnexal extension were not present and only one patient in this group had a tumour thickness of more than 0.4mm. Therefore, we did not see an association between these features and treatment failure or recurrence.

BCC nests can be the size of a few micrometers. Detection of BCC nests smaller than 200 μm can be missed with histological examination of only one level in a punch biopsy. However, the choice of cutting step section intervals of a few micrometers, to identify even the smallest BCC nest, would result in a too labour-intensive protocol. Moreover, the clinical implications of the smallest BCC nests are still controversial. Therefore, cutting step section intervals at 200 μm intervals was chosen because this was a more affordable protocol. According to this study, the misdiagnosis of punch biopsies was lower, compared to the current protocol, when using cutting step sections of approximately 440 μm after the initial H&E stained sections. This leads to examination of only the 2nd and 4th additional H&E stained sections (Table 3). The number of missed mixed type BCCs was even smaller when H&E stained sections were examined from the 1st and 4th or 3rd and 4th additional levels. Though, this would lead to varying cutting step section intervals that may be confusing and more difficult to apply in daily practice.

The whole punch biopsy was not cut up in sections entirely because the Radboudumc regulates that a part of the punch biopsy has to be available in case of future testing. Therefore, there is a possibility of missing a BCC nest in the remaining tissue. However, 9.5-18% tissue shrinkage can occur during formalin fixation and histological processing, embedding and mounting.[19-22] This leaves only a small section of tissue that remains unevaluated after additional sectioning.

Non-invasive skin imaging techniques such as the reflectance confocal microscopy (RCM) might be helpful for the diagnosis of the BCC subtype prior to treatment. Studies have shown

the ability of RCM to distinguish the BCC subtypes.[23,24] RCM was also able to detect subclinical BCC recurrences. [25] Peppelman *et al.* have set up a randomized controlled trial to determine the accuracy of RCM in identifying BCC subtypes and the cost-effectiveness compared to punch biopsy. In future, non-invasive techniques can aid in establishing a more accurate BCC diagnosis as evaluation of the whole tumour can be performed prior to treatment and possibly lead to a higher cost-effectiveness.[26]

In conclusion, this study shows that in more than 20% of the punch biopsies, diagnosed as sBCCs, more aggressive subtypes are missed with histological examination of only one level from a punch biopsy. We recommend step sectioning (such as 5 levels of 200 μm) in case of a diagnosis of sBCC to reduce the risk of missing more aggressive BCC subtypes and to prevent undertreatment. We plan to monitor the study patients for another two years to extend the follow up period in order to evaluate whether the detection of a more aggressive BCC subtype is associated with a higher number of recurrences.

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2.2

Is an one-day patient friendly methyl aminolevulinate photodynamic therapy illumination scheme for superficial basal cell carcinoma feasible? A randomised multicenter pilot trial.

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Submitted

Abstract

Background: Topical methyl aminolevulinate photodynamic therapy (MAL-PDT) is highly effective for the treatment of superficial basal cell carcinoma (sBCC). However, current European MAL-PDT protocol requires at least two hospital visits, which is costly and unpractical for patients. The aim of this study was to evaluate the effect of fractionated MAL-PDT on the same day, using two light fractions at 3 and 4 hours compared to illumination at 3 and 5 hours after MAL-application, in a randomised multicenter pilot trial.

Methods: Thirty patients were randomised into two groups. The first group received illumination at 3 and 4 hours (20 + 55 J/cm^2) after MAL-application. In the other group, two light fractions were performed at 3 and 5 hours (20 + 55 J/cm^2) after MAL-application. The lesion response was evaluated at 3 and 12 months after treatment.

Results: In the group, illuminated at 3 and 5 hours, 70.0% showed a complete response at 3 months compared to 63.6% in the other group. At 12 months, 80.0% showed a complete response in the group, illuminated at 3 and 5 hours, compared to 72.7% in the other group. A total of 5 clinical treatment failures and recurrences occurred, of which 3 appeared to be mixed type BCCs.

Conclusion: MAL-PDT, using two illumination sessions on the same day, is effective in the treatment of sBCC. A larger clinical study is needed to evaluate the benefit of fractionated MAL-PDT over the current protocol.

Introduction

Basal cell carcinoma (BCC) is the most common type of skin cancer with an increasing incidence worldwide, becoming an important health problem accompanied with rising health care costs.[1-3] There are different histological BCC subtypes including infiltrative (iBCC), micronodular (mnBCC), nodular (nBCC) and superficial BCC (sBCC). While nBCC is the most common type, a significant increase in the superficial subtype is noticed.[4] Although, surgery is an appropriate treatment option, sBCC is also suitable for non-surgical treatment modalities, since it is easy accessible with topical treatment. Non-surgical treatment options include photodynamic therapy (PDT), Imiquimod cream, 5-Fluorouracil (5-FU) cream, cryosurgery or electrodessication and curettage.[5] Photodynamic therapy has been recommended as a first-line treatment for sBCC by an international consensus.[6] PDT involves the application of a topical photosensitizer or its prodrug, in most cases aminolevulinic acid (ALA) or its methylated ester methyl aminolevulinate (MAL). In the heme biosynthetic pathway ALA and MAL are endogenously converted into protoporphyrin IX (PpIX). After application, an optimal time interval is needed for the production and accumulation of PpIX in the target cells. There is preferential production of PpIX in malignant cells. Thereafter, illumination of the lesion results in the formation of reactive oxygen species triggering apoptosis and necrosis of the target cells.[7]

Methyl aminolevulinate is the photosensitizing agent approved for PDT of sBCC and/or nBCC.[8] The current European protocol for MAL-PDT in sBCC consists of two light fractions (37 J/cm^2) one week apart, repeated at 3 months if required.[5,6,9,10] Three hours after application of MAL cream, the cream is wiped off and the tumour is illuminated with a light source with non-coherent red light (630 nm). However, the double procedure is unpractical for patients. Furthermore, the required day care visits result in high treatment costs, which are mainly attributed to general hospital overhead and personnel costs.[11] Therefore, a MAL-PDT protocol requiring two illumination fractions on the same day would be more practical for patients and result in reduced treatment costs.

In ALA-PDT, multiple studies have shown the benefit of splitting the illumination into two light fractions over a single illumination session.[12-14] This is due to re-synthesis of PpIX during the dark interval between two light fractions.[15-17] Also in MAL-PDT, there is re-synthesis of PpIX after illumination.[18] Further studies have tried to optimise fractionated ALA-PDT. Robinson *et al.* showed that a low dose light fraction followed by a high dose light fraction results in a higher efficacy of ALA-PDT compared to delivering two equal light fractions.[19] De Bruijn *et al.* showed a significant trend for increasing skin damage after PDT with increasing length of dark interval.[20] Though, a long dark interval would not be desirable when using fractionated PDT in clinical practice.

There is one clinical study on mouse skin that investigated the response of MAL-PDT using a single and a two-fold illumination scheme and compared that to ALA-PDT.[18] Four hours after ALA or MAL application, the skin was illuminated using either a single light fraction (100 J/cm^2) or a two-fold illumination scheme (5 + 95 J/cm^2) with a two hour interval. They showed that fractionated illumination did not enhance the clinical efficacy of MAL-PDT, as was the case when using ALA. However, the optimum illumination scheme for MAL-PDT is 3 hours after application and not 4, as is the case in ALA-PDT.[21] Furthermore, this study was performed on normal mouse tissue and not on human tumour tissue and MAL is known to be more tumour selective than ALA.[22,23]

Therefore, the aim of this study was to evaluate fractionated illumination of MAL-PDT in patients with sBCC lesions. We compared two light fractions (20 + 50 J/cm^2) of MAL-PDT with one or two hours interval: illumination at 3 and 4 hours compared to illumination at 3 and 5 hours after MAL-application. The total light dose was 75 J/cm^2, according to the standard MAL-PDT protocol for sBCC (two sessions of 37 J/cm^2, one week apart with a 630 nm lamp).

Materials and methods

Patients

This investigator-initiated, prospective, single-blinded, randomised multicenter pilot trial was conducted from June 2013 until October 2016 at the Radboud university medical center (Radboudumc), Nijmegen and Maxima Medical Center (MMC), Eindhoven, The Netherlands. The study was approved by the ethics committee (NL41859.091.12) and was conducted according to the Declaration of Helsinki. Due to the preliminary aspect of the study, a small sample size of 30 patients was used. All patients provided written informed consent. Patients above the age of 18 years with a histological proven (3 mm punch biopsy) primary sBCC were included. From each patient, one sBCC was included. Exclusion criteria were patients with a known allergy to MAL or related compounds, participation in other clinical studies, received treatments in the last 12 weeks for skin cancer in the area to be treated, usage of chronic immunosuppressive medication and patients who were pregnant or breastfeeding. Patients were randomized into two groups in a 1:1 ratio using a sealed envelope system generated by a research nurse. The first group received illumination at 3 and 4 hours after MAL cream application. The second group was illuminated at 3 and 5 hours after application of MAL. Randomisation occurred prior to pre-treatment of the lesion. The research physician, who enrolled the patients and assessed the lesion response, was blinded to the assigned treatment. Patients and treating physicians were not masked for the assigned therapy. Lesion sizes were determined clinically. An ellipse formula ($\pi ab/4$) was used to calculate the lesion area from the smallest (a) and largest dimension (b).

MAL-PDT treatment protocol

Salicylic acid 10% in vaseline daily for one week or an adhesive dressing (DuoDERM®, ConvaTec Inc.) was applied prior to PDT if necessary. A MAL cream was used (Metvix®, 160mg/g, Galderma). First, a layer of MAL cream (approximately 1 mm thick) was applied to the lesion and to the surrounding 10 mm of normal skin. The tumour site was covered with an adhesive, occlusive dressing (Tegaderm®, 3M Health Care Ltd) and tinfoil to prevent influence of light. Three hours after application, the cream was wiped off and the tumour was illuminated using the 630 nm Aktilite® CL 128 lamp (Galderma). The lesion, including a margin of at least 10 mm was illuminated. The first illumination session took around 4 minutes (20 J/cm^2) and the second session around 12 minutes (55 J/cm^2). The possibility of pain sensations during illumination was explained to the patients and they were given the choice of no medication or Acetaminophen (1000 mg the day before, in the morning of and/or one hour before treatment). The Visual Analogue Scale (VAS) score was used to assess the extent of pain that the patients endured during illumination.

Lesion response

The lesions were clinically evaluated at 3 and 12 months after treatment. At each visit, the clinical treatment response (complete, partial, no response), lesion reduction and possible adverse events were evaluated. Complete responses (CR) were defined as 100% clinical visual clearance of the sBCC. Partial responses (PR) were defined as $\geq$ 50% reduction in the greatest diameter. No responses (NR) were assessed as $<$ 50% reduction in the greatest diameter. Photographs were taken at each follow up visit, unless no change was observed. A punch biopsy was performed in case of suspicion of a residual or recurrent BCC. If necessary, the choice of an additional treatment was determined by the treating physician.

Histopathological examination process

During the study, all punch biopsies were routinely histologically examined with hematoxylin and eosin (H&E) stained tissue sections obtained from one level (at approximately 1000 μm). Superficial BCCs were histologically defined as nests of baseloid cells residing high in the dermis, usually in a multifocal pattern.[24] After the PDT study, all punch biopsies were sectioned in 4 additional levels with an interval of 200 μm, in order to evaluate whether more aggressive BCC subtypes might have been missed using the routine protocol. After every 200 μm, 10 sections of 4 μm each were sectioned of which two sections were stained with H&E and evaluated by a pathologist-in-training (GJK) and pathologist (WAMB). BCC subtype classification was based on the Dutch guideline.[25] Tumour thickness was evaluated by measuring the basaloid nest from the stratum granulosum up until the deepest point of invasion using a 0.1 mm precise ocular micrometer.

Statistical analysis

Descriptive statistics, including median and range for continuous variables and percentages for categorical data, were used to explore patient and tumour characteristics. The Mann-Whitney test and Fisher's exact test were used to compare continuous and categorical variables between groups, respectively. The Spearman's rank-order correlation was used to assess whether there was an association between the amount of Acetaminophen used prior to illumination and the VAS score after the first and second illumination. In case a more aggressive BCC subtype was detected in the punch biopsies, that were sectioned in additional levels, follow up time was calculated from date of MAL-PDT treatment to date of second treatment or last (post-study) follow up (until January 2017). A p-value of ≤ 0.05 was regarded statistically significant. Statistical analyses were performed using IBM SPSS Statistics 22.0 (SPSS Inc., NY, USA).

Results

Between June 2013 and July 2015, 30 patients with sBCCS were enrolled; 16 patients of the Radboudumc and 14 of the MMC. Eight patients were excluded due to non-adherence to the protocol (no punch biopsy obtained (n=6), more than one lesion per patient illuminated (n=2)) and one patient was lost to follow-up. The remaining 21 patients were included in the analyses. Patient and tumour characteristics were comparable in both groups (Table 1).

Three months post-treatment, both groups showed CR rates between 63.6-70.0% (Table 2). In the group, illuminated at 3 and 4 hours, 7 out of 11 sBCCs (63.6%) showed a CR after 3 months, while 3 sBCCs (27.3%) showed a PR (Table 2). A punch biopsy was obtained from one of the partial responsive lesions, which revealed a sBCC. This lesion was marked as a treatment failure (9.1%) and treated with Imiquimod with a good effect. One lesion (9.1%) remained the same size, but showed minimal erythema. This lesion was listed as NR and was followed up. Seven out of 10 lesions (70.0%) in the group, illuminated at 3 and 5 hours, showed CR after 3 months. From 2 of the 3 partial responsive lesions, punch biopsies were performed and both revealed sBCCs. However, after surgical excision, both lesions appeared to be mixed type BCCs. The other PR lesion was followed up.

The CR rate after 12 months was 8 (72.7%) and 8 (80.0%) for respectively the groups, illumination at 3 and 4 hours and at 3 and 5 hours (Table 2). In the group, illuminated at 3 and 4 hours, 2 BCCs showed PR (18.2%); one of these was detected at an extra visit 5 months after illumination. Punch biopsies from these PR lesions revealed sBCCs (n=2) of which one was a treatment failure and the other one a recurrence, since it showed a complete response earlier at an extra visit 6 months after illumination. After surgical excision of these lesions, one was diagnosed as a sBCC. However, the other appeared to be a nBCC.

Table 1. Patient and tumour characteristics separated by treatment group

Patient and tumour characteristics	Illumination at 3 and 4 hours (n=11)	Illumination at 3 and 5 hours (n=10)
Gender		
- Male	6 (54.5)	3 (30.0)
- Female	5 (45.5)	7 (70.0)
Age (years)	67 [49-81]	67 [45-86]
Fitzpatrick skin type		
- I	3 (27.3)	2 (20.0)
- II	8 (72.7)	7 (70.0)
- III	0	1 (10.0)
- IV	0	0
Location BCC		
- Head and neck area	0	0
- Upper extremities	1 (9.1)	2 (20.0)
- Trunk	7 (63.6)	5 (50.0)
- Lower extremities	3 (27.3)	3 (30.0)
sBCC lesion size (mm^2)	78.5 [39.3-176.7]	78.5 [47.1-188.5]
Pretreatment sBCC		
- No	3 (27.3)	2 (20.0)
- Salicylic acid 10% in vaseline	1 (9.1)	0
- Duoderm	7 (63.6)	8 (80.0)

n.a., not applicable; sBCC, superficial basal cell carcinoma.
Continuous variables are displayed by median and [range], categorical variables are displayed by absolute counts and (proportion).

No statistical differences were noted between the groups at 3 and 12 months with respect to lesion responses and median lesion size reduction. The median VAS scores after the first illumination were 3 and 4.5 and after the second illumination 4 and 4 for the group illuminated at 3 and 4 hours and 3 and 5 hours respectively (Supplementary table 1). No significant differences were found in the median VAS scores between the two illumination groups. There was no association between the Acetaminophen dose prior to illumination and the VAS score after illumination. Moreover, no serious adverse events were reported (Table 2).

Histopathological examination

Twenty-six punch biopsies (21 initial and 5 post-treatment biopsies) were available for additional sectioning (Table 3). In 4 punch biopsies (3 initial and 1 post-treatment biopsies), other BCC subtypes were detected after additional sectioning. They showed 3 nBCCs (Table 3; patients 6, 12, 17) and one mixed type BCC with nodular and infiltrative components (Table 3; patient 8). Two of these lesions (patients 6 and 8) were treatment failures which were surgically excised. The median follow up time for these lesions, until their second treatment, was 612 days [range 276-947 days]. The other two (patients 12 and 17) did not show any clinical signs of treatment failure or recurrence after treatment and therefore continued their (post-study) routine dermato-oncological follow up at their respective hospitals. The median follow up time (including time of follow up post-study until January 2017) for these lesions was 816 days [range 739- 892 days].

Table 2. Results of biopsy-proven superficial basal cell carcinomas

	At 3 months				At 12 months			
	Illumination at 3 and 4 hours	**Illumination at 3 and 5 hours**	***p*-value**	**Total**	**Illumination at 3 and 4 hours**	**Illumination at 3 and 5 hours**	***p*-value**	**Total**
Number of biopsy-proven sBCCs	11	10		21	10	8		18
Clearance of biopsy-proven sBCCs								
- Complete response (%)	7 (63.6)	7 (70.0)	0.757	14 (66.7)	8 (72.7[a])	8 (80.0[b])	0.110	16 (76.2[c])
- Partial response (%)	3 (27.3)	3 (30.0)	0.890	6 (28.6)	2 (18.2[a])	0	0.110	2 (9.5[c])
- No response (%)	1 (9.1)	0 (0)	0.329	1 (4.8)	0	0	n.a.	0
Histological confirmation of response:	1 (of the PR)	2 (of the PR)		3	2 (of the PR)	0		2
- Biopsy	sBCC (n=1)	sBCC (n=2)		3	sBCC (n=2)	0		2
- Excision	0	s/nBCC (n=1), s/iBCC (n=1)		2	sBCC (n=1)[d], nBCC (n=1)[e]	0		2
Treatment failure (%)	1[f] (9.1)	2 (20.0)	0.705	3 (14.3)	1[e] (9.1[a]) Excision: nBCC	0	0.762	1 (4.8[c])
Recurrence (%)	0	0	1.000	0	1[d] (9.1[a]) Excision: sBCC	0	0.918	1 (4.8[c])
Median lesion size reduction in mm^2 [range][g]	66.0 [0-176.7]	78.5 [18.9-188.4]	0.524	70.7 [0-188.4]	78.54 [39.3-176.7]	72.3 [47.1-106.8]	0.660	78.5 [39.3-176.7]
Median lesion size reduction in percentage [range][g]	100 [0-100]	100 [71.0-100]	0.645	100 [0-100]	100 [75.0-100]	100 [100-100]	0.346	100 [75-100]

iBCC, infiltrative basal cell carcinoma; n.a.: not applicable; nBCC, nodular basal cell carcinoma; sBCC, superficial basal cell carcinoma.

[a] Percentage of the total number sBCCs in the group of illumination at 3 and 4 hours (n=11).

[b] Percentage of the total number of sBCCs in the group of illumination at 3 and 5 hours (n=10).

[c] Percentage of the total number of sBCCs in both groups (n=21).

[d] Recurrence: presence of tumour tissue detected in follow-up after previous tumour clearance. This patient had a partial response at 3 months, complete response at an extra visit 6 months, and partial response at 12 months after illumination.

[e] In one patient the partial response and treatment failure were detected at an extra visit 5 months after illumination.

[f] Treated with Imiquimod, no excision occurred.

[g] Mean lesion reduction: compared to baseline.

Table 3. Histopathological evaluation

Prior to illumination			3 months				12 months			
Patients with punch biopsies	**BCC subtype detected in 1st punch biopsy**	**BCC subtype(s) detected in additional levels in 1st punch biopsy (with tumour thickness in mm)**	**Treatment effect**	**BCC subtype detected in 2nd punch biopsy**	**BCC subtype detected in additional levels in 2nd punch biopsy (with tumour thickness in mm)**	**Excision**	**Treatment effect**	**BCC subtype detected in 2nd punch biopsy**	**BCC subtype detected in additional levels in 2nd punch biopsy (with tumour thickness in mm)**	**Excision**
1	sBCC	sBCC (0.21)	CR	-	-	-	CR	-	-	-
2	sBCC	sBCC (0.33)	CR	-	-	-	CR	-	-	-
3	sBCC	sBCC (0.23)	CR	-	-	-	CR	-	-	-
4	sBCC	sBCC (0.22)	PR	sBCC	sBCC (0.31)	**s/nBCC**	-	-	-	-
5	sBCC	sBCC (0.38)	CR	-	-	-	CR	-	-	-
6	sBCC	**nBCC (0.63)**	PR	-	-	-	PR[a]	sBCC[a]	sBCC[a] (0.31)	**nBCC[a]**
7	sBCC	sBCC (0.20)	PR	-	-	-	PR	sBCC	sBCC (0.24)	sBCC
8	sBCC	sBCC (0.32)	PR	sBCC	**n/iBCC (0.63)**	**s/iBCC**	-	-	-	-
9	sBCC	sBCC (0.28)	CR	-	-	-	CR	-	-	-
10	sBCC	sBCC (0.45)	CR	-	-	-	CR	-	-	-
11	sBCC	sBCC (0.35)	CR	-	-	-	CR	-	-	-
12	sBCC	**nBCC (0.70)**	CR	-	-	-	CR	-	-	-
13	sBCC	sBCC (0.41)	CR	-	-	-	CR	-	-	-
14	sBCC	sBCC (0.20)	CR	-	-	-	CR	-	-	-
15	sBCC	sBCC (0.17)	CR	-	-	-	CR	-	-	-
16	sBCC	sBCC (0.25)	PR[b]	-	-	-	CR	-	-	-
17	sBCC	**nBCC (0.36)**	NR[b]	-	-	-	CR	-	-	-
18	sBCC	sBCC (0.18)	CR	-	-	-	CR	-	-	-
19	sBCC	sBCC (0.09)	CR	-	-	-	CR	-	-	-
20	sBCC	sBCC (0.15)	CR	-	-	-	CR	-	-	-
21	sBCC	sBCC (0.37)	PR	sBCC	sBCC (0.38)	- Treated with Aldara with good effect.	-	-	-	-

iBCC, infiltrative basal cell carcinoma; nBCC, nodular basal cell carcinoma; sBCC, superficial basal cell carcinoma.

[a] This punch biopsy was obtained at an extra visit 5 months after illumination.

[b] No additional treatments were given after the initial MAL-PDT treatment.

- Punch biopsy or surgical excision not performed.

Supplementary table 1. Results VAS-scores and adverse events after illumination

	After illumination		
	Illumination at 3 and 4 hours	**Illumination at 3 and 5 hours**	**p-value**
Median VAS score after 1st illumination [range]	3 [0-7.0]	4.5 [2.0-7.0]	0.173
Median VAS score after 2st illumination [range]	4 [0-8.0]	4 [2.0-8.0]	0.603
Adverse event[a]	1 (local itchiness)	0	

VAS score, Visual Analogue Scale score.

[a] Adverse event: related to MAL-PDT treatment, during or directly after illumination.

Discussion

Current MAL-PDT treatment for sBCC requires two treatment sessions on separate days, and repeated after 3 months if necessary. This requires at least two hospital visits for patients, which is unpractical and costly. Therefore, optimisation of this protocol might be more beneficial to patients and reduce treatment costs. Therefore, the purpose of this study was to evaluate two different MAL-PDT protocols in which two light fractions were performed on the same day, with one or two hours interval.

Overall, this pilot study shows that two sessions of illumination on the same day leads to CR rates between 72.7-80.0% at 12 months. Though, a comparison of our results with other studies is challenging because of differences among studies on key elements, such as PDT-procedure, selection of lesions, length and methods of follow up and endpoint assessment. There are two larger prospective studies that are more in line with our study design.[26,27] They show higher CR rates at 12 months compared to our study. However, the high CR rate in the study of Szeimies *et al.* is due to the fact that sBCCs, that showed incomplete response at 3 months, received 2 further MAL-PDT sessions (75 J/cm^2). Furthermore, Basset-Seguin *et al.* showed comparable results at 3 months when one single session of MAL-PDT (light dose 75 J/cm^2) was given after sBCCs were treated with MAL-cream 3 hours prior to illumination.[28] This leads to the question whether it might be easier to perform one illumination session of 75 J/cm^2 instead of re-illumination at 4 and 5 hours. However, the incomplete responders in the study of Basset-Seguin *et al.* were, thereafter, treated with 2 MAL-PDT illumination sessions one week apart at 3 months. For that reason, there is no information on the long term effect of a single illumination session. Therefore, a study with a larger population comparing a single MAL-PDT illumination session (75 J/cm^2) and fractionated MAL-PDT (20 + 50 J/cm^2) with a longer follow up period is recommended.

In this study, some lesions showed less erythema after PDT compared to before treatment, or mild remaining erythema possible due to scarring of the biopsy location or after PDT treatment. According to our strict definitions of 'lesion response', these lesions were marked PR or NR. However, not all these lesions were not clinically suspect for a treatment failure or recurrence. Therefore, not all of them were biopsied. This is also the reason why some lesions, which appeared as PR or NR at 3 months, showed CR at 12 months.

All adverse events that were reported in this study were in accordance with other studies.[26,29] Furthermore, no serious adverse reactions occurred. Arits *et al.* showed in their study that serious adverse events only occurred in patients treated with Imiquimod and 5-FU but not in the MAL-PDT treatment group.[26] More importantly, a generally better cosmetic outcome is observed after PDT treatment of sBCC compared to other treatment options.[26,27] Furthermore, the treatment regime for Imiquimod and topical 5-FU is intensive and long (4-6 weeks). [30] In daily practice,

not all patients will be motivated or able to apply a cream for such a long period. For these patients, hospital-based treatments such as MAL-PDT and surgical excision might be preferable. In case both treatments can take place during one visit, MAL-PDT may have the benefit over surgical excision, especially when cosmetic outcome and problematic healing sites are taken into consideration.

The 5 treatment failures and recurrences in the present study were partly due to the presence of a more aggressive BCC subtype in the excision specimen, most probably caused by sampling error or histological underdiagnosis of the punch biopsy. In 3 lesions, other BCC subtypes (nodular and infiltrative) were detected in the excision specimen. Although, MAL-PDT is also effective in nBCC, poorer clearance rates and higher recurrences were seen in these tumours compared to sBCCs.[27,31,32] Moreover, MAL-PDT is not registered for the treatment of iBCC. Histological underdiagnosis of the punch biopsies may have led to an increased number of treatment failures due to undertreatment. In order to reduce the risk of sampling error, additional sectioning of the punch biopsy can be performed.[33,34] This might result in a more accurate diagnosis for adequate treatment. In our study, additional sectioning of the punch biopsies yielded the detection of 4 BCCs with a more aggressive BCC component, of which 3 were from the primary punch biopsies. Two of the lesions (one from the 1st and one from the 2nd punch biopsy) were treatment failures and removed by surgical excisions. The other two did not show any clinical signs of treatment failure or recurrence after MAL-PDT (median follow up period more than 2 years). In these two lesions, MAL-PDT seemed to be an effective treatment. A hypothesis for this might be that MAL-PDT is more effective in nBCCs with a small tumour thickness (Table 3; patient 17) compared to nBCCs with a larger tumour thickness (patients 6 and 12). On the other hand, one nBCC lesion with a large tumour thickness (patient 12) had a good clinical effect after MAL-PDT (follow up approximately 2 years). There is a chance that this lesion might recur after an extended follow up period, like in the study of Roozeboom *et al*., where they noticed treatment failures 3 years after MAL-PDT.[35] Therefore, this patient remains in (post-study) routine clinical follow up. One mixed type BCC was not detected after additional sectioning of the primary punch biopsy (Table 3; patient 4). This may have been a result of sampling error of the punch biopsy. The usage of non-invasive diagnostic techniques, such as the reflectance confocal microscopy (RCM), may reduce sampling errors as they offer the possibility to image the whole lesion and distinguish different BCC subtypes.[36,37] Overall, various options are available to reduce sampling errors resulting in higher cost-effectiveness, because they prevent the need for repeated biopsies and subsequent treatment.

In conclusion, this study shows that MAL-PDT, given in a two-fold illumination scheme with one or two hours interval, shows promising results in the treatment of sBCC. Though, a larger clinical study is needed to evaluate the benefit of fractionated MAL-PDT over a single MAL-PDT session and the regular MAL-PDT protocol.

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3.1

The current role of reflectance confocal miscroscopy within the continuum of actinic keratosis and squamous cell carcinoma: a systematic review

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Abstract

Background: Clinical differentiation between actinic keratosis (AK), squamous cell carcinoma (SCC) *in situ*, invasive SCC and its variants can be difficult. Reflectance confocal microscopy (RCM) is a non-invasive technique for *in vivo* skin imaging.

Objectives: To explicate the diagnostic and monitoring use of RCM within the spectrum of AK and SCC by presenting the corresponding RCM features. Additionally, to evaluate the accuracy of RCM for these diagnoses compared to histopathology.

Materials & methods: A systematic literature search was performed in PubMed, EMBASE, Cochrane Library and Web of Science databases. The quality was assessed using the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) checklist.

Results: Twenty-five eligible studies were included. Different diagnostic RCM features have been described for AK, actinic cheilitis (AC), erythroplasia of Queyrat, Bowen disease, invasive SCC and keratoacanthoma (KA). The overall sensitivity and specificity of RCM for the diagnosis of SCC, AK, SCC *in situ* and KA ranged between 79-100% and 78-100%, respectively.

Conclusion: Current literature describes the use of RCM for diagnosing AK, AC, erythroplasia of Queyrat, Bowen disease, invasive SCC and KA, and for monitoring of AK treatments, with a good accuracy. Unfortunately, studies of high methodological quality are lacking. Pre-treatment of hyperkeratotic lesions and uniform definitions of RCM features are required to aid in the differentiation between AK, SCC *in situ*, SCC and its variants in clinical practice.

Introduction

Actinic keratoses (AKs) are common precancerous skin lesions. AKs may progress to invasive squamous cell carcinomas (SCCs) ranging from 0-0.075% per lesion per year, with a risk up to 0.53% per lesion in patients with a history of non-melanoma skin cancer (NMSC). [1] Therefore, they represent an early point on the continuum of malignancy. Early recognition of SCC is important, as it accounts for the majority of NMSC-related metastases and death.[2] Clinical differentiation between AK, SCC *in situ*, invasive SCC and its variants can be difficult, but is important because of the associated risk of mortality. Bowen disease (BD) and erythroplasia of Queyrat (EoQ) are considered SCCs *in situ*, whereas verrucous carcinoma (VC) is a rare low-grade variant of SCC.[3] Keratoacanthomas (KAs) are characterized by the proliferation of highly, differentiated squamous epithelia. Clear distinction from a highly differentiated SCC is often impossible.[3] Therefore, some regard them to be a variant of SCC. Dermoscopic features for AKs, SCCs *in situ*, invasive SCCs and its variants have been described but lacked diagnostic accuracy.[4-6] Therefore, routine histopathology remains the gold standard. However, a biopsy procedure is invasive, may cause local inflammatory reactions and might result in sampling error and scarring. For that reason, non-invasive diagnostic technologies such as the reflectance confocal microscopy (RCM) have been proposed. RCM uses a near infrared laser of 830 nm and can image the skin until a depth of approximately 250 μm. It offers *in vivo* imaging of the skin with cellular resolution and has the potential to overcome problems that are associated with biopsy sampling.

RCM has been described to be useful for the diagnosis of inflammatory skin conditions, melanocytic lesions and NMSC, especially basal cell carcinoma.[7-13] In the area of NMSC, particularly AK and SCC, the knowledge and experience with RCM is increasing.[14-17] However, due to lack of a systematic approach in the current reviews, which did not highlight all diagnoses within the continuum of AK and SCC, we aimed to systematically appraise and summarize the available primary literature reporting data on the diagnostic and monitoring use of RCM within the spectrum of AK and SCC by presenting the corresponding RCM features. Furthermore, this review evaluates the accuracy of RCM for these diagnoses compared to histopathology.

Methods

A search strategy was developed based on RCM, AK, SCC *in situ*, invasive SCC and its variants deducted from the classification scheme in *Dermatology.*[3] The literature search was performed in four electronic databases: PubMed, EMBASE, Cochrane Library and Web of Science using the search terms “confocal microscopy” or “confocal laser microscopy” or “confocal laser scanning microscopy” combined with “actinic keratosis”, “precancerous conditions”, “squamous cell carcinoma”, “Bowen disease”, “keratoacanthoma”, “verrucous carcinoma”, “erythroplasia of Queyrat” or “actinic cheilitis” and all synonyms. Currently, the most widely used RCM is the

Vivascope 1500. Other devices for *in vivo* imaging include the Vivascope 1000 (precursor of the Vivascope 1500) and the Vivascope 3000 (handheld device). Articles with patients that had a histopathologically proven diagnosis, of the Vivascope system only and with diagnostic and/or monitoring RCM features and/or the diagnostic accuracy compared to histopathology were included. Publications describing diagnoses in medical specialty fields other than dermatology, unavailable texts, reviews and conference abstracts were excluded. Studies were restricted to the English, Dutch or German language and limited to humans. All published studies until March 22, 2016 were included.

Titles, and when necessary abstracts and keywords, were scanned by a review author (KPN). Thereafter, two reviewers (KPN and MP) screened title and abstract, and if required, material and methods. Following this selection, the two reviewers critically assessed the full texts for their eligibility. Differences in assessment between the two reviewers were discussed with a third (PEJVE) and fourth investigator (MJPG). Data extraction for all studies meeting the inclusion criteria was performed by two authors. The STrengthening the Reporting of Observational studies in Epidemiology (STROBE) criteria was used to assess the quality of the research.[18] Modifications to this scale were made by excluding the items 'variables' and 'study size' to evaluate the quality of case reports and case series. Studies were graded into the following categories, A: fulfilling more than 80% of the STROBE criteria (high); B: 50-80% (moderate); C: less than 50% (low).

Results

The search strategy identified 2396 articles (Fig. 1). The first selection resulted in 169 articles on RCM and diagnoses within the spectrum of AK and SCC. The second selection resulted in 95 abstracts eligible for full text screening and yielded an overlap of 94% between the two reviewers. The full text screening resulted in 25 included articles and had an overlap of 87% between the two reviewers (Fig. 1). Twenty-five publications were included with articles on AK (n=15), AC (n=1), EoQ (n=1), BD (n=7), SCC (n= 8), and KA (n=2) (Table 1). The included studies consisted of case series (n=17), case-controls (n=5), cohort studies (n=2) and case reports (n=1). The number of scanned lesions varied between 1-46 (AK), 1-25 (BD), 1-11 (SCC) and 3 (KA). The methodological quality of the studies varied between category B (n=13) and C (n=12) with a mean STROBE criteria percentage of 44.8 and standard deviation of 14.7. Eight studies determined the sensitivity and specificity of different RCM diagnoses compared to histopathology. Definitions and descriptions of the reflectance confocal microscopic features used in the studies are presented in Supplementary table 1.

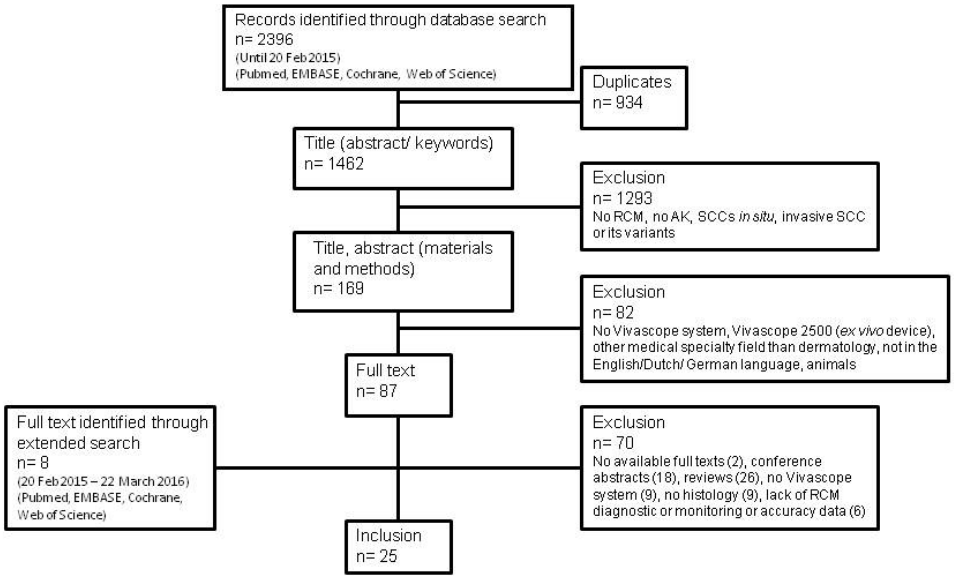

Figure 1: Flowchart selection procedure.
This flowchart shows the number of publications identified, screened and included or excluded at each stage of the review process.

Actinic keratosis and actinic cheilitis

Thirteen studies depicted diagnostic and monitoring RCM features in non-pigmented AKs (Table 1).[14-16,19-27] Specific diagnostic RCM features for AKs are shown in Table 2. These conclusions were based upon case series and case controls with low to moderate methodological quality (Table 1).[14] The same features were found for AC.[28] Ulrich *et al.* showed that RCM features of AKs correlated well with routine histology.[25] AKs predominantly had a focally disarranged or mildly atypical honeycomb pattern in the spinous-granular layer [15] and mostly showed an atypical honeycomb pattern or normal keratinocytes in the stratum granulosum.[14,17] Four studies determined the diagnostic accuracy of RCM features for AKs.[20,21,25,26] The sensitivity ranged between 91-100% and specificity between 78-100%. Ulrich *et al.* described a sensitivity of 86% and specificity of 100% for AC.[28] Careful interpretation of these results is warranted, since all studies included a small number of cases. Moreover, Horn *et al.* only performed biopsies in 50% of the clinically suspected AK lesions and Incel *et al.* combined data from AKs and BD because of lack of distinctive features between both diagnoses.[20,21] Two studies described RCM features for pigmented AKs (Table 1).[29,30] Most observed features were similar to non-pigmented AKs with addition of some RCM features that are listed in Table 2.

Bowen disease

Eight studies of low to moderate methodological quality described RCM features for (non-) pigmented BD (Table 1).[15,21,22,26,31-34] General features of non-pigmented BD are displayed in Table 2. Ulrich *et al.* described two types of targetoid cells, each with its own characteristic morphologic appearance, corresponding to dyskeratotic cells.[33] In addition to the RCM features found in non-pigmented BD, pigmented BD showed more RCM features (Table 2). These observations were all based on case series. The main study limitations were lack of a control group and a small population size. The highest number of scanned lesions per study did not exceed 25 BD lesions.[15] Moscarella *et al.* showed a sensitivity of 100% for the detection of BD in collision tumours.[32] Though the observers, who analysed the RCM images retrospectively, were blinded for the histopathological diagnosis, they were aware that the aim of the study was to evaluate collision tumours. This might have resulted in an information bias. Two studies depicted a sensitivity and specificity of respectively 93-97% and 97% for the combined diagnosis of AK and BD due to a lack of distinctive features between both diagnoses.[21,26]

Erythroplasia of Queyrat

EoQ was described by Arzberger *et al.* in a case control study (Table 1).[35] The characteristic RCM features for EoQ are shown in Table 2, with a sensitivity and specificity each of 100%.

Squamous cell carcinoma

RCM imaging of SCCs has been described in eight studies, consisting of case series and case controls (Table 1).[14,15,17,19,21,36-38] The most described RCM features are listed in Table 2. Compared to AKs, the spinous-granular layer in SCCs showed more extensive atypia[15], the severe architectural disarray was present in the stratum granulosum[14,17,21] and the number of blood vessels and diameter of the vessels were increased.[17] This latter can be explained by the higher metabolic need of the tumour.[39,40] Peppelman *et al.* demonstrated that the presence of architectural disarray in the stratum granulosum in combination with architectural disarray in the stratum spinosum and/or nest-like structures were the best predictors for SCCs.[17] Cinotti *et al*. reported a sensitivity of 100% to diagnose SCC with RCM, although, biopsies were only performed in 74% of the clinical suspicious lesions.[36] The non-biopsied lesions were monitored for at least 12 months and showed no changes during that period. Incel *et al.* described a sensitivity and specificity of 79% and 99% respectively for the diagnosis of SCC and KA.[21] However, both studies included a small number of SCCs and therefore the diagnostic accuracy must be carefully interpreted.

Keratoacanthoma and verrucous carcinoma

RCM features for KAs were described in two case series (Table 1).[15,21] General features are listed in Table 2. Incel *et al.* excluded lesions with prominent hyperkeratosis.[21] Both studies imaged a small number of lesions (n=3). There were no studies on the diagnostic accuracy of RCM in

diagnosing KA. Though, one study showed the sensitivity and specificity of 79% and 99% for the diagnosis of SCCs and KAs.[21] No studies on RCM imaging of VC were found.

RCM as a treatment monitoring tool

Monitoring RCM features for AKs and AC after treatment with topical 3% Diclofenac sodium with 2,5% hyaluronic acid and for AKs after shave biopsy have been described (Table 1).[16,23,28] A decrease in atypical honeycomb pattern in AKs and AC and changes in dermal collagen in AKs after treatment were observed. The reduction in epidermal atypia after topical treatment was confirmed by histopathological evaluation.[16] Limitations of the studies were the small sample size, being non-randomized, uncontrolled studies and the exclusion of hyperkeratotic AKs.

Discussion

Clinical differentiation between AK, SCC *in situ*, SCC and its variants can be challenging and taking a biopsy in these cases is an invasive method. Additionally, it inhibits monitoring of the same lesion after treatment. RCM may transcend these problems. To our knowledge, this is the first systematic review to explicate the use of RCM for all diagnoses within the continuum of AK and SCC, and to evaluate the accuracy of *in vivo* RCM imaging for these conditions compared to histopathology.

Unfortunately, depth penetration imposes a major limitation on RCM in hyperkeratotic lesions, that is often associated with invasive SCCs. The inability of RCM to image at the level of the dermo-epidermal junction and stratum basale in potentially malignant hyperkeratotic lesions excludes important information from the diagnostic process. Instead, a distinction between SCC and AK using RCM must rely on the fact that in SCC extensive keratinocytic atypia involves the entire epidermis, including the stratum granulosum.[14,15,17,20,31,33,41,42] Though, differentiation between SCCs and AKs remains challenging in hyperkeratotic lesions without visualization beyond the dermo-epidermal junction. In SCC, nestlike structures and pleomorphic cells can be detected in the dermis, while these RCM features are absent in AK. [15,17] Careful curettage of the hyperkeratotic scale or pre-treatment with keratinolytic agents may evade this problem.
Furthermore, it might be tough to differentiate between focal or extensive and mild or severe atypia. Therefore, experienced RCM observers and a high quality of image acquisitions are needed. A recent study of Pellecani *et al.* showed a significant correlation of the grading of keratinocyte atypia in AK between experienced, blinded RCM observers and histopathology.[43]

Two distinct types of targetoid cells corresponding to dyskeratotic cells were described for BD.[33] However, dyskeratotic cells were also seen in SCC and AK but lacked clear definitions of their morphological appearances. Therefore, the two types of targetoid cells mentioned by Ulrich *et al.* may not be specific for BD. This also shows that there is need for uniformity in the definitions of RCM features for image assessment.

Regarding KA, it is possible that there was a lack of power to detect significant differences between KA and AK and SCC due to the small number of KA in the limited number of publications. Furthermore, the differentiation between VC and KA may be challenging. While VC commonly involves the subcutaneous fat and beyond, KA characteristically does not extend beyond the depth of the eccrine sweat.[44] Therefore, a deep biopsy is essential. Due to the limited penetration depth of the RCM, it is unlikely that the diagnosis of VC can be made by this technique.

Other studies have described the reduction of epidermal atypia in AKs after treatment with Imiquimod and photodynamic therapy.[45,46] Ulrich *et al.* detected residual atypia in two AK lesion, one not showing a clinical treatment response and one showing clinical clearance, after treatment with Imiquimod.[45] This was suggestive of incomplete clearance. They also developed a RCM atypia scoring system to evaluate the cellular changes in AK after treatment to facilitate the use of RCM. The authors concluded that, by using RCM as a monitoring tool to detect early residual atypia, the recurrence rates might ultimately decrease.

In two case control studies, 'normal looking' skin adjacent to AK lesions[14] or non-lesional skin from the contralateral side[20] were used as controls. However, RCM imaging of these skin sites may not be adequate controls, due to the neoplastic changes across the entire sun field exposed field of skin caused by ultraviolet light.[47,48] Jafari *et al.* detected discrete keratinocyte atypia at the level of the stratum spinosum, presence of solar elastosis and dilated blood vessels in clinically uninvolved skin sites perilesional from AKs.[46] Therefore, a control group consisting of individuals with non-photodamaged skin and no history of UV-induced neoplasia may be more reliable.

Current studies are promising and show an overall sensitivity and specificity of 79-100% and 78-100%, respectively, for diagnosing AK, SCC *in situ*, SCC and its variants using RCM. However, no randomised controlled trials (RCTs) are reported on this subject. A RCT comparing RCM with punch biopsy or surgical excision in clinically suspected SCC can determine the diagnostic accuracy of the RCM for diagnosing SCC.

In conclusion, the current performed RCM studies are promising and show a variety of RCM features for the diagnosis of AK, BD, AC, EoQ, SCC and KA using RCM. Additionally, the monitoring RCM features after treatment of AKs and AC have been described. However, large, prospective, RCM observer-blinded RCTs, case-controls with a non-photodamaged skin controlgroup and cohort studies of high methodological quality are currently lacking. Curettage or pretreatment of hyperkeratotic lesions and uniform definitions of RCM features are necessary in order to identify RCM features that will aid in differentiating between AK, SCC *in situ*, SCC and its variants in clinical practice.

Table 1: Characteristics of included studies

First author (year)	Study design	STROBE criteria (%)	No. scanned skin sites	Histopatho-logical diagnosis	Aim: diagnostic or monitoring	RCM features per diagnosis (frequency in %)	Sens/ spec RCM diagnosis compared to histo-pathologic diagnosis	Type Viva-scope
Aghassi (2000)[14]	Case- control	C (25%)	21: Clinically suspected AK lesions (n=7) Non-lesional skin adjacent to AK lesions (n= 6) Non-lesional skin of healthy volunteers (n=8)	4: AK (n=3) SCC (n=1)	Diagnostic: To characterize AKs using in vivo RCM	AK: *SC*: - Hyperkeratosis (71%) SG: - Nuclei enlargement (14%) - Architectural disarray (14%) - Typical honeycomb pattern (86%) *SS:* - Nuclear enlargement and pleomorphism (100%) - Architectural disarray (57%) *Dermis:* - Elongated dermal papillae (29%) SCC: *Epidermis:* - Hyperkeratosis (100%), - Slight nuclei enlargement in SG (100%) - Architectural disarray in SG (100%)	-	1000
Agozzino (2014)[37]	Case report	C (21%)	1: Oral SCC (n=1)	1: Oral SCC (n=1)	Diagnostic: To determine the location with the most significant microscopic changes within an oral lesion suspected for oral SCC to take a biopsy	SCC: *Epidermis:* - Hyperkeratosis - Keratinocyte atypia and polymorphism - Targetoid-like structures	-	3000

First author (year)	Study design	STROBE criteria (%)	No. scanned skin sites	Histopatho-logical diagnosis	Aim: diagnostic or monitoring	RCM features per diagnosis (frequency in %)	Sens/ spec RCM diagnosis compared to histo-pathologic diagnosis	Type Viva-scope
Arzberger (2013)[35]	Case-control	C (39%)	20: Clinically suspected SCC in situ lesions (n=6) Clinically suspected balanitis lesions (n=9) Non-lesional skin at periphery of balanitis lesions (n=5)	15: SCC in situ (n=3) Zoon balanitis (n=6) Nonspecific balanitis (n=6)	Diagnostic: To identify in vivo morphologic criteria for balanitis and SCC in situ using RCM and to evaluate the ability of RCM to differentiate between balanitis and SCC in situ compared to histopathological methods	EoQ: *Epidermis:* - Atypical honeycomb pattern (100%) - Architectural disarray (100%) - Round nucleated cells (67%) - Scattered small bright cells (67%) *Dermis:* - Scattered small bright cells (67%) - Round papillary vessels (100%) - Vermicular blood vessels (33%)	EoQ: Sens: 100% Spec: 100%	1500
Bassoli (2012)[22]	Case series	B (50%)	54: *Among others:* Actinic keratosis (n=6) Pigmented SCC in situ (n=5)	54: *Among others:* Actinic keratosis (n=6) Pigmented SCC in situ (n=5)	Diagnostic: To identify RCM features of lichen planus- like keratosis (LPLK), correctly interpret the RCM features of LPLK by correlation to histopathology and compare them to RCM findings of skin neoplasms in the differential diagnosis of LPLK (AK, SCC, BCC, melanoma)	AK: *Epidermis:* - Scale (50%), - Typical honeycomb pattern (17%) - Atypical honeycomb pattern with broadened cell borders (83,3%), *Dermis*: - Dark areas in upper dermis (50%), - Isolated plump- bright cells (33%) - Convoluted blood vessels (66,7%) Pigmented BD Epidermis: - Atypical honeycomb pattern of SG (100%) - Isolated dendritic cells (80%) Dermis: - Sparse, isolated plump-bright cells (20%)	-	1500

Braga (2009)[31]	Case series	C (29%)	5: *Among others:* SCC in situ (n=1)	5: *Among others:* SCC in situ (n=1)	Diagnostic: To demonstrate the use of RCM as an adjunct to the bedside diagnosis of pink lesions	BD: *Epidermis:* - Scale crust, - Atypical honeycomb pattern with heterogeneity in brightness and width of the grid (cellular outlines) and in size of the nuclei in SG and SS - Single round cells with a bright rim and central darkness	-	1500
Cinotti (2014)[36]	Case-control	B (54%)	92: Clinically suspected eyelid margin tumor lesions (n=47) Non-lesional eyelid margins of the other eye (n=45)	35: *Among others:* SCC (n=3)	Diagnostic: To evaluate the suitability of in vivo confocal microscopy for eyelid margin tumours	SCC: *Epidermis:* - Atypical honeycomb pattern and/or architectural disarray in the spinous-granular layer *Dermis:* - Horizontal and dilated blood vessels	SCC: Sensitivity: 100% Spec:-	3000
Eichert (2010)[19]	Review including a case series	C (29%)	100: *Among others:* AK (n=3) SCC (n=5)	100: *Among others:* AK (n=3) SCC (n=5)	Diagnostic: To compare various published diagnostic in vivo RCM features for cutaneous tumours to their population of 100 patients with skin tumours and to discuss the differences	AK and SCC: *Epidermis:* - Hyperkeratosis - Atypical keratinocytes - Poorly defined and irregular keratinocyte cell boundaries	-	1500

First author (year)	Study design	STROBE criteria (%)	No. scanned skin sites	Histopatho-logical diagnosis	Aim: diagnostic or monitoring	RCM features per diagnosis (frequency in %)	Sens/ spec RCM diagnosis compared to histo-pathologic diagnosis	Type Viva-scope
Farnetani (2015)[27]	Case series	B (71%)	100: *Among others:* AK (n=3)	100: *Among others:* AK (n=3)	Diagnostic: To test interobserver reproducibility in recognition of previously published RCM descriptors	AK: - Nonedged dermal papillae (96.3%) - Aspecific DEJ (59.3%) - Plump bright cells (55.6%) - Collagen bundles (33.3%) - Atypical cells (29.6%) - Ringed DEJ (29.6%) - Irregular epidermal architecture (11.1%) - Disarray or noninvisible papillary contour (11.1%) - Meshwork DEJ (11.1%) - Junctional nests (11.1%) - Baseloid cord-like structures (7.4%) - Pagetoid cells (7.4%) - Ulceration (7.4%)	-	1500
Fraga-Braghiroli (2014)[34]	Case series	B (50%)	6: *Among others:* SCC in situ (n=1)	6: *Among others:* SCC in situ (n=1)	Diagnostic: To describe the utility of RCM handheld probe as a bedside adjunct for clinical diagnosis of solitary facial papules	BD: *Epidermis:* - Irregular honeycomb pattern in SG and SS	-	3000
Horn (2008)[20]	Case- control	B (61%)	60: Clinically suspected AK lesions (n=30) Non-lesional skin from the contralateral side (n=30)	15: AK (n=15)	Diagnostic: To validate RCM in diagnosing AK, to evaluate morphologic features determined by RCM and to determine the method's diagnostic performance and reliability	AK: *Epidermis:* - Inhomogenous, irregular stratum corneum - Irregular honeycomb pattern of keratinocytes - Loss of stratification of epidermal layers - Dyskeratotic areas - Different size and shape of nuclei of keratinocytes - Irregular keratinocyte borders - Irregular intercellular keratinocyte connections	AK: Sens: 91% Spec: 78%	1000

Incel (2014)[21]	Case series	B (50%)	122: *Among others:* AK (n=8) BD (n=7) SCC (n=11) KA (n=3)	122: *Among others:* AK (n=8) BD (n=7) SCC (n=11) KA (n=3)	Diagnostic: To determine the value of RCM on the vascularity of non-pigmented skin tumours	AK: *Epidermis:* - Surface squam - Cells with central dark nucleus in SC - Keratinocytes of varying size and shape - Atypical honeycomb pattern - Small refractile cells *Dermis:* - Net-like material around midrefractile collagen bundles - Small refractile cells - Round oval dark spaces filled with midrefractile cells - Curved linear vessels BD: *Epidermis:* - Corneocytes with nucleus - Variable atypical honeycomb pattern - Elongated keratinocytes *Dermis:* - Round vessels in dermal papillae - Curved linear vessels SCC: *Epidermis:* - Refractile squam/ crust in SC and nucleated cells with dark center in SC - Atypical honeycomb pattern, disarranged pattern at SG - Large round nucleated cells in SG - Dendritic cells in SG	AK/ BD: Sens: 93% Spec: 97% SCC/KA: Sens: 79% Spec: 99%	3000

First author (year)	Study design	STROBE criteria (%)	No. scanned skin sites	Histopatho-logical diagnosis	Aim: diagnostic or monitoring	RCM features per diagnosis (frequency in %)	Sens/ spec RCM diagnosis compared to histo-pathologic diagnosis	Type Viva-scope
						Dermis: - Small edged papillae - Dendritic cells - Curved linear vessels KA: *Epidermis:* - Refractile squam/ crust and nucleated cells with darker center in SC - Atypical honeycomb pattern, disarranged pattern in SG - Large round nucleated cells in SG - Dendritic cells in SG *Dermis:* - Small edged papillae - Curved linear vessels around centrally localized crust in radial distribution		
Longo (2013)[38]	Case series	B (67%)	140: *Among others:* SCC (n=6)	140: *Among others:* SCC (n=6)	Diagnostic: To assess whether the diagnostic accuracy of RCM was comparable to histopathology for the diagnosis of nodular lesions and to identify possible limitations of this technique	SCC: *Epidermis:* - Scales - Disarray of epidermis *Dermis:* - Prominent vascularity	-	1500

Malvehy (2015)[16]	Cohort	B (62%)	3 or more clinically visible AK lesions and non-lesional adjacent skin ('subclinical AK') per patient (Total number of scanned lesions unknown)	AK	Monitoring: To describe the changes in clinical and subclinical AK during and after topical treatment with 3% diclofenac sodium with 2,5% hyaluronic acid twice daily for 90 days and describe the correlation between RCM and histology	Clinical and subclinical AK: *After 2 weeks of treatment (t=2) and 6 weeks of treatment (t=3):* *Epidermis:* - Decrease of scaling, area of atypical honeycomb pattern and the degree of atypical honeycomb pattern - Increase in polygonal nucleated cells, detached corneocytes and inflammation - An elevated level of inflammation (presence of small bright inflammatory cells and dendritic cells in spinous layer and upper dermis) *At the end of the treatment (3 months; t=4):* *Epidermis:* - Decrease of scaling, area of atypical honeycomb pattern and the degree of atypical honeycomb pattern - Normalization of the number of polygonal nucleated cells, detached corneocytes and inflammation - An elevated level of inflammation (presence of small bright inflammatory cells and dendritic cells in spinous layer and upper dermis) *Dermis:* - Changes in dermal collagen thickening and arrangement of fibres (huddles of collagen and curled fibres were replaced by aligned thinner fibres)	-	*1500*

First author (year)	Study design	STROBE criteria (%)	No. scanned skin sites	Histopatho-logical diagnosis	Aim: diagnostic or monitoring	RCM features per diagnosis (frequency in %)	Sens/ spec RCM diagnosis compared to histo-pathologic diagnosis	Type Viva-scope
Moscarella (2013)[32]	Case series	B (50%)	24: *Among others:* Confirmed collision tumours with: SCC in situ (n=4)	24: *Among others:* Collision tumours with: SCC in situ (n=4)	Diagnostic: To see if RCM was a valuable tool when dealing with collision tumours	BD: *Epidermis:* - Superficial scaling - Atypical honeycomb pattern, disarranged pattern *Dermis:* - Round blood vessels	*BD:* *Sens: 100%* *Spec: -*	*1500*
Moscarella (2015)[29]	Case series	C (42%)	17: Pigmented AK (n=17)	17: Pigmented AKs (n=17)	Diagnostic: To define dermoscopic and RCM features of histopathologically confirmed pigmented AKs	Pigmented AK: *Epidermis:* - Epidermal thickness >25 micrometers (53%) with a mean thickness of 54 micrometers *SC:* - Scaling (52.9%) - Disruption/individual cells (47%) - Parakeratosis (24%) - Corneal pseudocysts (24%) - Keratin filled invagination (35%) *Suprabasal layer:* - Atypical keratinocytes (100%) - Disarranged epidermal pattern (29%) - Mottled pigmentation (47%) - Targetoid cells (6%) - Intra-epidermal dendritic cells (71%) *DEJ:* - Ringed areas/ small and bright papillae/ densely packed papillae (47%) - Polycyclic papillary contours (19%) - Cords (41%)	-	1500

						Upper dermis: - Plump bright cells (53%) - Huddled collagen bundles (24%) - Coarse collagen bundles (41%) - Curled fibres (6%) - Linear vessels (18%)		
Nascimento (2014)[30]	Case series	B (58%)	79: Among others: Pigmented AK (n=58), though RCM imaging performed in only n=9.	79: Among others: Pigmented AK (n=58)	Diagnostic: To evaluate sensitivity, specificity, and interobserver reproducibility of a novel dermoscopic feature, inner gray halo (IGH), and establish its histopathological and RCM correlations.	<u>Pigmented AK:</u> *Epidermis:* - Atypical honeycomb pattern (100%) - Numerous dendritic cells in the area between hair follicles, but not infiltrating the infundibulum - Irregular keratinocytes mainly located outside the infundibulum *DEJ:* - A ring of pigmented keratinocytes surrounding the hair follicles	-	1500
Peppelman (2014)[17]	Case series	B (54%)	30: AK (n=24) SCC (n=6)	30: AK (n=24) SCC (n=6)	Diagnostic: To determine in vivo RCM features that are specific for making a distinction between AK and SCC	<u>SCC:</u> *Epidermis:* - Architectural disarray SG and SS *Dermis:* - Nest-like structures - Increased blood vessel diameter and number of blood vessels compared to AK	-	1500

First author (year)	Study design	STROBE criteria (%)	No. scanned skin sites	Histopatho-logical diagnosis	Aim: diagnostic or monitoring	RCM features per diagnosis (frequency in %)	Sens/ spec RCM diagnosis compared to histo-pathologic diagnosis	Type Viva-scope
Richtig (2010)[23]	Cohort	B (59%)	10: Clinically suspect AK lesions (n=10)	10: AK (n=10)	Monitoring: To investigate the applicability of in vivo RCM for the follow up of AK before shave biopsy, after 3 and 12 months	AK Before shave biopsy: *Epidermis:* - Irregular, inhomogenous SC (100%) - Loss of regular stratification (100%) - Irregular size and nuclei of keratinocytes (100%), - Irregular cell borders (100%), - Dyskeratotic areas (100%) 3 months after shave biopsy: *Epidermis:* - Homogenous SC (80%), - Regular stratification of epidermis (80%) - Regular size of keratinocytes (80%) - Irregular, inhomogenous SC (20%) - Loss of regular stratification of epidermis (20%) - Irregular size and nuclei of keratinocytes (20%) - Irregular cell borders (20%) - Dyskeratotic areas (20%) *Dermis:* - Increase in collagen bundles (100%)	-	1000

						12 months after shave biopsy: *Epidermis:* - Irregular, inhomogenous SC (20%) - Loss of regular stratification of epidermis (20%) - Irregular size and nuclei of keratinocytes (20%) - Irregular cell borders (20%), - Dyskeratotic areas (20%)		
Rishpon (2009)[15]	Case series	C (36%)	38: AK (n=7) SCC in situ (n=25) Invasive SCC (n=3) Keratoacan-thoma (n=3)	38: AK (n=7) SCC in situ (n=25) Invasive SCC (n=3) Keratoacan-thoma (n=3)	Diagnostic: To identify criteria for the diagnosis of SCC and AK by RCM	<u>AK</u> *SC:* - Scale (100%) - Polygonal nucleated cells (14%) *SG and SS:* - Milder atypical honeycomb pattern or more focal architectural disarray than in SCC (100%) - Round nucleated cells (14%) *Dermis:* - Round vessels traversing dermal papillae (72%) <u>BD:</u> *SC:* - Scale (92%), - Polygonal nucleated cells (8%) *SG and SS:* - More extensive atypia and/or architectural disarrangement than in AK (100%), - Round nucleated cells (72%) *Dermis:* - Round vessels traversing dermal papillae (100%)	-	1500

First author (year)	Study design	STROBE criteria (%)	No. scanned skin sites	Histopatho-logical diagnosis	Aim: diagnostic or monitoring	RCM features per diagnosis (frequency in %)	Sens/ spec RCM diagnosis compared to histo-pathologic diagnosis	Type Viva-scope
						<u>SCC:</u> *SC* - Scale (100%), - Polygonal nucleated cells (33%) *SG and SS:* - More extensive atypia and/or architectural disarrangement than in AK (100%) - Round nucleated cells (33%) *Dermis:* - Round vessels traversing dermal papillae (100%) - Pleomorphic nucleated cells in superficial dermis (33%) <u>KA:</u> *SC:* - Scale (100%) *SG and SS:* - Atypical honeycomb pattern/ architectural disarray (100%) - Round nucleated cells (33%) *Dermis:* - Round vessels traversing dermal papillae (67%)		

Ulrich, Forschner (2007)[24]	Case series	C (38%)	20: *Among others:* AK (n=10)	20: AK (n=10) DSAP (n=10)	Diagnostic: To determine relevant RCM criteria for identification of DSAP and to define distinguishing criteria for DSAPs compared with AK	AK: *Epidermis:* - Parakeratosis - Separation of individual corneocytes - Impetiginisation of SC - More severe architectural disarray in SG and SS and keratinocytes with nuclear and cell polymorphism than in DSAP - Inflammatory cells (exocytosis) - Spongiosis - No observable sharp border surrounding the AK lesion *Dermis:* - Dilatation of blood vessels - Solar elastosis	-	1500
Ulrich, Gonzalez (2011)[28]	Case series	C (34%)	10: Clinically suspect actinic cheilitis lesions (n=10)	10: *Among others:* Actinic cheilitis (n=7)	Diagnostic and monitoring: To describe the RCM features of actinic cheilitis and the monitoring response to 3% Diclofenac in 2,5% hyaluronic acid applied twice daily for 90 days	Actinic cheilitis: *Before treatment (t=1):* *Epidermis:* - Hyperkeratotic scale (71%) - Disruption SC with thereby single detached corneocytes and parakeratosis (100%) - Atypical honeycomb pattern in SG and SS (86%) - Absence of typical honeycomb pattern (14%) *Dermis:* - Solar elastosis (86%) - Dilated and round blood vessels (100%) - Multiple small, bright round cells in upper dermis and the DEJ (43%) - Cells of dendritic appearance in the dermis and DEJ (29%)	Actinic cheilitis: Sens: 86% Spec: 100%	1500

First author (year)	Study design	STROBE criteria (%)	No. scanned skin sites	Histopatho-logical diagnosis	Aim: diagnostic or monitoring	RCM features per diagnosis (frequency in %)	Sens/ spec RCM diagnosis compared to histo-pathologic diagnosis	Type Viva-scope
						4 weeks after cessation of treatment (t=2): *Epidermis:* - Loss of cellular atypia (83%) - Typical honeycomb pattern (83%) - Atypical honeycomb pattern (17%) - Keratinocyte atypia (17%)		
Ulrich, Kanitakis (2012)[33]	Case series	C (21%)	10: BD (n=10)	10: BD (n=10)	Diagnostic: To report RCM features of BD and correlate them to the corresponding histological features	<u>BD:</u> *SC*: - Superficial epidermal disruption (100%) - Parakeratosis (90%) - Neutrophils (70%) *SG and SS:* - Atypical honeycomb pattern with cell and nuclei polymorphism (100%) - Targetoid cells type 1 and targetoid cells type 2 within the atypical honeycomb pattern - Large cells with aggregated bright nuclei (50%) *Dermis*: - Round- to- oval blood vessels in centre of dermal papillae with increased tortuosity within the superficial papillary dermis (100%) mostly becoming S-shaped vessels in the lower papillary dermis (70%) - Small round bright cells (50%)	-	1500

Ulrich, Maltusch (2008)[25]	Case series	B (54%)	56: Clinically suspected AK lesions (n=46) Non-lesional, non-sun-exposed skin on the forearms (n=10)	46: AK (n=46)	Diagnostic: To evaluate the applicability of RCM in the diagnosis of AK in correlation with histopathology	AK: *SC:* - Hyperkeratosis - Parakeratosis - Individual corneocytes - Impetiginization *SG and SS:* - Nuclear and cellular pleomorphism - Architectural disarray - Spongiosis - Exocytosis *Dermis:* - Blood vessel dilatation - Lymphocyte rolling - Solar elastosis - Dermal inflammatory infiltrate	AK: Sens: 98-100% Spec: 100%	1500
Ulrich, Maltusch (2007)[26]	Case- control	C (43%)	88: Clinically suspect AK lesions (n=44) Non-lesional skin (n=44)	44: AK (n=44), of which: KIN I (n=30) KIN II n=7) KIN III (n=7)	Diagnostic: To evaluate the RCM morphology of clinically diagnosed AK and to correlate the findings with routine histopathology	AK/ BD: *Epidermis:* - Hyperkeratosis - Parakeratosis - Keratinocyte atypia - Nuclear and cellular pleomorphism - Inflammatory cells *Dermis:* - Blood vessel dilatation - Solar elastosis	AK/BD: Sens: 98% Spec: -	1500
Wurm (2012)[42]	Case series	C (25%)	4: *Among others:* Pigmented AK with adjacent seborrheic keratosis (n=1)	4: *Among others:* Pigmented AK with adjacent seborrheic keratosis (n=1)	Diagnostic: To show the value of RCM for facial lesions and to increase knowledge of RCM morphologic features	Pigmented AK with adjacent seborrheic keratosis: *Epidermis:* - Atypical honeycomb pattern - Nucleated bright round cells - Anastomosing epithelial cords at the level of DEJ	-	1500

Abbreviations: AK, actinic keratosis; BD, Bowen disease; EoQ, erythroplasia of Queyrat; SCC, squamous cell carcinoma; BCC, basal cell carcinoma; KA, keratoacanthoma; SK, seborrheic keratosis; DSAP, disseminated superficial actinic porokeratosis; SC, stratum corneum; SG, stratum granulosum; SS, stratum spinosum; DEJ, dermo-epidermal junction.

Table 2: Most frequently described diagnostic reflectance confocal microscopic features

Diagnosis	RCM features
Non-pigmented actinic keratosis and actinic cheilitis	Scale Hyperkeratosis Parakeratosis Atypical keratinocytes **Normal or atypical honeycomb pattern in SG** Architectural disarray in SG Atypical honeycomb pattern or architectural disarray in SS Poorly defined and irregular keratinocyte cell boundaries Exocytosis Dilated blood vessels Dermal solar elastosis
Pigmented actinic keratosis	With addition of: Mottled pigmentation Targetoid cells Epidermal dendritic cells Cords Densely packed, small and bright papillae Ring of pigmented keratinocytes surrounding the hair follicle Dermal plump bright cells
Non-pigmented Bowen disease	Scale Hyperkeratosis Parakeratosis Atypical keratinocytes Atypical honeycomb pattern and/or architectural disarray in SG and SS (Epi)dermal small bright cells
Pigmented Bowen disease	With addition of: Epidermal dendritic cells Dermal plump bright cells
Erythroplasia of Queyrat	Atypical honeycomb pattern and/or architectural disarray in SG and SS Round nucleated cells (Epi)dermal small bright cells Round papillary vessels
Squamous cell carcinoma	Scale Hyperkeratosis Parakeratosis **Architectural disarray in SG** Atypical honeycomb pattern in SG Atypical honeycomb pattern or architectural disarray in SS Round nucleated cells Dilated blood vessels Increased number of blood vessels **Nest-like structures in superficial dermis** **Pleomorphic nucleated cells in superficial dermis**
Keratoacanthoma	Scale Hyperkeratosis Atypical honeycomb pattern and/or architectural disarray in SG and SS Round nucleated cells

Abbreviations: RCM, reflectance confocal microscopy; SC, stratum corneum; SG, stratum granulosum; SS, stratum spinosum. Bold: most important RCM features to distinguish between actinic keratosis and squamous cell carcinoma.

Supplementary table 1: Definitions and descriptions of the reflectance confocal microscopic features used in the studies

RCM feature	Definitions and descriptions
Anastomosing epithelial cords	Corresponding to anastomosing bridges of basaloid cells in histopathology.[42]
Architectural disarray or disarranged pattern	Severe disarranged epidermal pattern, formed by atypical keratinocytes, in which the honeycomb pattern is no longer visible.[15,16]
Atypical keratinocytes	Pleomorphic keratinocytes different in cell size and nucleus.[19]
Atypical or irregular honeycomb patterns	Deviation from the normal honeycomb pattern formed by irregular shaped keratinocytes.[15,34]
Cells with central dark nucleus in SC	Correlating to parakeratosis.[21]
Coarse collagen bundles	Bright fibrillar structures in the upper dermis forming a web-like arrangement.[29]
Cords	Elongated bright tubular structures in the DEJ.[29]
Corneal pseudocysts	Well circumscribed large, round, highly refractile intra-epidermal structures.[29]
Curled fibres	Highly refractive thick and short fibres, sometimes forming compact masses in presence of solar elastosis.[29]
Curved linear vessel	Curved linear dark spaces within dermal papillae that do not branch.[21]
Densely packed small and bright papillae	Round to polymorphous dermal papillae that are densely packed.[29]
Disruption/ individual cells or disruption of SC	Single, detached corneocytes in SC.[28,29]
Exocytosis	Highly refractive, round structures in the epidermis corresponding to inflammatory cells.[17]
Huddled collagen bundles	Large hyporefractive blotches of amorphous material in the upper dermis and in which individual collagen fibres are no longer visible.[29]
Impetiginisation	The presence of very small bright structures corresponding to inflammatory cells in the stratum corneum.[28]
Keratin filled invaginations	Round to longitudinal invaginations of the lesion surface, containing structureless amorphous material of various brightness.[29]
Large cells with aggregated bright nuclei	Corresponding to multinucleated keratinocytes.[33]
Lymphocyte rolling	Lymphocytes within blood vessels appearing as round, high refractive cells.[17]
Mottled pigmentation	Corresponding to clustered bright keratinocytes detectable in a honeycomb pattern.[29]
Nest-like structure	Round, demarcated structures in the dermis that are often surrounded by fibrosis.[17]
Normal honeycomb pattern	Uniform, regular spaced, broad keratinocytes forming a pattern resembling a honeycomb.[17]
Parakeratosis or polygonal nucleated cells in SC	Single, detached nucleated cells at the SC with sharp outlines and dark centre.[29,33]
Plump- bright cells	Large, irregular shaped, bright cells with ill-defined borders and usually no visible nucleus corresponding to melanophages.[22,29]
Poorly defined and irregular keratinocyte cell borders	Poorly demarcated individual keratinocytes with broad and blurred cell borders.[19]
Round nucleated cells	Cells with sharply refractive cell borders surrounding a dark nucleus corresponding to atypical or dyskeratotic keratinocytes.[15]

Round papillary vessels	Blood vessels that run perpendicular to the horizontal confocal imaging, producing a round appearance.[15]
Scale	Increased thickness of SC seen as refractive amorphous material.[29]
Single round cells with a bright rim and central darkness	Most consisting with dyskeratotic keratinocytes.[31]
Small bright cells	Bright cells without visible nuclei corresponding to inflammatory cells.[35]
Solar elastosis	Bright dense bundles in the dermis with a lace-like appearance.[28]
Targetoid cell type 1	Large cell with a bright centre and a dark peripheral halo, correlating to a dyskeratotic keratinocyte separated from adjacent cells by a clear retraction halo.[33]
Targetoid cell type 2	A large cell with a dark centre and a bright rim surrounded by a dark halo, correlating to a dyskeratotic keratinocyte containing a pycnotic nucleus.[33]
Targetoid-like structures	Large cell with a bright centre and a dark peripheral halo (targetoid cell 1) or large cell with a dark centre and a bright rim surrounded by a dark halo (targetoid cell 2) corresponding to dyskeratotic cells.[33]
Vermicular blood vessels	Blood vessels that run parallel to the surface, producing a tortuous appearance.[15]

Abbreviations: SC: stratum corneum; SG: stratum granulosum; SS: stratum spinosum; DEJ: dermo-epidermal junction.

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3.2

Reflectance confocal microscopy: non-invasive distinction between actinic keratosis and squamous cell carcinoma

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Abstract

Background: Early recognition of squamous cell carcinoma (SCC) is difficult. Non-invasive reflectance confocal microscopic (RCM) imaging of the skin is a promising diagnostic technique. Although several RCM features for SCC and actinic keratosis (AK) have been described, it is not determined whether RCM has the ability to distinguish between SCC and AK.

Objective: To determine *in vivo* reflectance confocal microscopic features that are specific for making a distinction between AK and SCC.

Methods: In 24 patients, 30 lesions clinically suspicious for AK or SCC were selected for RCM imaging. Following the imaging procedure, a 3 mm skin biopsy was obtained for confirmation of the histopathological diagnosis. Two observers evaluated the RCM images according to a literature based list of RCM features. The obtained data were evaluated by an univariate and forward multivariate logistic regression analysis, kappa analysis and independent T-test.

Results: The univariate logistic regression showed statistically significant odds ratios for several RCM features, including architectural disarray in the stratum granulosum, architectural disarray in the spinous layer and nest-like structures in the dermis. The forward multivariate logistic regression analysis showed that the combination of these features increased the ability to make the correct diagnosis AK and SCC non-invasively. The interobserver agreement between a starting and an experienced RCM observer ranged from poor to no agreement.

Conclusion: This study revealed specific RCM features that can distinguish between AK and SCC, stimulating further prospective large cohort research in this field. This will result in correct, efficient and adequate diagnosis and treatment of clinically difficult to distinguish AK and SCC lesions.

Introduction

Skin cancer is the most commonly diagnosed type of cancer in the Caucasian population, with rapid increasing incidence rates.[1] Squamous cell carcinoma (SCC) and basal cell carcinoma are considered non-melanoma skin cancers (NMSC). The incidence ratio between those two NMSC types is approximately 1:4.[2, 3] Despite the lower frequency, SCC accounts for the majority of NMSC related metastatic disease, making early recognition important.[4]

SCC arises out of epidermal keratinocyte dysplasia. These atypical keratinocytes penetrate the basal membrane in order to involve the dermis and deeper tissues. Actinic keratosis (AK) are commonly considered as premalignant skin lesions, which act as precursor to SCC. It is demonstrated that 0.1%–20% of all AK lesions can progress to invasive SCC. However, it is not possible to predict which lesion is at risk.[5-7] The development of AK lesions is induced by e.g. ultraviolet radiation, causing damage to keratinocytes and their proliferation. In contrast to SCC, the basal membrane is not disrupted in AK lesions.[4-6]

The diagnosis of AK is mainly made upon clinical evaluation. In contrast, a lesion clinically suspected for SCC is confirmed by histological evaluation of a skin biopsy. However, the clinical distinction between AK and SCC can be difficult and is not always reliable.[8] Dermoscopy can be useful in determining the diagnosis non-invasively. Although, in SCC and SCC *in situ*, glomerular or dotted vessels are often visible, but the absence of these vessels will not exclude the presence of a SCC.[9-11] In addition, there may be some overlay between dermoscopic features of AK and SCC.[10] Lastly, SCC is often difficult to visualize by dermoscopy because the scaly surface might obscure the underlying morphology.[9] Therefore, routine histopathology remains the gold standard, although this entails patient discomfort, time and expenses. Moreover, the feasibility of obtaining biopsies from affected and surrounding skin sites is sometimes limited and can result in a sampling error. For these reasons, the interest in the development of non-invasive diagnostic methods to distinguish between AK and SCC is increasing.

Reflectance confocal microscopy (RCM) is a non-invasive technique for *in vivo* imaging of the skin that uses near-infrared laser light. This technique produces horizontal images of the skin in shades of grey, with a resolution comparable to conventional histology.[12] Non-invasive RCM is painless, can evaluate a larger area or the whole tumour, can image the exact same location over time and will not induce any kind of skin damage or inflammatory response. Further, artefacts caused by tissue processing during histopathological assessment can be avoided.[13]

Currently, RCM is used for several dermatological purposes such as diagnosing and monitoring of inflammatory skin diseases, melanoma and NMSC, including SCC and its precursor AK.[14-25] Several RCM features for AK and SCC have been described previously.[9, 26-37] However, to the best of our

knowledge, there are no studies that have determined the ability of RCM to distinguish between AK and SCC *in vivo*. Therefore, the aim of this study is to determine, based on statistical evaluation, whether there are RCM features that are specific for making an *in vivo* distinction between AK and SCC.

Materials and methods

Subjects

In 24 patients (12 men and 12 female), lesions clinically suspicious for AK or SCC were included for RCM imaging. The age of the patients ranged from 53 to 80 years, with a mean age of 67 years. In 19 patients, a history of NMSC was documented. Four of these patients used chronic immunosuppressive drugs after kidney transplantation and one patient was treated with radiotherapy. Within all patients, the skin type varied between I and III, according to the Fitzpatrick scale. The patients were recruited from the department of Dermatology, Radboud university medical center, Nijmegen, The Netherlands. Skin lesions with significant hyperkeratosis, extensive crusts, ulcerations or lesions located on body sites that were inaccessible for the RCM probe were excluded. A small control group of two subjects without a skin condition was included to compare vascular RCM features. This study was approved by the local medical ethics committee and was conducted according to the principles of the Declaration of Helsinki.

RCM imaging and analysis

For navigation during RCM imaging, pictures with a lower quality than dermoscopy were taken using a Vivacam macro camera (Vivacam; Lucid Inc., Rochester, NY, USA). *In vivo* RCM imaging was performed using the Vivascope 1500 system (Lucid Inc.). A detailed description of this technique has been published previously.[38, 39] Vertical mapping (Vivastack) was performed by capturing a series of images of 0.5 x 0.5 mm with steps of 4.5 μm in depth. The mapping started at the stratum corneum until the papillary dermis. Horizontal mapping of 4 x 4 mm (Vivablock) were made at different levels of the skin. In this study, the first appearance of nucleated cells, independent of the cell size and shape, was considered as the granular layer. Since the granular layer is only a few cell layers thick, two steps in depth below this point was considered the spinous layer. In most lesions, a movie was made at the level of the dermo– epidermal junction in order to visualize capillary blood flow. Images were obtained using Vivascan 7.0 software (Lucid Inc.). For image analysis, a list of diagnostic RCM features for AK and SCC was composed according to literature (Table 1).[26-33, 35] RCM images were retrospectively evaluated for these features by an experienced RCM user (2.5 years), and a starting RCM user who was instructed in the basic interpretation of RCM imaging for approximately 2 weeks. Both observers were not blinded for the final diagnosis and evaluated the lesions systematically for the presence or absence of individual RCM features. Further, the mean blood vessel diameter and number of vessels per confocal image (0.5 mm x 0.5 mm) were determined for both AK and SCC lesions. These measures were compared to the control group. An increased vascular dilatation was defined as a diameter of more than 5 μm and an increased number of blood vessel as more than 5 vessels per confocal image.

Histopathology

Following RCM imaging, punch biopsies with a diameter of 3 mm were obtained under local anaesthesia with 1% xylocaine/adrenaline. After 4h fixation in formaldehyde, the skin samples were embedded in paraffin and thereafter sectioned and stained with haematoxylin-eosin (HE) for histopathological evaluation by a pathologist.

Table 1. List of RCM features for actinic keratosis (AK) and squamous cell carcinoma (SCC) with the description used in the evaluation and analysis of this study. This list was composed according to literature.[26-33, 35, 40]

RCM features	Described for AK	Described for SCC
Stratum corneum		
SC disruption, detached corneocytes[a]	x	x
Hyperkeratosis[b]	x	x
Parakeratosis[c]	x	
Orthokeratosis[d]	x	
Inflammatory cells[e]	x	x
Stratum granulosum		
Normal honeycomb pattern[f]	x	
Atypical honeycomb pattern[g]	x	
Architectural disarray[h]		x
Cellular and nuclear pleomorphism[i]	x	x
Targetoid cells 1[j]	x	
Targetoid cells 2[k]	x	
Multinucleated keratinocytes[k]	x	
Stratum spinosum		
Atypical honeycomb pattern	x	x
Architectural disarray	x	x
Cellular and nuclear pleomorphism	x	x
Targetoid cells 1	x	
Targetoid cells 2	x	
Multinucleated keratinocytes	x	
Spongiosis[m]	x	x
Exocytosis[n]	x	
Dermo-epidermal junction		
Increased blood vessel dilatation[o]	x	
Increased number of blood vessels[p]		x
Increased capillary flow		x
Lymphocyte rolling[q]	x	
Dermis		
Solar elastosis[r]	x	x
Inflammatory cells	x	
Keratin pearl[s]		x
Nest-like structure[t]		x

[a] Detached corneocytes: white, highly refractive polygonal structure of approximately 30-40 μm in diameter in the stratum corneum. [b]Hyperkeratosis: thickening of the stratum corneum of more than 15 μm. [c]Parakeratosis: nucleated cells appearing as bright oval nuclei centrally within corneocytes in the stratum

corneum. [d]Orthokeratosis: hyperkeratosis without parakeratosis. [e]Inflammatory cells: highly refractive structures of 8-10 μm in diameter. [f]Normal honeycomb pattern: uniform, regular spaced, broad keratinocytes forming a grid resembling a honeycomb. [g]Atypical honeycomb pattern: irregular shaped cells deviating from the normal honeycomb pattern. [h]Architectural disarray: severe disarranged epidermal pattern in which the honeycomb pattern is no longer visible. [i]Cellular and nuclear pleomorphism: variation in cellular and nuclear shape and size. [j]Targetoid cells 1: large cell with a bright centre and a dark peripheral halo. [k]Targetoid cells 2: large cell with a dark centre and a bright rim surrounded by a dark halo. [l]Multinucleated keratinocytes: large cells with tight aggregates of bright nuclei. [m]Spongiosis: enlargement of the bright intercellular spaces due to fluid accumulation between keratinocytes. [n]Exocytosis: inflammatory cells appearing as highly refractive structures in the epidermis. [o]Increased blood vessel dilatation: blood vessel diameter of more than 5 μm. [p]Increased number of blood vessels: more than 5 blood vessels per 0.5 x 0.5 mm RCM confocal image. [q]Lymphocyte rolling: lymphocytes in blood vessels appearing as round, highly refractive cells. [r]Solar elastosis: network of thick, highly refractive collagen bundles intermixed with moderately refractive, lace-like elastic fibres. [s]Keratin pearl: whorl-shaped accumulation of keratin appearing as highly refractive, speckled structure in the dermis. [t]Nest-like structure: round, demarcated structures in the dermis that are often surrounded by fibrosis.

Statistical analysis

An univariate logistic regression analysis was performed on each individual RCM feature (predictor) for the diagnosis of AK, on the data set as obtained by the experienced RCM user. The same analysis was performed for SCC. The predictors were expressed in odds ratios (OR) with *p*-values. Predictors with a statistically significant OR (with a *p*-value <0.05) were evaluated in a forward multivariate logistic regression analysis in order to make a prediction model for the diagnosis of AK and SCC. The difference in blood vessel diameter and number of vessels per RCM confocal in SCC and AK lesions were analysed using an independent T-test. The interobserver agreement between the experienced observer and the starting RCM user was determined by kappa analysis (κ). The concordance was assessed by calculating the kappa value for each individual RCM parameter. A kappa value between 1 and 0.81 corresponded with an excellent interobserver agreement, values with high concordance included those features with $\kappa = 0.8–0.61$, moderate concordance with $\kappa = 0.6–0.41$, poor concordance with $\kappa = 0.4–0.1$ and a kappa of ≤ 0 corresponded to no interobserver agreement. All data analyses were conducted using computer software (SPSS Inc. version 20, Chicago, IL, USA).

Results

A total of 30 biopsy proven lesions were evaluated with RCM, of which 24 AK and 6 invasive non-pigmented SCC. The lesions were located on the head and neck area in 37% (n = 11), thorax in 27% (n = 8), upper extremities in 27% (n = 8) and lower extremities in 10% (n = 3).

RCM features for distinction between AK and SCC

Univariate logistic regression analysis resulted in statistically significant OR values for architectural disarray in the stratum granulosum, architectural disarray in the stratum spinosum and nest-like

structures in the dermis (Table 2, Figure 1). The forward multivariate logistic regression analysis with these parameters showed that the presence of architectural disarray in the granular layer would result in a correct diagnosis in 84.6% of the SCC cases. The combination of architectural disarray in the granular layer with architectural disarray in the stratum spinosum and/or dermal nest-like structures had a correct prediction of 88.5% of the SCC cases (Table 2). All other evaluated RCM features were not statistically significant and were therefore not able to distinct between AK and SCC.

Increased vascularisation in SCC and AK lesions

Comparing healthy skin with AK and SCC lesions, the mean blood vessel diameter and number of blood vessels per RCM confocal were increased in both AK and SCC lesions. The blood vessel diameter and number of blood vessels were highest in SCC. However, the vascular differences between AK and SCC were not statistically significant when analysed by an independent T-test (Table 3).

Interobserver agreement between independent observers

The interobserver agreement for the RCM parameters between an experienced and starting RCM user ranged from poor to no agreement. The highest concordance was reached for parakeratosis in the stratum corneum ($\kappa = 0.33$), architectural disarray in the stratum granulosum ($\kappa = 0.34$) and inflammation in the superficial dermis ($\kappa = 0.36$).

Table 2. RCM features for the diagnosis of SCC and AK with significant odds ratios (p-value < 0.05) based on the univariate logistic regression analysis. Further, the prediction model for diagnosis of SCC based on these RCM features is shown by the forward multivariate logistic regression analysis.

Univariate logistic regression analysis			
RCM parameter	**Odd ratio for diagnosis SCC**	**Odds ratio for diagnosis AK**	**p- value**
SG architectural disarray	24.0	0.042	0.013
SS architectural disarray	15.0	0.067	0.023
DERMIS nest-like structure	11.0	0.091	0.029

Forward multivariate logistic regression analysis	
RCM parameter	**Predicted percentage correctly diagnosed SCC**
SG architectural disarray	84.6%
SG architectural disarray SS architectural disarray	88.5%
SG architectural disarray DERMIS nest-like structure	88.5%
SG architectural disarray SS architectural disarray DERMIS nest-like structure	88.5%

All selected parameters are shown in ascending order, according to p-value.
SS, stratum spinosum; SG, stratum granulosum

Table 3. Blood vessel characteristics of healthy skin and patients with squamous cell carcinoma (SCC) and actinic keratosis (AK)

	Mean blood vessel diameter ± SD (μm)	Mean number of blood vessel ± SD (μm)
Control	4.26 ± 0.00	3.5 ± 2.1
AK	13.52 ± 9.89	7.8 ± 4.9
SCC	27.62 ± 32.25[1]	8.6 ± 4.2[2]

[1] *p*-value when compared to AK was 0.386 [2] *p*-value when compared to AK was 0.739.
The mean number of blood vessels per 0.5x0.5 mm RCM confocal image.

Discussion

RCM has been proven to be a useful, non-invasive tool for the *in vivo* diagnosis of melanocytic lesions and inflammatory skin conditions.[14-16, 23-25] Further, RCM knowledge and experience in the field of NMSC is increasing.[17-19, 22, 36] Due to often clinical similar appearance, distinction between SCC and AK can be challenging. Currently, the diagnostic distinction between these skin lesions, especially when solely based on clinical aspects, may not always be reliable. Whereas, obtaining biopsies is an invasive method and the feasibility is sometimes limited, mainly because of the risk of sampling errors. Therefore, the purpose of this study was to assess *in vivo* RCM features that are specific enough to make a distinction between AK and SCC using RCM as a non-invasive *in vivo* diagnostic method.

We demonstrated that, in clinically suspicious AK or SCC lesions, the presence of architectural disarray in the stratum granulosum in combination with architectural disarray in the spinous layer and/or tumour nest in the dermis were the main RCM features to distinguish SCC from AK. This result is in agreement with other studies that found architectural disarray in the granular layer in SCC, while the stratum granulosum in AK showed either normal keratinocytes or an atypical honeycomb pattern.[9, 26, 28, 32, 33, 35, 40] However, architectural disarray in the stratum spinosum was not only described in SCC but also in AK.[9, 27, 28, 37] Therefore, architectural disarray in the spinous layer alone is not a good predictor for SCC. Although we found differences in the granular and spinous layer, it should be mentioned that it might be hard to make the distinction between the granular and spinous layer *in vivo*. A good definition of the layers and experience in RCM image analysis are required.

The observed nest-like structures in the dermis correlate to aggregates of atypical keratinocytes corresponding to the diagnosis of invasive SCC. However, we also detected these nest-like structures in two AK lesions. This observation might have resulted from sampling error, whereby the biopsy was taken at a different site than where the nest-like structures were seen with RCM. This demonstrates the great advantage of RCM in evaluation of the total lesion, and therefore can prevent sampling errors. Further, we found an increased mean vascular diameter and a larger number of vessels for SCC and AK. Our results are in line with other studies and can be explained by the high metabolic needs of a tumour, which leads to vascular dilatation and neovascularisation.[29, 41]

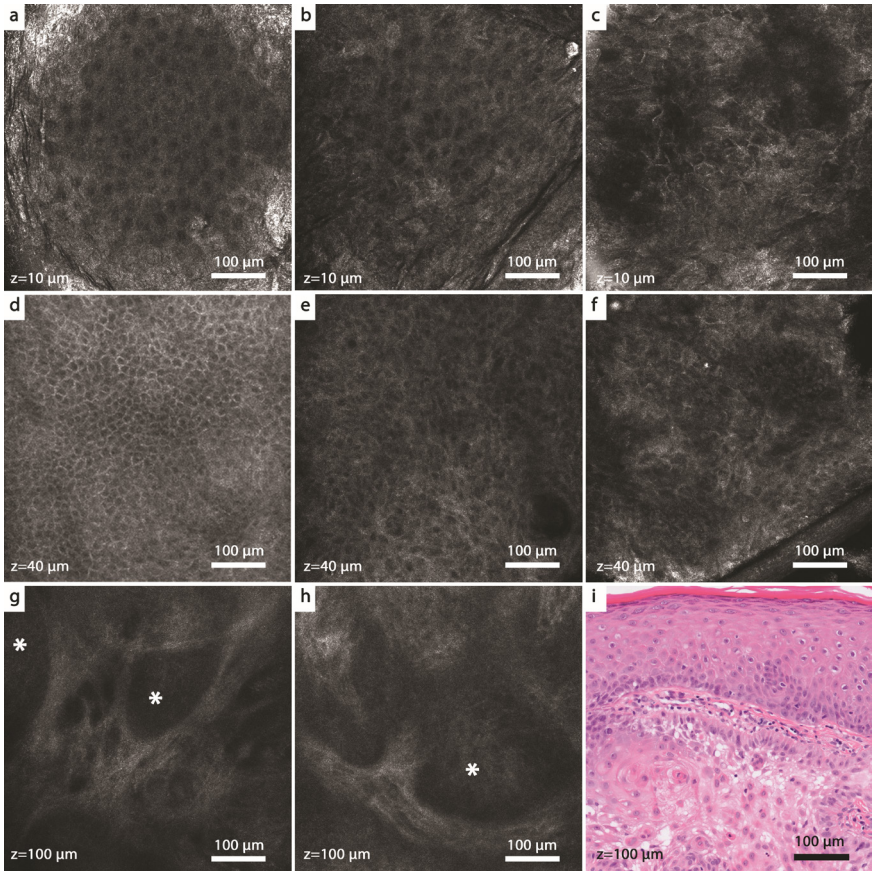

Figure 1. Representative images of actinic keratosis (AK) and squamous cell carcinoma (SCC). a) RCM image of AK showing a normal honeycomb pattern of the stratum granulosum. b) Atypical honeycomb pattern of the granular layer, visualized in an AK lesion. c) Architectural disarray of the granular layer in a SCC. d) Normal honeycomb pattern at the level of the stratum spinosum. e) RCM image at the level of the stratum spinosum, showing an atypical honeycomb pattern, which can be present in either AK or SCC. f) Architectural disarray of the spinous layer that is mainly observed in SCC. g and h) In SCC lesions at the dermal level, tumour nests (white asterisk) with surrounding fibrosis were visualized by RCM. i) Haematoxylin-eosin stained tissue section displaying an invasive SCC nest.

AK can be categorized according to the Keratinocyte Intraepithelial Neoplasia (KIN) with subdivision into three histopathological grades. In KIN 1, the keratinocytic atypia is limited to the lower third of the epidermis, whereas in KIN II, the lower two-thirds of the epidermis is involved. In KIN III, including Bowens disease, cell atypia is found in the full thickness of the epidermis without infiltration of atypical cells into the dermis. Although Bowens disease develops as epidermal carcinoma *in situ*, it may progress into invasive SCC.[4, 32, 34, 42] Therefore, it would be very interesting and useful to evaluate in a larger cohort whether there are specific RCM features

that allow distinction between KIN grades Bowens disease and invasive SCC. Despite the fact that RCM has some limitations in depth, this study shows that there are epidermal RCM features that might allow *in vivo* distinction between clinical similar appearing AK and SCC lesions.

The overall poor to no interobserver agreement in this study showed that RCM features for AK and SCC were difficult to learn and assess for an inexperienced RCM user. This illustrates the learning curve, which evaluation of RCM images is associated with. In contrast, the interobserver agreement between experienced RCM users is higher.[26, 37, 43] Horn *et al.* showed a moderate to poor interobserver agreement for RCM features of AK between two dermato-oncologists with previous experience in RCM.[26] In addition, Ulrich *et al.* also found a higher concordance for AK features among two independent experts in the field of RCM.[37] However, this kind of data are not available for the diagnosis of SCC lesions.

Dermoscopy is another commonly used non-invasive technique that improves the diagnostic accuracy of pigmented and non-pigmented skin lesions.[10] Several dermoscopic features for AK an SCC are described,[10, 11] of which some can be observed by RCM. Fraga-Braghiroli *et al.* observed with RCM the appearance of round circles with a bright white rim at the level of the dermo-epidermal junction corresponding to the small brown circles that can be observed with dermoscopy in pigmented SCC.[44] Unfortunately, we were not able to confirm this observation since this study only revealed non-pigmented lesions. In a larger prospective study, it would be interesting to include pigmented lesions as well. Although dermoscopy is a useful technique, it is not always conclusive due to similar appearing features between AK and SCC and the limitations of the surface examination. Especially in these cases real-time *in vivo* RCM, that can image the skin at morphological level until the papillary dermis, is of additional value.

It needs to be mentioned that for SCC, it remains difficult to include a large number of lesions. The often hyperkeratotic scale of a SCC is hard to evaluate with either dermoscopy or RCM. However, the major advantage of both techniques cannot be found in these clinical evident SCC. The major challenge lies in the field of clinical similar appearing lesions and to distinct between *in situ* SCC and invasive SCC. Our goal was to evaluate these similar appearing lesions, explaining the unequal number included AK and SCC.

In conclusion, this study revealed specific epidermal and dermal RCM features that can distinguish between AK and SCC *in vivo*. This stimulates further prospective, large cohort investigation in this field, which will contribute to development of protocols, resulting in correct, efficient and adequate diagnosis and treatment of clinically similar appearing AK and SCC. Furthermore, we have shown that extensive training and experience in RCM is required in order to correctly differentiate AK from SCC by RCM.

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3.3

Diagnosis of basal cell carcinoma by reflectance confocal microscopy: study design and protocol of a randomised controlled multicenter trial

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Abstract

Background: Skin cancer, including basal cell carcinoma (BCC), has become a major health care problem. The limitations of a punch biopsy (at present the gold standard) as a diagnostic method together with the increasing incidence of skin cancer points out the need for more accurate, cost-effective and patient friendly diagnostic tools. *In vivo* reflectance confocal microscopy (RCM) is a non-invasive imaging technique that has great potential in skin cancer diagnosis.

Objective: To investigate whether *in vivo* RCM can correctly identify the subtype of BCC and to determine the cost-effectiveness of RCM compared to punch biopsy (usual care) in a randomised controlled multicenter trial.

Methods: Based on a power of 80% and an alpha of 0.05, 329 patients with lesions clinically suspicious for BCC will be included in this study. Patients will be randomised for RCM or for a punch biopsy. When a BCC is diagnosed, surgical excision will follow and a follow-up visit will be planned 3 months later. Several questionnaires will be filled (EQ-5D, EQ-5D VAS, iMTA PCQ and TSQM-9). We will perform statistical analyses, cost-effectiveness and patient outcome analyses after data collection.

Results: This research started in January 2016 and is ethically approved. We expect to finish this study at the end of 2018.

Conclusion: In this study, we will investigate whether RCM is at least as good in identifying BCC subtypes as conventional pathological investigation of skin biopsies. Anticipating that RCM is found to be a cost-effective alternative, it saves on direct medical consumption (e.g. labour of the pathologist and other medical personnel) as well as materials related to treatment failure with at least equal effectiveness.

Trail registration:
ClinicalTrails.gov: NCT02623101 (https://clinicaltrials.gov/ct2/show/NCT02623101)

Introduction

Skin cancer is a common type of cancer and its incidence is increasing rapidly in Western countries.[1-3] This cancer comprises two types: melanoma (MM) and non-melanoma skin cancer (NMSC). NMSC is further divided into basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and its precursor lesions; actinic keratosis (AK) and Bowen disease. In the Netherlands, the registry of NMSC is poor. However, based on recent literature and guidelines, it is estimated that the incidence of malignant skin tumours and the premalignant AK is around 235,000 in 2015. This will have a major impact on our health care system. Moreover, it is predicted that numbers will rise at the rate of 4.5-8% per year, depending on the type of skin cancer.

Currently, in case of suspicion on NMSC, the pathological examination of a punch biopsy is the gold standard, according to the Dutch guidelines. In case of clinical suspicion of AK, the diagnosis is made à vue, without pathological confirmation. Already in 2003 in the United States, skin cancer was found to be among the most costly of all cancers to treat. Therefore, it is evident that skin cancer places an enormous burden on healthcare systems with increasing costs.[4] In case of suspicion of skin cancer, it is important to diagnose and treat it in an early phase, preferable in a patient friendly manner. As BCC is the most common skin cancer (about 75% of all skin cancers), this study will focus on this type of skin cancer.

Clinically, BCC can vary in appearance but is often characterised by small, translucent, or pearly papules with telangiectasias.[5] In the past, the diagnosis was mainly made clinically. However, non-invasive therapies have become available. Therefore, determination of the BCC subtype has become more important. For this reason, pathological analysis of a punch biopsy is currently the gold standard to confirm the clinical diagnosis and determine the subtype of BCC. The following subtypes of BCC can be distinguished: superficial (sBCC), nodular (nBCC), aggressive BCC (micro-nodular (mnBCC) and infiltrative (iBCC)).[6] It is experienced that there is a sample error in 29% of the cases with the conventional diagnostic procedure, resulting in an incorrect subtype diagnosis.[7] For this reason, and because of the increasing incidence of skin cancer, more accurate, cost-efficient and patient friendly diagnostic tools are desirable.

Reflectance confocal microscopy

Reflectance confocal microscopy (RCM) is a non-invasive imaging technique. It provides real time images of cell and tissue structures and *in vivo* dynamics, without the need for *ex vivo* tissue samples. RCM visualises human skin up to a depth of around 250 μm.[8-12] Refractive index differences between cells and surrounding tissue provide the contrast. The contrast of RCM imaging of the skin is mainly provided by melanin and keratin.[10] Most, but not all tumours can be visualised. For thicker tumours, RCM may help to find the optimal localisation to perform a

biopsy, as superficial features in these tumours may help to spot these lesions.[13] Moreover, RCM can image the whole tumour.

RCM features for NMSC have been described and showed a high correlation with conventional histopathological features.[13-15] These features aid in diagnosing AK and SCC, and BCC.[13-15] For the nodular and micronodular BCC subtypes, the following RCM characteristics are described: tumour nests with peripheral palisading, branch-like structures, fibrotic septa and increase of vascular diameter. The size and shape of the tumour nests allow further distinction between these BCCs. Solar elastosis and tumour nests connected with the basal cell layer characterise sBCCs.[14] Infiltrative BCCs are more challenging to visualise due to their histological complex appearance and deeper location.[16]

Only few studies report data on the diagnostic accuracy of RCM for a primary BCC diagnosis.[14,16-19] These studies show a high sensitivity and specificity for RCM as a diagnostic tool for BCC. Although they show the potential of RCM in the diagnosis of BCC, prospective large-scale studies are lacking. Furthermore, no diagnostic accuracy data is reported on determining the BCC subtype using RCM. These kind of studies are required for implementation of RCM in the routine patient care and incorporation into the health insurance system. Implementation of RCM in the routine patient care settings has the advantage of diagnosis a BCC at the first consultation and therefore, the patient can be treated at a short term. A second consultation for explaining the diagnosis and performing the treatment might be unnecessary. Therefore, time saved by using RCM can be used for other new patients.

Objectives

The primary objective of this study is to investigate whether *in vivo* RCM can identify the BCC subtype at least as correct as a skin punch biopsy. We hypothesise that RCM imaging allows correct identification of the BCC subtype (nBCC, mnBCC, sBCC, iBCC and mixed type BCC), and true and false positive results are equal compared to conventional pathological investigation of skin biopsies (gold standard). It is postulated that RCM is more cost-effective and patient friendly compared to the current procedure. Therefore, the quality of life (Qol), costs and quality adjusted life years (QALY's) will be evaluated as secondary outcome measures. Overall, with implementation of RCM in dermatological skin cancer care, it is aimed to contribute to cost-effective, non-invasive and patient friendly diagnostic methods.

Methods

Recruitment, inclusion and study design

Patients with lesions clinically suspicious (diagnosis à vue) for BCC, eligible for RCM, visiting the dermatological departments of the Radboud university medical center, Nijmegen; the Canisius Wilhelmina Hospital, Nijmegen; and the Rijnstate Hospital Arnhem-Velp, in The Netherlands will be asked to join this study.

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Patients must be 18 years old and above.
- Patients must be able to adhere to all requirements of the study.
- Patients must be willing to give written informed consent.
- There must be a clinical diagnosed or clinical suspicion of a basal cell carcinoma.

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Patients who currently participates in another investigational research or has been in the previous 28 days before the study.
- Patients having a medical condition which excludes participating in the study, according to the investigator.
- Incapacitated subjects.
- Subjects with lesion(s) on parts of the body that do not allow adequate imaging of the tumour with RCM.

When a patient meets these criteria and has given informed consent, he or she will be assigned to a randomisation arm according to a computer-generated block randomisation using Castor EDC, CIWIT B.V., Amsterdam, The Netherlands (Figure 1).

Power calculation

The primary outcome in this study is the percentage of correctly identified BCC subtypes after excision (gold standard in this study). We assume that this is 71% when a biopsy is used and 85% when RCM is used (based on an ongoing study). In this case, 148 patients are needed per group to obtain a power of 80% (Fisher-exact, two-sided, alpha=0.05). We expect that 10% of the patients with a clinically suspected BCC will not have a histopathologically confirmed BCC. Therefore, we will include approximately 329 patients with a clinical suspicion of BCC. In this multicenter randomised controlled trial (RCT), it is also possible to obtain empirical estimates on the (cost-)effectiveness in the daily clinical practice. The expected benefit of the RCM is anticipated at 92 euro per patient. Based on a conservative choice of the SD of 100 euro and the CI of 95%, 146 patients per group are required. Counting a 10% possible dropouts, around

322 patients need to be included. To answer both questions, a total of 329 patients with a clinical suspected BCC will be included in this study.

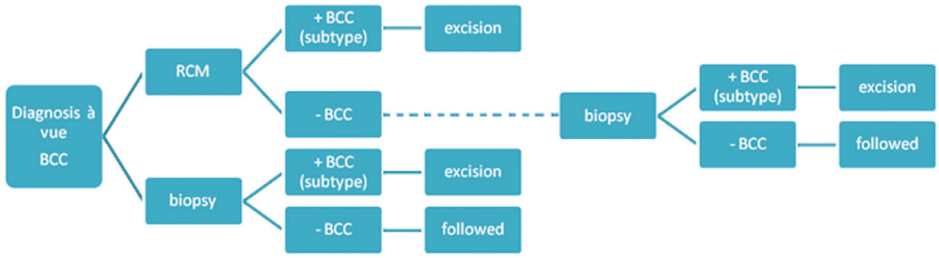

Figure 1: Randomisation procedure. Two randomisation arms are designed. After inclusion, a patient with a clinical diagnosis of BCC (diagnosis à vue) will be randomised over two arms. One arm contains the standard procedure of a skin punch biopsy, the other arm contains the diagnostic tool to be investigated, RCM. A punch biopsy will also be obtained when there is no suspicion of a BCC using RCM.

Outcome measures

The primary outcome measure is defined as correct subtyping of the BCC after excision. The histopathological diagnosis of the excision specimen will be compared to the diagnosis made by RCM or by punch biopsy. Secondary outcome measures are QoL, cost and QALY's.

Procedure

Patients will be assigned to either the RCM or the biopsy study arm (Figure 1). When a BCC is diagnosed using RCM or a punch biopsy, surgical excision will follow according to standard care time schedule at the centre where the BCC is diagnosed with margins according to the Dutch guidelines (3 mm for sBCC, 5 mm for aggressive BCC subtypes). In case of a BCC diagnosis, a follow-up visit will be planned 3 months after surgery. In the absence of a BCC diagnosis, based on either RCM or a punch biopsy, the patients will again be followed up after 3 months. During visit 1 (diagnostic procedure) several questionnaires will be filled (EQ-5D, EQ-5d VAS, iMTA PCQ and TSQM-9). At the follow-up visit (after treatment), the questions about satisfaction of the diagnostic procedure will be asked again. In order to establish the added monetary value of RCM, a contingent valuation method (CVM) was used. Patients that belong to the RCM arm but also had a punch biopsy, and in which both times no BCC was observed, were interviewed according to the CVM.

RCM will be performed using the commercially available Vivascope 1500 (Caliber Imaging & Diagnostics, Rochester, NY, USA) according to a standardised protocol. Vivablocks of 4 x 4 mm will be made at the level of the stratum corneum, stratum spinosum, dermo-epidermal junction and dermis in order to find RCM features for BCC and the subtype. Vivastacks will be made in

the areas of interest. Movies will be made to document vascularisation. When indicated, the Vivascope 3000 handheld device will be used. The RCM user is working for 4 years with the device. If a punch biopsy needs to be obtained according to the randomisation scheme, this will occur after local anaesthesia (1% Xylocaine/Adrenaline) and the punch biopsy will have a diameter of 3 mm. The punch biopsy will be taken from the most clinically suspected area of the lesion.

Statistical analysis

After data collection, statistical analyses will be performed. The Fisher's-exacts test will be used to test the differences in the primary outcome between the two study arms (punch biopsy, RCM) for statistical significance. Multivariable logistic regression will be used to study possible differences between the subtypes and the effects of possible other variables. This will be performed in order to evaluate variables or sets of variables for its discriminative character that can be used to develop protocols and guidelines for future RCM users in the Netherlands.

Cost-effectiveness analysis

The cost analysis comprises two main parts. First, on patient level, volumes of care will be measured prospectively over the time path of the clinical trial using the iMCQ (a generic instrument for measuring medical costs [20]) complemented with procedure specific cost information like cost of RCM equipment and patient out-of-pocket expenses, such as over-the-counter drugs (e.g. pain related). Relevant, (missing) entries will be verified or completed by data from the medical records or inpatient treatment facility's administration system. Second, per modality (RCM or usual care) standard cost prices will be determined using the Dutch guideline [21] or else real/full cost prices via activity-based costing. Productivity losses will be estimated using a patient-based questionnaire. [22] The friction cost-method will be applied following the Dutch guidelines.[21]

Patient outcome analysis

The effect analysis adheres to the design of an equivalent RCT and measures diagnostic performance and QoL at baseline and at fixed points along the follow-up of the RCT. To measure the quality of the health status of the patients, a validated so-called health-related quality of life (HRQoL) instrument will be used, the EuroQol-5D-3L (EQ-5D).[23] This HRQoL instrument will be completed by the patients and is available in a validated Dutch translation.[24] The EQ-5D is a generic HRQoL instrument comprising five domains: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. The EQ-5D index is obtained by applying predetermined weights to the five domains. This index gives a societal-based global quantification of the patient's health status on a scale ranging from 0 (death) to 1 (perfect health). Patients will also be asked to rate their overall HRQoL on a visual analogue scale (EQ- 5D VAS) consisting of a line ranging from 0 (worst imaginable health status) to 100 (best imaginable). The patient outcome

analysis will be complemented with a CVM questionnaire and measures of satisfaction and pain related to diagnosing subtype BCC.

Results

This investigator initiated RCT is conducted according to the principles of the Declaration of Helsinki (2013) and in accordance with the medical Research Involving Human Subjects Act (WMO). The study is funded by ZonMw, a Dutch organisation that finances health science, and thereby, stimulates the use of obtained knowledge to improve healthcare. The medical ethics committee approved the study protocol in December 2015 (NL 54549.091.15). The study will start in January 2016, and is expected to finish at the end of 2018. This trial had also been registered at ClinicalTrails.gov (nr: NCT02623101).

Discussion

Considering the increasing skin cancer problem, including BCC, and the disadvantages of the current diagnostic gold standard, histopathological diagnosis of a punch biopsy, there is a need for more cost- and time-efficient diagnostic tools with a high accuracy for diagnosing skin malignancies. These tools should be able to distinct between different skin cancer types and should be able to determine the correct BCC subtype, as different subtypes of BCCs are treated differently. Punch biopsies often result in sampling errors, as only a small part of the tumour is investigated, resulting in potentially inappropriate chosen therapies. As a sample error may lead to treatment failures or recurrences, other subsequent treatments are needed. This will eventually lead to increasing costs. In addition, the conventional method is unfriendly for patients, as it is invasive, painful and might result in scarring. Furthermore, the diagnosis cannot be made instantly.

To contribute to implementation of RCM as a non-invasive skin cancer diagnostic tool, this study will investigate whether RCM is at least as good in identifying BCC subtypes as conventional histopathological investigation of skin biopsies. Hypothesising that RCM is a cost-effective alternative to the present care, it saves on direct medical consumption like labour of the pathologist and other medical personnel as well as materials related to treatment failure with at least equal effectiveness.

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4.1

The value of (video)dermoscopy in the diagnosis and monitoring of common inflammatory skin diseases: a systematic review

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Submitted

Abstract

Clinical diagnosis of inflammatory skin disorders (ISD), including hair and nail disorders, is not always straightforward. Not uncommonly, a punch biopsy is required. Dermoscopy and videodermoscopy (VD) are non-invasive techniques that are used for *in vivo* examination of the skin, hair and nails. Both techniques can contribute to determining the accurate diagnosis and can be promising in assessing the treatment effects. We systematically searched and reviewed the current published literature on ISD evaluated by dermoscopy and VD in the electronic databases PubMed, Embase, Cochrane Library and Web of Science. All studies were assessed for their quality using the Strengthening the Reporting of Observational studies in Epidemiology and Cochrane checklist. Finally, 82 studies were eligible for inclusion. An overview is presented of the (video)dermoscopic features for common ISD diagnoses, with the corresponding accuracies, and monitoring features for treatment effects. Although, (video)dermoscopy is a promising technique, studies of high methodological quality are necessary to evaluate the value of VD over conventional dermoscopy in common ISD.

Introduction

The need for more objective evaluation of the skin within the dermatology is increasing. Dermoscopy is a simple and affordable non-invasive imaging technique that suits the need for more objective dermatological features. It generally allows 10x magnification, offering the possibility to assess skin structures in more detail and is gaining increasing importance in the diagnosis and monitoring of skin diseases. The many improvements in digital camera and computer technology between 1980 and 2000 have led to the development of digital VD.[1] VD is performed by a video camera equipped with optic fibers and lenses that allow high-resolution imaging at magnifications up to 1000x. The images obtained are visualised on a monitor and can directly be stored digitally, to identify and compare changes over time.[2] VD allows higher magnification, compared to conventional dermoscopy, which potentially offers visualisation of more specific features. However, there is a lack of studies comparing these two techniques.[3,4]

Currently, dermoscopy is used more often in the daily clinical practice than VD, mainly in the evaluation of neoplastic lesions, including melanoma and non-melanoma skin cancers.[5,6] In contrast, inflammatory skin diseases (ISD), including hair and nail disorders, have been studied less frequently by dermoscopy. Moreover, there is scarce knowledge on the possible additional value of VD, compared to dermoscopy.

Clinical diagnosis of ISD can sometimes be difficult. In these cases histopathology can contribute to the accurate diagnosis. However, biopsy is an invasive method with risks of developing local inflammatory reactions and scar tissue with a possibility of sampling errors and the inability to follow dynamic processes over time. Furthermore, a biopsy is unfavourable in the facial area in which ISD are often localised.[7] Therefore, in general, the clinical diagnosis of ISD is the leading factor in many cases. This means that diagnostic uncertainty is often accepted, resulting in therapeutic uncertainty. Dermoscopy has shown to aid in assessing the clinical diagnosis of ISD.[8] Moreover, dermoscopy might be promising in assessing the treatment effects.[2] As dermoscopy and VD are both non-invasive and patient friendly techniques, they are preferred over invasive methods. This makes them interesting techniques for daily use in the clinical setting.

A systematic review on both VD and dermoscopy is essential for an objective evaluation of the current literature in order to facilitate the increasing use of both techniques in the daily clinical practice. Furthermore, the value of VD over conventional dermoscopy can be assessed. Therefore, current literature was evaluated for the following purposes:

i To present an overview of the most prevalent ISD, including hair and nail disorders, that can be evaluated by dermoscopy and/or VD.

ii To assess diagnostic and monitoring features of dermoscopy and VD, including the accuracy.

iii To evaluate the current value of VD over conventional dermoscopy in ISD.

Methods

A systematic literature review following the PRISMA statement checklist[9] was conducted in four electronic databases: PubMed, Embase, Cochrane Library and Web of Science. The search was based on dermoscopy, VD and a list of all relevant synonyms. ISD included a broad spectrum of diagnoses, requiring a systematic approach based on the classification scheme of inflammatory dermatological disorders by Billings *et al.*[10] The focus of this review lies in the applicability of dermoscopy and VD in the most prevalent ISD with a primary inflammatory component. Publications on oral and genital dermatoses, genodermatoses, vascular diseases, pigmentary diseases, infections and infestations, non-cicatricial alopecias or studies focusing exclusively on (video)dermoscopy of hair shafts were excluded. Publications on (video)capillaroscopy were also excluded, because this specific tool is solely used for the visualisation of the microcirculation whereas (video)dermoscopy can be used for viewing a broader range of features. We included several bullous dermatoses with a genetic origin and cicatricial alopecias.

The search was restricted to publications on humans and the English, German and Dutch language. All published studies until the date of 03.10.2016 were included (Supplementary table 1). After the initial search, titles, and when necessary abstracts and/or materials and methods were screened (MHEV). After this selection, two reviewers (MHEV and MP) independently screened titles and abstracts according to a checklist specifying inclusion and exclusion criteria. Where the abstract was unavailable, full-text articles were screened for specific criteria and if relevant retrieved for further evaluation. Subsequently, the full texts were critically assessed for their eligibility by two reviewers (MHEV and MP). Differences in assessment between the two reviewers were either resolved by consensus or discussed with a third reviewer (KPN). Studies had to fulfill the following three criteria for inclusion: (i) the use of (video)dermoscopy; (ii) description of ISD features and (iii) description of (video)dermoscopy features. Abstracts with unavailable full texts, articles concerning skin diseases different from ISD according to Billings *et al*.[10] and papers regarding other imaging techniques were excluded. Excluded papers were reviews, editorials, opinions, *ex vivo* or animal studies. To ensure all relevant studies were included, the reference lists of all included articles for the full text screening were checked for additional relevant studies not identified by the initial search.

The final selected articles were evaluated for the following study characteristics; type of ISD, study design, number of participants, number and localisation of evaluated lesions, aim of the study, used technique (dermoscopy or VD), (video)dermoscopic features, their accuracy and the reference test to (video)dermoscopy.

Methodological quality of the included studies was assessed using the STrenghtening the Reporting of OBservational studies in Epidemiology (STROBE) criteria.[11] This scale was modified to assess the quality of case reports and case series by exclusion of the terms 'variables' and 'study size'. Studies are classified by three categories. Category A was defined as fulfilling $\geq$ 80% of the STROBE criteria, category B as 50-80% and category C as $<$ 50% of the STROBE criteria. Randomised controlled trials (RCTs) could not be evaluated using the STROBE checklist, and were assessed using the Cochrane checklist.

Results

In total, 13.978 articles were found after the literature search (Figure 1). This total consisted of 9489 unique titles that were assessed for further screening. The first screening process identified 983 articles concerning *in vivo* (video)dermoscopy of the skin. The screening of the two reviewers showed an overlap of 98% and resulted in the inclusion of 193 articles that were eligible for full-text screening. Twenty-four full texts were unavailable and 87 articles did not met the inclusion criteria. The full-text screening had an overlap of 100% between the two reviewers. The additional check of the reference lists of the included studies did not result in the selection of additional valuable articles.

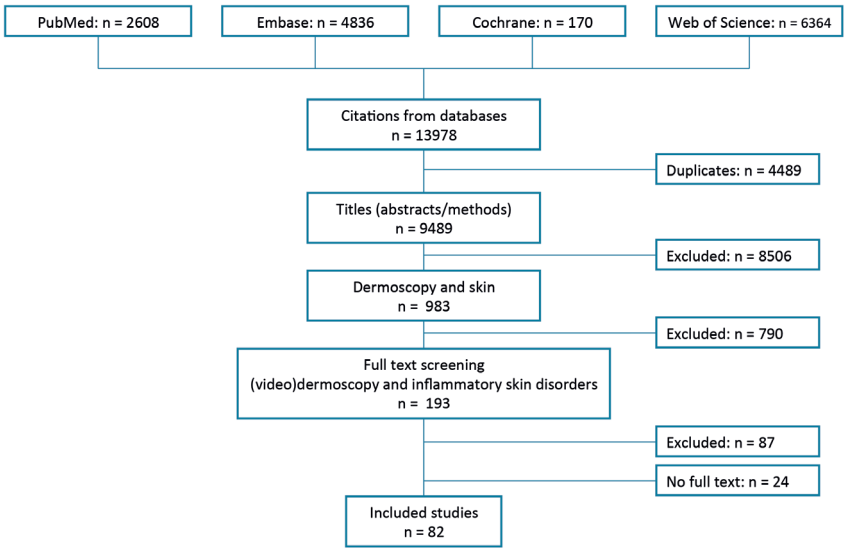

Figure 1. Flow chart: selection process of included studies.

Eventually, 82 publications were included. Table 1 provides an overview of the ISD categories and number of included studies. Ten studies were included in several categories as they described multiple inflammatory diagnoses.[7,12-20] There were 12 articles on videodermoscopy (Table

2)[12,16,18,21-29], 68 articles concerning dermoscopy (Table 3)[7,13-15,17,19,20,30-90] and 2 studies using both dermoscopy and VD (Table 2 and 3).[91,92] Four studies presented monitoring features[23,39,58,63], whereas 73 articles had a diagnostic aim.[7,12-22,24-28,30-38,40-57,59-62,64-71,73-82,84-86,88,90-92] Five articles described both diagnostic and monitoring features.[29,72,83,87,89]

Table 1. Category and number of included studies

Spongiotic dermatitis (n = 11)*
Pityriasis rosea[17,19,56] Various forms of eczematous/erythematous dermatitis (allergic and contact dermatitis, atopic dermatitis, seborrheic dermatitis, chronic hand eczema, drug rashes, erythema multiforme and annular erythema).[7,13-19,57,58]
Psoriasiform dermatitis (n = 24)*
Psoriasis[12-21,23,25,29,52,55,59-63,92] Pityriasis rubra pilaris[61,64] Prurigo nodularis[19,65] Lichen simplex chronicus[66]
Interface dermatitis (n = 11)*
- Lupus erythematosus (cutaneous) and lichen planus[12,14,17,19,20,67-72]
Perivascular dermatitis (n = 2)*
- Common urticaria[19,30]
Nodular and diffuse dermatitis (n=4)*
- Sarcoidosis (cutaneous) [7,19,31,32]
Pallisading granulomatous dermatitis (n = 5)*
- Granuloma annulare and necrobiosis lipoidica[19,33-36]
Sclerosing dermatitis (n=16)*
Morphea/scleroderma (systemic sclerosis) [19,28,37-44,53,54] Lupus (systemic lupus erythematosus) [19,38]
- Lichen sclerosus (extragenital) [43-48]
Bullous dermatitis (n = 8)
- Darier's disease, dermatitis herpetiformis, pemphigus and pemphigoid and variants, Grover's disease[19,26,49,50,73-75,90]
Alopecia (cicatricial) (n = 20)*
- Frontal fibrosing alopecia, discoid lupus erythematosus, lichen planopilaris, central centrifugal cicatricial alopecia, folliculitis decalvans, alopecia mucinosa, pseudopelade of Brocq.[7,18,22,24,27,51,76-88,91]
Miscellaneous inflammatory and reactive disorders (n = 2)*
- Rosacea[7,89]
Total n = 82

Most included studies in this review were case reports (n=25) and case series (n=15), followed by cross-sectional studies (n=35), cohort studies (n=4), case-control studies (n=2) and RCT (n=1). The methodological quality assessment identified that 72% (59/82) studies were classified as category C and the remaining 28% (23/82) were classified as category B (Table 2 and 3). The majority of the studies lacked description of the setting, variables, potential sources of bias, description of statistical methods and generalisability. We included one RCT that had

a moderate quality according to the Cochrane checklist. An overview of the most frequently described features of all included ISD is given in Table 4. Because of the extensive spectrum of imaged ISD, four common ISD, of which the (video)dermoscopic accuracy was available, will be discussed in detail.

Plaque type psoriasis

Eight studies revealed VD features for diagnostic and monitoring applications in psoriasis.[12,16,18,21,23,25,29,92] The most observed VD features were arborising, torturous, glomeruli-like or 'bushy' capillaries.[12,16,18,23,25,29] The majority of the studies (n=13) used dermoscopy to evaluate psoriasis.[13-15,17,19,20,52,55,59-63] Using dermoscopy, the most observed vascular features were homogeneous red dots and globules, red lines and twisted red loops. Although present in every psoriatic plaque, red dots are not a specific dermoscopic feature for psoriasis, as they can also be found in other papulosquamous dermatoses. However, the uniform, symmetrical, regular distribution of these red dots throughout the lesion is suggestive for psoriasis.[17] When the presence of marked hyperkeratosis impedes the view of underlying dermoscopic features, scale removal may be useful to display the above-mentioned vascular pattern and possible tiny red blood drops ("Auspitz sign").[62] Vazquez-Lopez *et al*.[20] showed a 100% sensitivity for the feature red globules in the differentiation of plaque psoriasis (PP) and lichen planus (LP). Pan *et al*.[55] introduced a dermoscopic diagnostic model for the differentiation of solitary psoriatic plaques from intra-epidermal carcinoma and superficial basal cell carcinoma. They concluded that red dots, homogeneous vascular pattern and light red background were significant dermoscopic features for psoriasis, yielding a diagnostic probability of 99% if all three features were present. Lallas *et al.*[17] showed a diagnostic sensitivity of 84,9% and specificity of 88,0% for the combination of regularly distributed dotted vessels over a light-red background associated with diffuse white scales. Several studies showed the monitoring capacity of (video)dermoscopy to examine therapeutic effect.[23,29,63] Overall, both dermoscopy and VD can be considered as a diagnostic and monitoring tool in psoriasis.

Lichen planus

Videodermoscopic imaging of LP was described in one study[12], whereas 9 studies used conventional dermoscopy.[14,17,19,20,67,68,70-72] With VD, wickham's striae can be observed.[12] The most described dermoscopic features for LP include radial capillaries or red lines and Wickham's striae. Wickham's striae are described as round, linear, reticular or annular pearly-whitish structures and may develop thin or broad arboriform projections, surrounded by dotted or linear vessels that highlight them.[17,71] Wickham's striae correspond histopathologically to compact orthokeratosis above the zones of wedge-shaped hypergranulosis.[20] Vazquez-Lopez *et al*.[20] showed a 92% sensitivity of Wickham's striae in the diagnostic differentiation of LP and PP. Furthermore, dermoscopy is able to discriminate between two types of hyperpigmentation in LP: a brownish diffuse pattern, that is probably related to pigmentation in the epidermis, and a

deeper grey blue dotted pattern, which corresponds to pigment in dermal melanophages.[12,72] These features have potential prognostic value, as lesions with the largest numbers of blue dots seem to be more persistant.[72]

Systemic sclerosis

Dermoscopy and VD are used to visualise vascular features of systemic sclerosus (SSc) in the nailfolds. Seven studies of low to moderate methodological quality were included, of which one research group used VD to evaluate SSc.[28,37,38,40,42,53,54] Videodermoscopy showed dilated capillaries, nailfold bleeding and avascular areas.[28] The most important dermoscopic features are similar to the features observed by VD. These findings confirm clinical suspicion of SSc justifying further investigation. Muroi *et al*.[54] revealed a sensitivity and specificity of resp. 83,1% and 100% for the presence of two or more enlarged capillaries in one or more fingers. Ohtsuka *et al*.[28] showed that the distribution of dilated capillaries and/or nail fold bleeding in SSc was significantly elevated compared to the normal controls, with a sensitivity of 40.8% and specificity of 93.5% respectively. Mazzotti *et al*.[53] showed a sensitivity and specificity for the scleroderma pattern (occurrence of avascular areas and/or the presence of dilated capillaries) using dermoscopy. The authors also concluded that these results are comparable to traditional capillaroscopy. In general, dermoscopy allows a fast and feasible recognition of vascular abnormalities of the nail fold. Moreover, it offers a promising alternative to more expensive and complicated (video) capillaroscopy that is not widely available.[42,53]

Cicatricial alopecia

Dermoscopy and VD are widely used in the evaluation of hair and scalp disorders. Five studies described VD features for different primary cicatrical alopecias (PCA).[18,22,24,27,91] Sixteen studies used dermoscopy to evaluate PCA.[7,24,51,76,78-88,91] The main VD and dermoscopic feature of cicatricial alopecia is the reduction or absence of hair follicles.[18,22,51,80,81,84-88,91] Abedini *et al*.[51] showed that the presence of non-follicular red dots, enlarged and tortuous branching vessels, follicular keratotic plugging, speckled pigmentation, perifolliculair erythema, peripilar white halo, and hair tufting had 100% specificity for the diagnosis of PCA. There are (video)dermoscopic features that may help dermatologists to differentiate between non-cicatricial alopecia and PCA.[18,51,80] Furthermore, (video)dermoscopy shows promising features in the monitoring of treatment effects in scalp disorders.[87,93]

Value of videodermoscopy (VD)

With VD, more specific and detailed features can be visualised compared to dermoscopy due to the higher magnification. In psoriatic lesions, low magnification (10x) by dermoscopy shows the presence of a red-dotted pattern. However, using VD with a higher magnification (100-400x), typical elongated and convoluted 'bushy' capillaries can be detected that are homogeneously distributed within the plaque.[16,25] Unlike dermoscopy, VD was also able to visualise dilated,

tortuous, elongated, and irregular distributed capillaries in the hyponychium of nail psoriasis patients.[29] After treatment, a significant reduction in the number of visible capillaries was seen with the VD. Therefore, VD can also be used to monitor treatment effects.

Discussion

Dermoscopy is a well-established technique used within dermatology, mainly in dermato-oncology. However, its indications has expanded to include e.g. scalp and hair diseases[93,94], nail and nail fold abnormalities[95,96] and inflammatory and infectious skin diseases.[8,97,98] Videodermoscopy is relatively 'modern' in this field with multiple benefits over 'common' dermoscopy and therefore gaining more interest. (Video)dermoscopic features of various ISD are widely described and, in a lesser degree, they are used to describe the follow up of treatments. However, the spectrum of ISD is extensive. Therefore, there is a need for a systematic review to address the current value and applications of both VD and dermoscopy in common ISD.

This review presents the broad application of (video)dermoscopy in common ISD. However, the methodological quality of the included studies is relatively low. There were 9 studies describing the sensitivity and specificity of dermoscopic features in diagnosing ISD. They show that dermoscopy is promising in aiding the clinical diagnosis of ISD. An advantage of VD is the availability of multiple magnifications, which makes it a promising technique to evaluate more specific and detailed features compared to dermoscopy.[16,25,29] Though, more studies of high methodological quality on VD is recommended to determine its value over conventional dermoscopy. These studies should also address attention to the interobserver variability, and level of training, which is currently sparsely reported [37,42]

Although costs of VD are higher compared to dermoscopy, the use of this technique might contribute to standardisation of dermoscopic imaging. Compared to dermoscopy, VD is less influenced by external factors, such as environmental light. Colours can be calibrated, maintaining the same environmental conditions which can lead to obtaining more reproducible images.[99] This can enhance the clinical judgment, in particular in the monitoring of lesions.

Recent development has led to development of software programs that quantify geometric and colorimetric parameters of videodermoscopic images. They introduce an objective element to the diagnosing making process, and can therefore be an useful tool, particular for clinicians (dermatologist as well as general practitioners) with minimal training in dermoscopy.[100-102] Moreover, quantitative assessment, using computer programs, can be useful in the monitor of treatment effects.[29] Using VD, the skin images are directly visualised on a monitor which can be used as a didactic modality to patients and students, aiding in the explanation of skin diseases to others. Moreover, VD can overcome the embarrassment that may occur in some cases during

dermoscopic examination when there is close contact between the dermoscopist's head and patient's skin surface.

In conclusion, this systematic review provides an overview of ISD imaged by (video)dermoscopy. (Video)dermoscopy is a promising tool for diagnosing and monitoring common ISD. However, as dermatological imaging is developing quickly, it is important to evaluate the value of VD over conventional dermoscopy. Therefore, it is recommended to perform more research on VD or comparative studies on VD and dermoscopy in common ISD.

Table 2. Overview of the included studies per inflammatory skin disease category for videodermoscopy

First author + Year	Study design	STROBE category	Participants (n)	Evaluated lesions (n)	Location of evaluated lesions	Diagnosis	Technique	Aim (diagnostic or monitoring)	Dermoscopic features	Sens/spec features	Reference test
Spongiotic dermatitis											
Ross* 2006[18]	Cross-sectional	C	235: 23 psoriasis; 26 SD; 30 PCA; 15 control	-	Scalp	(scalp) psoriasis, SD, DLE, LPP, FD	VD x20-x70	Diagnostic: to (1) characterise features of several nontumoural scalp and hair conditions using videodermoscopy; and (2) assess the potential usefulness of videodermoscopy in the clinical evaluation of these conditions.	Psoriasis: simple and twisted red loops; arborising red lines; yellow scales SD: simple and twisted red loops; arborising red lines; yellow scales DLE: arborising red lines ; hyperkeratotic perifollicular white scales; absence of follicular ostia LPP: simple red loops; arborising red lines; honeycomb pigment pattern; white dots; hyperkeratotic perifollicular white scales; absence of follicular ostia FD: simple and twisted red loops; arborising red lines ; white dots; white scales; absence of follicular ostia	-	-
Lacarrubba* 2016[16]	Letter: cohort	C	205: 105 PP; 50 AD; 50 ACD/ICD	177 PP	58 arms; 55 trunk; 64 legs (psoriasis)	PP, ACD, ICD	VD x150	Diagnostic: to assess the prevalence of 'bushy' capillaries in lesional skin	PP: monomorphic, homogeneously distributed, 'bushy' capillaries AD, ACD, ICD: normal looking capillaries, slightly dilated capillaries and/or isolated 'bushy'capillaries	-	-

First author + Year	Study design	STROBE category	Participants (n)	Evaluated lesions (n)	Location of evaluated lesions	Diagnosis	Technique	Aim (diagnostic or monitoring)	Dermoscopic features	Sens/spec features	Reference test
Psoriasiform dermatitis											
Ross* 2006[18]	Cross-sectional	C	235: 23 psoriasis; 26 SD; 30 PCA; 15 control	-	Scalp	(scalp) psoriasis, SD, DLE, LPP, FD	VD x20-x70	Diagnostic: to (1) characterise features of several nontumoural scalp and hair conditions using videodermoscopy; and (2) assess the potential usefulness of videodermoscopy in the clinical evaluation of these conditions.	Psoriasis: simple and twisted red loops; arborising red lines; yellow scales SD: simple and twisted red loops; arborising red lines; yellow scales DLE: arborising red lines ; hyperkeratotic perifollicular white scales; absence of follicular ostia LPP: simple red loops; arborising red lines; honeycomb pigment pattern; white dots; hyperkeratotic perifollicular white scales; absence of follicular ostia FD: simple and twisted red loops; arborising red lines ; white dots; white scales; absence of follicular ostia	-	-

Iorizzo 2008[29]	Letter: cohort	C	30 nail psoriasis; 5 nail LP; 15 control	-	Nails	Nail psoriasis	VD x40	Diagnostic + monitoring: to evaluate the capillary network of the fingernail hyponychium in patients with nail bed psoriasis in an attempt to find a tool aiding clinicians in the diagnosis and follow-up of this disorder	Nail psoriasis: dilated, tortuous, elongated and irregulary distributed capillaries of hyponychium; correlation between capillary density and disease severity LP: no visible capillaries Control: visible capillaries, limited to second, third and fourth digit of dominant hand (regulary distributed, reduced in number and less tortuous) After 3 month treatment: significant decrease in number of visible capillaries	-	-
Musumeci 2014[25]	Cross-sectional	C	60: 24 PP; 36 other erythemato-squamous disorders	124	-	PP	VD x150	Diagnostic: to assess the correlation between the vascular pattern evaluated using videodermatoscopy and the clinical diagnosis of psoriasis and other erythematous desquamative disorders	Bushy, homogenous capillaries;	-	-
Chandravathi* 2015[12]	Cross-sectional	B	80: 40 psoriasis; 40 LP	80	-	Psoriasis, LP	VD x20-x75	Diagnostic: to determine and compare dermoscopic patterns of PP and LP and correlate the dermoscopic images with histopathology of PP and LP in clinical difficult cases and compare the findings with previous studies	Psoriasis: red globules; glomeruli like vessels; Light red background; grey blue background; silvery white, greasy yellow or grey blue scales LP: wickham's striae; grey-blue dots; grey-blue background	-	Histopathology

First author + Year	Study design	STROBE category	Participants (n)	Evaluated lesions (n)	Location of evaluated lesions	Diagnosis	Technique	Aim (diagnostic or monitoring)	Dermoscopic features	Sens/spec features	Reference test
Kibar 2015[21]	Cross-sectional	C	243: 31 scalp psoriasis; 112 SD; 100 control	-	-	Scalp psoriasis, SD	VD	Diagnostic: to evaluate the trichoscopic figures that may help to differentiate scalp psoriasis and seborrheic dermatitis	Scalp psoriasis: Atypical red vessels; red dots; globules; signet ring vessels; structureless red areas; hidden hairs SD: twisted red loops; comma vessels	-	-
Yadav 2015[92]	Cross-sectional	C	68	-	Nails	Nail psoriasis	Dermoscopy x10 and VD	Diagnostic: to study the dermoscopic features of nails in patients of chronic plaque psoriasis	Irregular pits; onycholysis; dilated globose vessels at the onychodermal band; oil drop sign; splinter hemorrhages	-	-
Lacarrubba* 2016[16]	Letter: cohort	C	205: 105 PP; 50 AD; 50 ACD/ICD	177 PP	58 arms; 55 trunk; 64 legs (psoriasis)	PP, ACD, ICD	VD x150	Diagnostic: to assess the prevalence of 'bushy' capillaries in lesional skin	PP: monomorphic, homogeneously distributed, 'bushy' capillaries AD, ACD, ICD: normal looking capillaries, slightly dilated capillaries and/or isolated 'bushy'capillaries	-	-
Micali 2016[23]	Cohort	B	42	42	-	PP	VD x150	Monitoring: to evaluate changes in vascular pattern using VD, skin thickness by ultrasound, along with clinical observation, during treatment with biologicals	Baseline: comparable diameters of capillary bushes 60 days: reduction in diameter of capillary bushes in all groups	-	Ultrasound

Interface dermatitis											
Chandravathi* 2015[12]	Cross-sectional	B	80: 40 psoriasis; 40 LP	80	-	Psoriasis, LP	VD x20-x75	Diagnostic: to determine and compare dermoscopic patterns of PP and LP and correlate the dermoscopic images with histopathology of PP and LP in clinical difficult cases and compare the findings with previous studies	Psoriasis: red globules; glomeruli like vessels; Light red background; grey blue background; silvery white, greasy yellow or grey blue scales LP: wickham's striae; grey-blue dots; grey-blue background	-	Histopathology
Sclerosing dermatitis											
Ohtsuka 2012[28]	Cross-sectional	C	118: 62 SSc; 56 control	-	Dorsum of the fourth finger	SSc	VD x40	Diagnosis: to find the distribution of nail fold capillary abnormality in SSc	Dilated capillaries; nail fold bleeding; avascular areas	+	Histopathology
Bullous dermatitis											
Sar-Pomian 2014[26]	Case series	C	19: 9 pemphigus vulgaris; 10 pemphigus foliaceus	-	Scalp	pemphigus vulgaris, pemphigus foliaceus	VD x20 and x70	Diagnostic: to analyse whether trichoscopy may be useful in aiding differential diagnosis of scalp lesions in patients with pemphigus vulgaris and pemphigus foliaceus	Pemphigus vulgaris: extravasations; yellow hemorrhagic crusts; linear serpentine vessels; lace-like vessels; linear helical vessels, glomerular vessels and dotted vessels with whitish halo Pemphigus foliaceus: extravasations; yellow hemorrhagic crusts; linear serpentine vessels; linear helical vessels; glomerular vessels; white diffuse scaling; white polygonal structures; yellow diffuse scaling; tubular perifollicular scaling and hair casts; yellow dots with whitish halo	-	-

First author + Year	Study design	STROBE category	Participants (n)	Evaluated lesions (n)	Location of evaluated lesions	Diagnosis	Technique	Aim (diagnostic or monitoring)	Dermoscopic features	Sens/spec features	Reference test
Alopecia (cicatricial)											
Ross* 2006[18]	Cross-sectional	C	235: 23 psoriasis; 26 SD; 30 PCA; 15 control	-	Scalp	(scalp) psoriasis, SD, DLE, LPP, FD	VD x20-x70	Diagnostic: to (1) characterise features of several nontumoural scalp and hair conditions using videodermoscopy; and (2) assess the potential usefulness of videodermoscopy in the clinical evaluation of these conditions.	Psoriasis: simple and twisted red loops; arborising red lines; yellow scales SD: simple and twisted red loops; arborising red lines; yellow scales DLE: arborising red lines ; hyperkeratotic perifollicular white scales; absence of follicular ostia LPP: simple red loops; arborising red lines; honeycomb pigment pattern; white dots; hyperkeratotic perifollicular white scales; absence of follicular ostia FD: simple and twisted red loops; arborising red lines ; white dots; white scales; absence of follicular ostia	-	-
Tosti 2009[27]	Case series	C	5	-	Scalp	Scalp DLE	VD x20 to x70	Diagnostic: to describe morphologic and pathologic features of a new dermoscopic pattern	Follicular red dots	-	Histopathology

Duque-Estrada 2010[91]	Cross-sectional	C	14: 4 LPP; 5 FFA; 5 DLE	-	Scalp	DLE, LPP, FFA	VD and dermoscopy x10	Diagnostic: to describe dermoscopic findings in patients with clinical and histopathological characteristics of cicatricial alopecia	DLE: white patches, branching capillaries, keratin plugs and areas of reduced follicular ostia LPP: perifollicular scales, white dots; reduced follicular ostia FFA: reduced follicular ostia, perifollicular scales, perifollicular erythema; branching capillaries	-	-
Lacarrubba 2013[22]	Letter: cross-sectional	C	34	-	Scalp	FFA	VD x20 to x100	Diagnostic: to evaluate the videodermoscopic features of FFA	Perifollicular scaling; absence of follicular openings; brown halos/ white dots; absence of vellus hairs; pili torti; black dots/broken hairs	-	-
Miteva 2014[24]	Cross-sectional	B	51	153: each patient 3 images	Posterior; middle; anterior part of affected area on scalp	CCCA	VD x20	Diagnostic: to establish the spectrum of dermoscopic features and their frequency in CCCA	Honeycomb pigmented network; peripilar white/ gray halo; erythema; white patches; pin-point white dots; broken hairs; asterisk-like brown blotches; hair shaft variability; scales	+	Histopathology

* Studies that were included in multiple categories as they evaluated multiple diagnoses; ACD, allergic contact dermatitis; AD, atopic dermatitis; AM, alopecia mucinosa; CCCA, central centrifugal cicatricial alopecia; CHE, chronic hand eczema; CLE, chronic lupus erythematosus; CM, cutaneous mastocytosis; CS, cutaneous sarcoidosis; CU, common urticaria; DC, dissecting cellulitis; DLE, discoid lupus erythematosus; ER, erythematoteleangiectatic rosacea; FD, folliculitis decalvans; FFA, frontal fibrosing alopecia; GA, granuloma annulare; GF, granuloma faciale; GP, gutatte psoriasis; ICD, irritant contact dermatitis; LE, lupus erythematosus; LP, lichen planus; LPP, lichen planopilaris; LS, lichen sclerosus; LSC, lichen simplex chronicus; LV, lupus vulgaris; NL, necrobiosis lipoidica; NPCD, nonpolarised light contact dermoscopy; NVC, nailfold videocapillaroscopy; PCA, primary cicatricial alopecia; PLC, pityriasis lichenoides chronic; PN, prurigo nodularis; PNCD, polarised light noncontact dermoscopy; PP, plaque psoriasis; PR, pityriasis rosea; PRP, pityriasis rubra pilaris; RCM, reflectance confocal microscopy; SD, seborrhoeic dermatitis; SLE, systemic lupus erythematosus; SNFC, stereomicroscope nailfold capillaroscopy; SSc, systemic sclerosis; TMEP, teleangiectasia macularis eruptive perstans; UP, urticaria pigmentosa; VCAP, videocapillaroscopy; VD, videodermoscopy.

Table 3. Overview of the included studies per inflammatory skin disease category for dermoscopy

First author + Year	Study design	STROBE category	Participants (n)	Evaluated lesions (n)	Location of evaluated lesions	Diagnosis	Technique	Aim (diagnostic or monitoring)	Dermoscopic features	Sens/spec features	Reference test
Spongiotic dermatitis											
Chuh 2001[56]	Case series	C	3: 1 PR; 2 control	-	-	PR	Dermoscopy	Diagnostic: to present the role of digital epiluminescene dermoscopy in demonstrating collarette scaling	Collarette scaling	-	-
Vazquez-Lopez, Kreusch* 2004[19]	Cross-sectional	C	414	-	-	Psoriasis, PP, LP, eczema, PR, PN, CU, erythema multiforme, drug rashes; annular erythema, LE morphea, pemphigus, pemphigoid, sarcoidosis, NL, GA	Dermoscopy x10	Diagnostic: to evaluate and classify the dermoscopic vascular structures seen in nontumoural dermatoses	Psoriasis, spongiotic psoriasiform dermatitis: homogeneous red globules Erythema multiforme, drug rashes, CU, erythema annulare, morphea, NL, LP, LE, sarcoidosis: homogeneous red lines Drug rashes, PR, LP, LE, scaling spongiotic dermatitis: mixed vascular findings PP: red globules-rings LP: radial capillaries Advanced lesions LP: mixed vascular/ pigmented features	-	-

Murrell 2007[58]	RCT		200	-	-	AD	Dermoscopy	Monitoring: to assess the efficacy and safety of pimecrolimus cream 1% in patients with AD of the face and neck who are either dependent on, or intolerant of, TCS	Baseline: more skin atrophy and telangiectasia present in pimecrolimus group compared to vehicle arm Week 6: significant improvement of skin atrophy and teleagniectasia pimecrolimus compared to vehicle arm. Non-significant improvement of teleangiectasia.	-	-
Kim* 2011[15]	Cross-sectional	B	96: 55 scalp psoriasis; 41 SD	96	Scalp	Scalp psoriasis, SD	Dermoscopy x10	Diagnostic: to evaluate the usefulness of dermoscopy in the clinical differentiation of scalp psoriasis and seborrhoeic dermatitis	Scalp psoriasis: scales; red dots and globules; twisted red loops; glomerular vessels SD: scales; arborising vessels; atypical red vessels; featureless areas	-	-
Lallas, Kyrgidis* 2012[17]	Cross-sectional	B	169: 83 PP; 41 dermatitis; 25 LP; 20 PR	169	-	PP, dermatitis, LP, PR	Dermoscopy x10	Diagnostic: to determine and compare the dermoscopic patterns associated with PP, dermatitis, LP and PR and to assess the validity of certain dermoscopic criteria in the diagnosis of PP	PP: dotted vessels in regular arrangement over light red background and white scales; dermatitis: yellow scales and dotted vessels in patchy arrangement; PR yellowish background, dotted vessels and peripheral scales; LP wickham striae	+	Histopathology

First author + Year	Study design	STROBE category	Participants (n)	Evaluated lesions (n)	Location of evaluated lesions	Diagnosis	Technique	Aim (diagnostic or monitoring)	Dermoscopic features	Sens/spec features	Reference test
Lallas, Argenziano, Apalla* 2014[7]	Case series	B	115	115	-	SD, ER, sarcoidosis, DLE	Dermoscopy x10	Diagnostic: to describe and compare the dermoscopic patterns of common facial inflammatory skin diseases including SD, ER, sarcoidosis, LV, DLE and GF	SD: dotted vessels in patchy distribution; yellow scales ER: linear vessels in polygonal pattern Sarcoidosis: orange-yellowish structureless areas; linear, focused branching vessels DLE: perifollicular whitish halo; follicular keratotic plugs; white scales; linear branching vessels	-	-
Goncharova 2015[14]	Cross-sectional	B	74	74	-	PP, CLE, SD, LP	Dermoscopy x10	Diagnostic: to correlate dermoscopic findings with histopathologic reaction patterns of inflammatory dermatoses	Psoriasiform reaction pattern: intense red background; mostly regulary distributed red dots and globules Lichenoid reaction pattern: dull-red background; red lines, dots and globules; coma-shaped vessels Spongiotic reaction pattern: fading or light red background; regular or irregular vascular dots	+	Histopathology

Errichetti , Stinco* 2016[13]	Cross-sectional	C	21: 10 palmar psoriasis; 11 CHE	-	Hands	Palmar psoriasis, CHE	Dermoscopy x10	Diagnostic: to investigate palmar psoriasis and CHE using dermoscopy and to assess significance of specific dermoscopic features in order to improve their non-invasive differentiation	Palmar psoriasis: white scales; erythematous background; diffuse or patchy distribution; dotted vessels CHE: focally distributed yellowish scales; brownish-orange dots and/or globules; erythematous background; yellowish-orange crusts; patchily distributed white scales; focally distributed dotted vessels	-	Histopathology
Errichetti, Pegolo 2016[57]	Case report	C	2	-	-	Morbilliform drug eruption	Dermoscopy x10	Diagnostic: to describe 2 cases of resp. acute generalised exanthematous pustulosis and exanthematous (morbilliform) drug eruption and correlate dermoscopic and histologic features	Dotted/linear irregular vessels; pinkish-reddish background	-	Histopathology
Psoriasiform dermatitis											
Vazquez-Lopez, Manjon-Haces* 2003[20]	Cross-sectional	C	45: 25 LP; 20 PP	-	-	LP, PP	Dermoscopy x10	Diagnostic: dermoscopic features of PP and LP are investigated to determine both vascular and nonvascular features	LP: whitish striae, gray-blue dots; comedo; milium-like cysts; red lines PP: homogenous red globules	+	Histopathology

First author + Year	Study design	STROBE category	Participants (n)	Evaluated lesions (n)	Location of evaluated lesions	Diagnosis	Technique	Aim (diagnostic or monitoring)	Dermoscopic features	Sens/spec features	Reference test
Vazquez-Lopez, Kreusch* 2004[19]	Cross-sectional	C	414	-	-	Psoriasis, PP, LP, eczema, PR, PN, CU, erythema multiforme, drug rashes; annular erythema, LE morphea, pemphigus, pemphigoid, sarcoidosis, NL, GA	Dermoscopy x10	Diagnostic: to evaluate and classify the dermoscopic vascular structures seen in nontumoural dermatoses	Psoriasis, spongiotic psoriasiform dermatitis: homogeneous red globules Erythema multiforme, drug rashes, CU, erythema annulare, morphea, NL, LP, LE, sarcoidosis: homogeneous red lines Drug rashes, PR, LP, LE, scaling spongiotic dermatitis: mixed vascular findings PP: red globules-rings LP: radial capillaries Advanced lesions LP: mixed vascular/ pigmented features	-	-
Vazquez-Lopez, Marghoob 2004[63]	Cohort	C	20	20	-	Chronic PP	Dermoscopy x10	Monitoring: to investigate the value of the dermoscope for monitoring the long term safety of high potency topical steroids in patients with chronic psoriasis	Baseline: red globules, white areas End of study: red globules, red lines	-	-
Pan 2008[55]	Cross-sectional	B	225	300: 100 psoriasis	-	PP	Dermoscopy x10	Diagnostic: to describe the most significant morphologic findings seen on dermatoscopy of IEC, sBCC, and psoriasis, and formulate a diagnostic model based on these features	Homogenous vascular pattern; red dots; red globular rings; radial capillaries; dull-pink background; red globules; light-red background	+	Histopathology

Kim* 2011[15]	Cross-sectional	B	96: 55 scalp psoriasis; 41 SD	96	Scalp	Scalp psoriasis, SD	Dermoscopy x10	Diagnostic: to evaluate the usefulness of dermoscopy in the clinical differentiation of scalp psoriasis and seborrhoeic dermatitis	Scalp psoriasis: scales; red dots and globules; twisted red loops; glomerular vessels SD: scales; arborising vessels; atypical red vessels; featureless areas	-	-
Lallas, Apalla 2012[52]	Case report	C	3	-	-	Psoriasis	Dermoscopy	Diagnostic: to report and highlight the significant role of dermoscopy in the diagnosis of three clinically atypical and heterogeneous cases of psoriasis	Regularly distributed red dots; light/pinkish-red background	-	-
Lallas, Kyrgidis* 2012[17]	Cross-sectional	B	169: 83 PP; 41 dermatitis; 25 LP; 20 PR	169	-	PP, dermatitis, LP, PR	Dermoscopy x10	Diagnostic: to determine and compare the dermoscopic patterns associated with PP, dermatitis, LP and PR and to assess the validity of certain dermoscopic criteria in the diagnosis of PP	PP: dotted vessels in regular arrangement over light red background and white scales; dermatitis: yellow scales and dotted vessels in patchy arrangement; PR yellowish background, dotted vessels and peripheral scales; LP wickham striae	+	Histopathology
Lallas, Apalla, Karteridou 2013[61]	Case report	C	2: 1 PRP; 1 psoriasis	-	Abdomen; anterior trunk	PRP, psoriasis	Dermoscopy	Diagnostic: to observe dermoscopic findings in two patients suffering from PRP and psoriasis	PRP: round/oval yellowish areas surrounded by vessels of mixed morphology Psoriasis: regulary arranged dotted vessels; white scales	-	Histopathology

First author + Year	Study design	STROBE category	Participants (n)	Evaluated lesions (n)	Location of evaluated lesions	Diagnosis	Technique	Aim (diagnostic or monitoring)	Dermoscopic features	Sens/spec features	Reference test
Lallas, Apalla 2014[60]	Cross-sectional	C	85	139	48 scalp; 38 folds; 22 palmoplantar; 20 face	PP	Dermoscopy x10	Diagnostic: to describe the dermoscopic characteristics of psoriatic plaques located on the scalp, face, folds, palms, soles and genitalia	dotted vessels regular distributed; white diffuse scaling	-	-
Vazquez-Lopez 2014[62]	Letter: case report	C	-	-	-	PP	Dermoscopy	Diagnostic: to present the dermoscopic sequence of a psoriatic lesion before and after the development of Auspitz's sign (AS)	Before scraping: regularly distributed; homogeneous papillary vessels; light-red background; round red globules or dots/ simple, twisted, coiled capillary loops After scraping: Auspitz's sign (appearance of punctate bleeding spots)	-	-
Errichetti, Lacarrubba2015[59]	Cross-sectional	C	17: 9 GP		Trunk, extremities	GP	Dermoscopy x10	Diagnostic: To examine PLC and GP lesions using dermoscopy, and to investigate the significance of specific dermoscopic findings in order to facilitate their differentiation and decrease the number of cases requiring biopsy	Guttate psoriasis: dotted vessels diffuse distributed	-	-

Errichetti, Piccirillo, 2015[65]	Case series	C	14	-	12 upper limb; 10 lower extremities and trunk	PN	Dermoscopy x10	Diagnostic: to describe for the first time the dermoscopic features of PN and the useful contribution of dermoscopy in the differential diagnosis of such dermatoses	white starburst pattern; hyperkeratosis/ scales; crusts; erosions; follicular plugging; hemorrhagic spots; dotted/glomerular vessels	-	-
Goncharova* 2015[14]	Cross-sectional	B	74	74	-	PP, CLE, SD, LP	Dermoscopy x10	Diagnostic: to correlate dermoscopic findings with histopathologic reaction patterns of inflammatory dermatoses	Psoriasiform reaction pattern: intense red background; mostly regulary distributed red dots and globules Lichenoid reaction pattern: dull-red background; red lines, dots and globules; coma-shaped vessels Spongiotic reaction pattern: fading or light red background; regular or irregular vascular dots	+	Histopathology
Lopez-Gomez 2015[64]	Case report	C	1	-	Knees; elbows	PRP	Dermoscopy	Diagnostic: to describe: dermosocopic features of circumscribed juvenile PRP	Multiple whitish keratotic plugs with a yellow peripheral keratotic ring which coalesce into a orange-yellow plaque; papules surrounded by erythema with some linear vessels, centered by a hair	-	Histopathology
Yadav 2015[92]	Cross-sectional	C	68	-	Nails	Nail psoriasis	Dermoscopy x10 and VD	Diagnostic: to study the dermoscopic features of nails in patients of chronic plaque psoriasis	Irregular pits; oycholysis; dilated globose vessels at the onychodermal band; oil drop sign; splinter hemorrhages	-	-

First author + Year	Study design	STROBE category	Participants (n)	Evaluated lesions (n)	Location of evaluated lesions	Diagnosis	Technique	Aim (diagnostic or monitoring)	Dermoscopic features	Sens/spec features	Reference test
Errichetti, Stinco* 2016[13]	Cross-sectional	C	21: 10 palmar psoriasis; 11 CHE	-	Hands	Palmar psoriasis, CHE	Dermoscopy x10	Diagnostic: to investigate palmar psoriasis and CHE using dermoscopy and to assess significance of specific dermoscopic features in order to improve their non-invasive differentiation	Palmar psoriasis: white scales; erythematous background; diffuse or patchy distribution; dotted vessels CHE: focally distributed yellowish scales; brownish-orange dots and/or globules; erythematous background; yellowish-orange crusts; patchily distributed white scales; focally distributed dotted vessels	-	Histopathology
Quaresma 2016[66]	Letter: case series	C	3	-	Scalp	LSC	Dermoscopy x20 and x12	Diagnostic: dermoscopy as a useful tool to the diagnosis of LSC and correlation of dermoscopic signs with pathological features	Before treatment: Red and scaly scalp; hair breakage: broom hair fibers and hamburger sign After treatment: absence of scalp erythema and lichenification; improved hair density; normal short hair shafts	-	histopathology
Interface dermatitis											
Vazquez-Lopez 2001[71]	Case report	C	1	-	Elbows; knees	LP	Dermoscopy x10	Diagnostic: to investigate if the handheld dermatoscope improves the recognition of wickham striae and capillaries in LP lesions	Wickham striae surrounded by radial capillaries	-	-

Vazquez-Lopez, Maldonado-Seral, Lopez-Escobar 2003[72]	Letter: case series	C	10	50	-	LP	Dermoscopy x10	Diagnostic + monitoring: to investigate whether dermoscopy can provide data that are useful for assessing and monitoring patients with pigmented lichen planus	Diffuse pigmented pattern: lesions showing diffuse, structureless, brownish areas; Dotted pigmented pattern: lesions demonstrating fine or coarse grey-blue or brown dots or globules; Mixed pigmented pattern: diffuse brownish areas with dotted structures	-	-
Vazquez-Lopez, Manjon-Haces* 2003[20]	Cross-sectional	C	45: 25 LP; 20 PP	-	-	LP, PP	Dermoscopy x10	Diagnostic: dermoscopic features of PP and LP are investigated to determine both vascular and nonvascular features	LP: whitish striae, gray-blue dots; comedo; milium-like cysts; red lines PP: homogenous red globules	+	Histopathology
Vazquez-Lopez, Kreusch* 2004[19]	Cross-sectional	C	414	-	-	Psoriasis, PP, LP, eczema, PR, PN, CU, erythema multiforme, drug rashes; annular erythema, LE morphea, pemphigus, pemphigoid, sarcoidosis, NL, GA	Dermoscopy x10	Diagnostic: to evaluate and classify the dermoscopic vascular structures seen in nontumoural dermatoses	Psoriasis, spongiotic psoriasiform dermatitis: homogeneous red globules Erythema multiforme, drug rashes, CU, erythema annulare, morphea, NL, LP, LE, sarcoidosis: homogeneous red lines Drug rashes, PR, LP, LE, scaling spongiotic dermatitis: mixed vascular findings PP: red globules-rings LP: radial capillaries Advanced lesions LP: mixed vascular/ pigmented features	-	-

First author + Year	Study design	STROBE category	Participants (n)	Evaluated lesions (n)	Location of evaluated lesions	Diagnosis	Technique	Aim (diagnostic or monitoring)	Dermoscopic features	Sens/spec features	Reference test
Lallas, Kyrgidis* 2012[17]	Cross-sectional	B	169: 83 PP; 41 dermatitis; 25 LP; 20 PR	169	-	PP, dermatitis, LP, PR	Dermoscopy x10	Diagnostic: to determine and compare the dermoscopic patterns associated with PP, dermatitis, LP and PR and to assess the validity of certain dermoscopic criteria in the diagnosis of PP	PP: dotted vessels in regular arrangement over light red background and white scales; dermatitis: yellow scales and dotted vessels in patchy arrangement; PR yellowish background, dotted vessels and peripheral scales; LP wickham striae	+	-
Iga 2013[68]	Letter: case report	C	1	-	-	Annular LP	Dermoscopy	Diagnostic: to report the dermoscopic and histological observations of a case of annular LP	Ring-form whitish striae, surrounding capillaries	-	Histopathology
Lallas, Apalla, Lefaki 2013[69]	Cross-sectional	B	37	55	33 Face; 12 trunk; 10 extremities	DLE (not located on scalp)	Dermoscopy x10	Diagnostic: to describe the dermoscopic criteria observed in a series of patients with DLE located on areas other than the scalp, and to correlate them to the underlying histopathological alterations	Perifollicular whitish halo; follicular keratotic plugs; telangiectatic vessels; white scales; pigmentation; structureless whitish areas; follicular red dots	-	Histopathology

Nakamura 2013[70]	Case series	B	11	79	Nails	Nail LP	Dermoscopy	Diagnostic: evaluation of dermoscopic characteristics of nail LP in order to facilitate diagnosis and to assess prognosis	Nail matrix: pitting, trachyonychia, red lunulae, dorsal pterygium Nail bed: onycholysis, subungual keratosis, chromonychia, splinter hemorrhage, nail plate fragmentation Peronychium: paronychia Matrix, nailbed, paronychium: longitudinal streaks; anonychia	-	Histopathology
Coelho de Sousa 2015[67]	Case report	C	1	-	Legs	Hypertrophic LP	Dermoscopy x10	Diagnostic: to describe dermoscopic features of a specific case of hypertrophic LP	Contact dry dermoscopy: round and reticular whitish structures (some with arboriform projections) Contact immersion dermoscopy: large comedolike openings filled with round, yellowish keratin material ('oil drops within a brown background'); chalk-white structureless areas	-	Histopathology
Goncharova* 2015[14]	Cross-sectional	B	74	74	-	PP, CLE, SD, LP	Dermoscopy x10	Diagnostic: to correlate dermoscopic findings with histopathologic reaction patterns of inflammatory dermatoses	Psoriasiform reaction pattern: intense red background; mostly regulary distributed red dots and globules Lichenoid reaction pattern: dull-red background; red lines, dots and globules; coma-shaped vessels Spongiotic reaction pattern: fading or light red background; regular or irregular vascular dots	+	Histopathology

First author + Year	Study design	STROBE category	Participants (n)	Evaluated lesions (n)	Location of evaluated lesions	Diagnosis	Technique	Aim (diagnostic or monitoring)	Dermoscopic features	Sens/spec features	Reference test
Perivascular dermatitis											
Vazquez-Lopez, Maldonado-Seral, Soler-Sanchez 2003[30]	Case series	B	20: 10 CU	-	-	CU	Dermoscopy x10	Diagnostic: to determine if skin surface microscopy can aid in the clinical differentiation between common urticaria and urticarial vasculitis in daily practice	red-lined vascular pattern (papules or wheals); negative areas	-	Histopathology
Vazquez-Lopez, Kreusch* 2004[19]	Cross-sectional	C	414	-	-	Psoriasis, PP, LP, eczema, PR, PN, CU , erythema multiforme, drug rashes; annular erythema, LE morphea, pemphigus, pemphigoid, sarcoidosis, NL, GA	Dermoscopy x10	Diagnostic: to evaluate and classify the dermoscopic vascular structures seen in nontumoural dermatoses	Psoriasis, spongiotic psoriasiform dermatitis: homogeneous red globules Erythema multiforme, drug rashes, CU, erythema annulare, morphea, NL, LP, LE, sarcoidosis: homogeneous red lines Drug rashes, PR, LP, LE, scaling spongiotic dermatitis: mixed vascular findings PP: red globules-rings LP: radial capillaries Advanced lesions LP: mixed vascular/ pigmented features	-	-

Nodular and diffuse dermatitis											
Vazquez-Lopez, Kreusch* 2004[19]	Cross-sectional	C	414	-	-	Psoriasis, PP, LP, eczema, PR, PN, CU, erythema multiforme, drug rashes; annular erythema, LE morphea, pemphigus, pemphigoid, sarcoidosis, NL, GA	Dermoscopy x10	Diagnostic: to evaluate and classify the dermoscopic vascular structures seen in nontumoural dermatoses	Psoriasis, spongiotic psoriasiform dermatitis: homogeneous red globules Erythema multiforme, drug rashes, CU, erythema annulare, morphea, NL, LP, LE, sarcoidosis: homogeneous red lines Drug rashes, PR, LP, LE, scaling spongiotic dermatitis: mixed vascular findings PP: red globules-rings LP: radial capillaries Advanced lesions LP: mixed vascular/pigmented features	-	-
Pellicano 2010[32]	Case series	C	6	7	-	CS	Dermoscopy x10	Diagnostic: to evaluate the usefulness of dermoscopy in the differential diagnosis of CS	Small grouped, translucent orange globular structures associated with linear vessels of variable diameter; central scar-like areas	-	-
Hadj 2014[31]	Case report	C	1	1	Nose	Sarcoidosis	Dermoscopy	Diagnostic: to describe dermoscopic findings of sarcoidosis	Orange-yellowish patch; branching vessels	-	Histopathology

First author + Year	Study design	STROBE category	Participants (n)	Evaluated lesions (n)	Location of evaluated lesions	Diagnosis	Technique	Aim (diagnostic or monitoring)	Dermoscopic features	Sens/spec features	Reference test
Lallas, Argenziano, Apalla* 2014[7]	Cross-sectional	B	115	115	-	SD, ER, sarcoidosis, DLE	Dermoscopy x10	Diagnostic: to describe and compare the dermoscopic patterns of common facial inflammatory skin diseases including SD, ER, sarcoidosis, LV, DLE and GF	SD: dotted vessels in patchy distribution; yellow scales ER: linear vessels in polygonal pattern Sarcoidosis: orange-yellowish structureless areas; linear, focused branching vessels DLE: perifollicular whitish halo; follicular kera-totic plugs; white scales; linear branching vessels	-	-
Palisading granulomatous dermatitis											
Vazquez-Lopez, Kreusch* 2004[19]	Cross-sectional	C	414	-	-	Psoriasis, PP, LP, eczema, PR, PN, CU, erythema multiforme, drug rashes; annular erythema, LE morphea, pemphigus, pemphigoid, sarcoidosis, NL, GA	Dermoscopy x10	Diagnostic: to evaluate and classify the dermoscopic vascular structures seen in nontumoural dermatoses	Psoriasis, spongiotic psoriasiform dermatitis: homogeneous red globules Erythema multiforme, drug rashes, CU, erythema annulare, morphea, NL, LP, LE, sarcoidosis: homogeneous red lines Drug rashes, PR, LP, LE, scaling spongiotic dermatitis: mixed vascular findings PP: red globules-rings LP: radial capillaries Advanced lesions LP: mixed vascular/ pigmented features	-	-

Bakos 2012[33]	Letter: case report	C	2	-	Left leg; legs and arms	NL	Dermoscopy x20 and x50	Diagnostic: to describe dermatoscopic features of early-onset NL lesions in two patients	multiple branching thick telangiectasias; superficial thin hairpin-like vessels, regularly distributed; yellowish background; well-distributed arborising telangiectasias associated to a yellowish halo or background	-	Histopathology
Conde-Montero 2013[34]	Letter: case report	C	3	-	-	NL	Dermoscopy	Diagnostic: to report the cases of 3 patients with NL lesions at different stages with a view to establishing a correlation between dermoscopic and histologic findings	1: comma-shaped vessels; whitish structures on a pink background; orange-brown areas; fine network of vessels; 2: fine network of abundant vessels on a pink background; homogeneous orange-yellow areas 3: irregular arborising vessels on a light-brown background, whitish areas; patchy pigmented reticulum	-	Histopathology
Lallas, Zaballos 2013[35]	Letter: cross-sectional	C	47: 24 GA, 23 NL	47 GA; 37 NL	-	GA, NL	Dermoscopy	Diagnostic: to identify the dermoscopic patterns of GA and NL	GA: dotted vessels; linear vessels; absence of vessels; red-white, red, white background colour; pigmented structures NL: prominent network of linear arborising vessels; yellow, yellow-white, yellow-red background colour ; yellow crusting; ulceration	-	-

First author + Year	Study design	STROBE category	Participants (n)	Evaluated lesions (n)	Location of evaluated lesions	Diagnosis	Technique	Aim (diagnostic or monitoring)	Dermoscopic features	Sens/spec features	Reference test
Pellicano 2013[36]	Cross-sectional	C	24: 12 GA, 12 NL	-	-	GA, NL	Dermoscopy x10	Diagnostic: to evaluate the dermoscopic patterns of NL and GA and to compare these findings with other granulomatous skin disorders	GA: peripheral, structureless orange-reddish borders; isolated, unfocussed small vessels NL: evident, sharply focused, elongated and serpentine telangiectasias, typically located over a whitish, structureless background; structureless, yellow-orange areas	-	Histopathology
Sclerosing dermatitis											
Bergman 2003[38]	Cross-sectional	C	276: 106 patients; 170 control	-	Nails	Scleroderma, SLE	Dermoscopy x10	Diagnostic: to investigate the potential use of the unmodified common handheld dermatoscope as a capillaroscopic instrument	Scleroderma: enlargement of capillary loops; loss of capillaries; disorganization of normal distribution of capillaries; budding capillaries; extravasates SLE: SD pattern; twisted unenlarged capillaries; extravasates	-	-

Vazquez-Lopez, Kreusch* 2004[19]	Cross-sectional	C	414	-	-	Psoriasis, PP, LP, eczema, PR, PN, CU, erythema multiforme, drug rashes; annular erythema, LE morphea, pemphigus, pemphigoid, sarcoidosis, NL, GA	Dermoscopy x10	Diagnostic: to evaluate and classify the dermoscopic vascular structures seen in nontumoural dermatoses	Psoriasis, spongiotic psoriasiform dermatitis: homogeneous red globules Erythema multiforme, drug rashes, CU, erythema annulare, morphea, NL, LP, LE, sarcoidosis: homogeneous red lines Drug rashes, PR, LP, LE, scaling spongiotic dermatitis: mixed vascular findings PP: red globules-rings LP: radial capillaries Advanced lesions LP: mixed vascular/ pigmented features	-	-
Baron 2007[37]	Cross-sectional	B	8: 6 SSc; 2 control	32 (8x4)	Nailfolds of two fingers of each hand	SSc	Dermoscopy x10	Diagnostic: to assess the reliability of two office techniques, the ophthalmoscope and the Dermlite® dermatoscope, and to detect nailfold capillaroscopy abnormalities in SSc	Dilated capillaries; giant capillaries; avascular areas	-	-

First author + Year	Study design	STROBE category	Participants (n)	Evaluated lesions (n)	Location of evaluated lesions	Diagnosis	Technique	Aim (diagnostic or monitoring)	Dermoscopic features	Sens/spec features	Reference test
Campione 2009[39]	Case report	C	2	2	Right leg; right arm	Morphea	Dermoscopy	Monitoring: to investigate the effectiveness of dermoscopic assessment in localised morphea treated with imiquimod 5%	Before treatment: accentuated fibrotic beams crossed by spreading telangiectases in centre of lesion; pronounced erythematous border During treatment (2wk): noticeable improvement in atrophic area; slight reduction in fibrotic beams and blood vessels extension and thickness After treatment (16wk):complete remission (disappearance of neovascularisation and fibrosis)	-	-
Kimura 2011[46]	Letter: case report	C	1	1	Upper back	Extragenital LS	Dermoscopy	Diagnostic: to describe typical signs of extragenital LS in a specific case	Scales; comedo-like openings; telangiectasia; whitish-pink background; red-violet to red-brown perifollicular ovoid structures	-	Histopathology
Muroi 2011[54]	Cross-sectional	B	151: 83 SSc; 68 control	151 (x10 images each patient)	Nails	SSc	Dermoscopy x10	Diagnostic: to assess the practical utility of dermatoscope for assessment of capillary morphology in patients with SSc	Enlarged capillaries; hemorrhages	+	Histopathology and immuno-histochemistry

Shim 2012[44]	Letter: cross-sectional	C	39: 18 LS; 21 morphea	-	-	LS, morphea	Dermoscopy x10	Diagnostic: to investigate the diagnostic usefulness of dermatoscopy in differentiating lichen sclerous et atrophicus from morphea	LS: comedo-like openings; whitish patches; fibrotic beams; pigment network-like structures; linear branching vessels; commalike, hairpin and dotted vessels Morphea: comedo-like openings; whitish patches; fibrotic beams; pigment network-like structures; linear branching vessels	-	-
Dogan 2013[40]	Cross-sectional	C	39	382		SSc	Dermoscopy x10	Diagnostic: to compare the diagnostic value of dermatoscopy and VCAP which are widely used to determine changes in the nailfold capillary pattern in SSc patients	Early phase: few enlarged capillaries; few hemorrhages Active phase: frequent giant capillaries; hemorrhages Late phase: irregular enlargement of capillaries; severe loss of capillaries; avascular areas	-	VCAP
Giampetruzzi 2013[41]	Letter: cross-sectional	C	31	-	-	SSc	Dermoscopy x25 and x40	Diagnostic: to better appreciate vascular features of telangiectases on selected anatomic sites (e.g., face, chest, hands, forearms) and to provide a morphological description of altered skin microcirculation in patients with SSc from a novel perspective	Spot pattern (uniform rounded patches); reticular pattern (a reticular network, “mini-spider”)	-	NVC x200

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Larre Borges 2013[48]	Cross-sectional	B	26: 12 extra-genital LS	29	-	LS	Dermoscopy	Diagnostic: to evaluate and compare dermoscopic and histopathologic patterns of genital and extragenital LS	Patchy white to yellowish structures, scales, keratotic plugs, chrysalis structures; additional features: gray dots, erosions, brownish lines	-	Histopathology
Mazzotti 2014[53]	Cross-sectional	B	45	45 (x3 images each patient)	Fourth finger of the left hand	SSc	Dermoscopy: PNCD and NPCD	Diagnostic: to compare conventional capillaroscopy, using the gold-standard method SNFC, PNCD and NPCD, and to evaluate their accuracy in diagnosing characteristic SSc-related alterations	Presence of capillary deletion, haemorrhage, ectasia, megacapillaries, crossover, bushes, bizarre morphology; general pattern (occurrence of avascular areas and/or the presence of definitely dilated capillaries)	+	SNFC
Horcajada-Reales 2015[45]	Case report	C	2	-	1:Limbs and trunk; 2: lower eyelid	LS	Dermoscopy	Diagnostic: description of features of LS	Whitish background with comedo-like openings	-	-
Hughes 2015[42]	Cross-sectional	B	32	320	Nailbed	SSc	Dermoscopy x10	Diagnostic: to examine the ability to classify capillaries and to evaluate abnormality (severity), by both NVC and dermoscopy, to determine whether these differ between general and specialist rheumatologists, and to compare intra- and interrater reliability of both techniques	Enlarged capillaries; haemorrhages; areas of avascularity	-	NVC x200

Lacarrubba, Pellacani 2015[47]	Case report	C	1	-	Upper trunk	Extragenital LS	Dermoscopy	Diagnostic: to describe: clinical, dermoscopic, confocal microscopy and histologic correlations of extragenital LS	comedo-like openings	-	Histopathology and RCM
Nobrega 2016[43]	Case report	C	1	-	Hand, feet and knees	Extragenital LS with localised scleroderma	Dermoscopy	Diagnostic: to report a case of lichen sclerosus associated with scleroderma in children, highlighting the importance of dermoscopy in diagnosis	multiple whitish areas, some surrounded by an erythematous halo; whitish amorphous areas; pseudocomedones	-	-
Bullous dermatitis											
Vazquez-Lopez, Kreusch* 2004[19]	Cross-sectional	C	414	-	-	Psoriasis, PP, LP, eczema, PR, PN, CU, erythema multiforme, drug rashes; annular erythema, LE morphea, pemphigus, pemphigoid, sarcoidosis, NL, GA	Dermoscopy x10	Diagnostic: to evaluate and classify the dermoscopic vascular structures seen in nontumoural dermatoses	Psoriasis, spongiotic psoriasiform dermatitis: homogeneous red globules Erythema multiforme, drug rashes, CU, erythema annulare, morphea, NL, LP, LE, sarcoidosis: homogeneous red lines Drug rashes, PR, LP, LE, scaling spongiotic dermatitis: mixed vascular findings PP: red globules-rings LP: radial capillaries Advanced lesions LP: mixed vascular/ pigmented features	-	-

First author + Year	Study design	STROBE category	Participants (n)	Evaluated lesions (n)	Location of evaluated lesions	Diagnosis	Technique	Aim (diagnostic or monitoring)	Dermoscopic features	Sens/spec features	Reference test
Vazquez-Lopez, Lopez-Escobar 2004[75]	Case series	C	5	-	-	Darier's disease	Dermoscopy x10	Diagnostic: to in investigate the value of the handheld dermoscope for improving the recognition of pseudocomedones in Darier's disease papules	variable vascular structures (red dots, red lines, or erythema); giant pseudocomedones	-	-
Criado 2013[49]	Case report	C	1	1	Volar surface of the fingers	Dermatitis herpetiformis	Dermoscopy x10	Diagnostic: to describe dermoscopic features of a specific case of dermatitis herpetiformis with palmoplantar manifestation	Erythematous and violaceous dots; erythematous and brown dots	-	Histopathology
Lacarrubba, Verzi 2015[73]	Case report	C	2	-	-	Darier's disease	Dermoscopy	Diagnostic: to describe: clinical, dermoscopic, confocal microscopy and histologic correlations of Darier's disease	polygonal, starlike or roundish-oval-shaped yellowish/brownish areas of various size surrounded by a thin whitish halo	-	Histopathology and RCM
Errichetti, De Francesco 2016[90]	Case series	B	7	7	-	Grover's disease	Dermoscopy x10	Diagnostic: to evaluate the dermoscopic features of Grover's disease in a larger series of patients and correlate dermoscopy with histopathological findings	Darier-like subtype: central brownish area surrounded by a whitish halo, star-like shape; roundish oval shape; branched polygonal appearance; linear/ irregular vessels; dotted vessels spongiotic subtype: whitish scales; linear/ irregular vessels; subtle telangiectatic vessels; dotted vessels	-	Histopathology

Errichetti, Stinco, Lacarrubba 2016[50]	Letter: case series	C	11	11	-	Darier's disease	Dermoscopy x10	Diagnostic: to evaluate the dermoscopic features of Darier's disease in a group of patients, and to compare them with those of the main dermatoses which enter into the differential diagnosis	Centrally located yellowish/ brownish area with a polygonal or starlike morphology, surrounded by a more or less thin whitish halo, overlying a pinkish homogeneous structureless area; whitish scales; dotted and/or linear vessels often with whitish halo, surrounding the central yellow-brown areas	-	-
Sadayasu 2016[74]	Case report	C	1	-	Trunk; lower extremities	Grover's disease	Dermoscopy	Diagnostic: to report a case of Grover's disease associated with multiple sclerosis and discuss the potential correlation between dermoscopy and pathology	irregular brown crypts edged with a white band-like area surrounded by an erythematous halo; whitish scaly areas in patches within the brown crypts	-	Histopathology
Alopecia (cicatricial)											
Inui 2008[78]	Case report	C	4	-	Scalp	FFA	Dermoscopy x10	Diagnostic: to describe dermoscopic findings of FFA and to investigate the possibility of utilizing dermoscopy as a diagnostic tool for FFA	The loss of orifices, perifollicular erythema or scale was seen in all the four cases (4/4), in three cases (3/4) or in two cases (2/4)	-	Histopathology and immuno-histochemistry

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Duque-Estrada 2010[91]	Cross-sectional	C	14: 4 LPP; 5 FFA; 5 DLE	-	Scalp	DLE, LPP, FFA	VD and dermoscopy x10	Diagnostic: to describe dermoscopic findings in patients with clinical and histopathological characteristics of cicatricial alopecia	DLE: white patches, branching capillaries, keratin plugs and areas of reduced follicular ostia LPP: perifollicular scales, white dots; reduced follicular ostia FFA: reduced follicular ostia, perifollicular scales, perifollicular erythema; branching capillaries	-	-
Rubegni 2010[84]	Case report	C	1	-	Scalp	FFA	Dermoscopy	Diagnostic: to discuss the relevance of dermoscopy in the differential diagnosis of LPP	Absence of follicular openings; perifollicular scale; feeble perifollicular erythema	-	Histopathology
Karadag Kose 2012[80]	Case-control	B	288: 144 alopecia; 144 control	-	Scalp	PCA	Dermoscopy x10	Diagnostic: to evaluate the potential benefit of a handheld dermatoscope in the clinical diagnosis of alopecia	Absence of follicular ostia; tufted hairs; follicular hyperkeratosis; pili torti; pink-white appearance	-	-
Miteva 2012[81]	Letter: case series	B	141: 11 black patiens	-	Scalp	FFA	Dermoscopy	Diagnostic: to evaluate the distribution of black patients among patients with FFA and to describe features of FFA in black patients	Absence of follicular openings; white patches	-	Histopathology

Tsai 2012[87]	Case report	C	1	2	Scalp; right cheek	DLE	Dermoscopy x10	Diagnostic + monitoring: description of features of DLE and to assess changed features after treatment with mometasone furoate	Scalp: branching red lines; white and brown dyschromia; reduction of follicular ostia Right cheek: erythematous and brown dyschromia; central white macules; peripheral red lines After treatment: clearance of active auricular lesion, follicular keratin plugs and scales; residual red dyschromia and teleangiectasia	-	Histopathology and immuno-histochemistry
Inui 2014[77]	Letter: case report	C	2	2	-	DLE	Dermoscopy x10	Diagnostic: to describe dermoscopic features of specific cases of DLE	DLE: follicular keratotic plug (black triangle); polymorphous telangiectatic vessels; white scales; peripheral radial pigment streaks; structureless whitish area; perifollicular pigmentation	-	-
Lallas, Argenziano, Apalla* 2014[7]	Cross-sectional	B	115	115	-	SD, ER, sarcoidosis, DLE	Dermoscopy x10	Diagnostic: to describe and compare the dermoscopic patterns of common facial inflammatory skin diseases including SD, ER, sarcoidosis, LV, DLE and GF	SD: dotted vessels in patchy distribution; yellow scales ER: linear vessels in polygonal pattern Sarcoidosis: orange-yellowish structureless areas; linear, focused branching vessels DLE: perifollicular whitish halo; follicular kera- totic plugs; white scales; linear branching vessels	-	-

First author + Year	Study design	STROBE category	Participants (n)	Evaluated lesions (n)	Location of evaluated lesions	Diagnosis	Technique	Aim (diagnostic or monitoring)	Dermoscopic features	Sens/spec features	Reference test
Shim 2014[85]	Cross-sectional	B	148: 8 LPP; 7 DLE; 1 pseudo-pelade of Brocq	-	Scalp	LPP, DLE, pseudo-pelade of Brocq	Dermoscopy x10	Diagnostic: to investigate clinical usefulness of dermoscopy for diseases with small round or oval hairless patch on the scalp	LPP: lack of follicular ostia; perifollicular hyperkeratosis; perifollicular erythema DLE: lack of follicular ostia; keratin plugs Pseudopelade of Brocq: lack of follicular ostia without any other specific findings	-	-
Qi 2014[83]	Cross-sectional	B	59: 13 DLE; 2 LPP; 24 FD; 2 AM; 9 pseudopelade of brocq; 9 DC	-	Scalp	DLE, LPP, FD, AM, pseudo-pelade of brocq, DC	Dermoscopy	Diagnostic + monitoring: to study the clinical, pathological and dermoscopic characteristics of primary cicatricial alopecia in a Chinese population	Diagnostic: Follicular openings in all diagnoses except alopecia mucinosa; epidermal atrophy in all cases; FD: tufted hair; FD, dissecting cellulitis: pustules; alopecia mucinosa: patulous follicular openings Monitoring: increase in short vellus hairs; gradual decrease of teleangiectasia, epidermal scale, follicular hyperkeratosis, pustules and hair diameter diversity	-	Histopathology
Fernandez-Crehuet 2015[76]	Letter: Cross-sectional	C	238	-	Scalp	FFA	Dermoscopy	Diagnostic: to describe the trichoscopic features of FFA in a large series of patients and to correlate these findings with several relevant parameters of FFA	Follicular hyperkeratosis; perifollicular erythema; lonely hair; hair diameter diversity; cicatricial white patches; yellow dots	-	-

Kaliyadan 2015[79]	Case report	C	1	-	-	Linear LPP	Dermoscopy x10	Diagnostic: to present case of localised LPP over the face, extending to the scalp and to demonstrate the peculiar dermoscopic patterns of active lichen planopilaris when occurring over the face	Wickham's striae; prominent pigment clumps	-	Histopathology
Pirmez 2015[82]	Letter: case series	C	16	-	Scalp	FFA	Dermoscopy	Diagnostic: to describe cases of FFA in which an unusual retention of the hairline produced a misleading pseudo 'fringe sign'	Pseudo 'fringe sign'; discrete perifollicular erythema; scaling	-	-
Soares 2015[86]	Cross-sectional	B	80	-	Scalp	LPP	Dermoscopy	Diagnostic: to describe clinical, dermoscopic and histopathological findings of LPP in public and private practices	Reduction of follicles; perifollicular hyperkeratosis; polytrichia; white dots; visible vessels; black dots; pigmented halo; isolated terminal stalk	-	Histopathology
Wutte 2015[88]	Case report	C	1	-	-	FFA associated with DLE	Dermoscopy	Diagnostic: to report a case of FFA in association with DLE and to correlate dermoscopic and histologic features	Loss of follicular openings; perifollicular erythema; some perifollicular scaling	-	Histopathology

First author + Year	Study design	STROBE category	Participants (n)	Evaluated lesions (n)	Location of evaluated lesions	Diagnosis	Technique	Aim (diagnostic or monitoring)	Dermoscopic features	Sens/spec features	Reference test
Abedini 2016[51]	Case control	B	300: 100 PCA; 100 non-cicatricial alopecia; 100 control	-	-	PCA	Dermoscopy x10	Diagnostic: to assess the trichoscopic features in these patients and to find sensitive and/or specific trichoscopic findings that can be helpful in their diagnosis	non-follicular red dots/twisted red loops; enlarged branching; tortuous branching; follicular white and yellow keratotic plugging; red, white, yellow and black dots; comedonal openings; blue-gray dots; perifollicular pigmentation; speckled pigmentation; perifollicular erythema; perifollicular scale; peripilar white halo; perifollicular pustules; absence of follicular opening; scalp erythema; pili torti; hair tufting; reduced hair density	+	Histopathology
Miscellaneous inflammatory and reactive disorders											
Lallas, Argenziano, Apalla* 2014[7]	Cross-sectional	B	115	115	-	SD, ER, sarcoidosis, DLE	Dermoscopy x10	Diagnostic: to describe and compare the dermoscopic patterns of common facial inflammatory skin diseases including SD, ER, sarcoidosis, LV, DLE and GF	SD: dotted vessels in patchy distribution; yellow scales ER: linear vessels in polygonal pattern Sarcoidosis: orange-yellowish structureless areas; linear, focused branching vessels DLE: perifollicular whitish halo; follicular kera- totic plugs; white scales; linear branching vessels	-	-

Lallas, Argenziano, Longo 2014[89]	Letter: case series	C	12	-	Face	ER	Dermoscopy x10	Diagnostic + monitoring: to investigate if the vascular alterations of rosacea are highlighted by dermoscopy and whether the technique provides additional morphologic information on treatment monitoring	Before treatment: polygonal vessels; follicular plugs; superficial scales After treatment: disappeared, less prominent or persisting polygonal vessels	-	-

* Studies that were included in multiple categories as they evaluated multiple diagnoses; ACD, allergic contact dermatitis; AD, atopic dermatitis; AM, alopecia mucinosa; CCCA, central centrifugal cicatricial alopecia; CHE, chronic hand eczema; CLE, chronic lupus erythematosus; CM, cutaneous mastocytosis; CS, cutaneous sarcoidosis; CU, common urticaria; DC, dissecting cellulitis; DLE, discoid lupus erythematosus; ER, erythematoteleangiectatic rosacea; FD, folliculitis decalvans; FFA, frontal fibrosing alopecia; GA, granuloma annulare; GF, granuloma faciale; GP, gutatte psoriasis; ICD, irritant contact dermatitis; LE, lupus erythematosus; LP, lichen planus; LPP, lichen planopilaris; LS, lichen sclerosus; LSC, lichen simplex chronicus; LV, lupus vulgaris; NL, necrobiosis lipoidica; NPCD, nonpolarised light contact dermoscopy; NVC, nailfold videocapillaroscopy; PCA, primary cicatricial alopecia; PLC, pityriasis lichenoides chronic; PN, prurigo nodularis; PNCD, polarised light noncontact dermoscopy; PP, plaque psoriasis; PR, pityriasis rosea; PRP, pityriasis rubra pilaris; RCM, reflectance confocal microscopy; SD, seborrhoeic dermatitis; SLE, systemic lupus erythematosus; SNFC, stereomicroscope nailfold capillaroscopy; SSc, systemic sclerosis; TMEP, teleangiectasia macularis eruptive perstans; UP, urticaria pigmentosa; VCAP, videocapillaroscopy; VD, videodermoscopy.

Table 4. Overview of frequently described (video)dermoscopic features in the diagnosis of all included inflammatory skin diseases

Skin disorder	(Video)dermoscopic features
Pityriasis rosea	Yellowish background, peripheral white scales (collarette scaling), dotted vessels with patchy distribution
Dermatitis (eczematous, morbilliform, atopic, contact)	Dotted vessels with patchy distribution, yellow scales
Psoriasis	Homogeneous red dots and globules, red lines and twisted red loops, white scales and a light-red/dull background
Pityriasis rubra pilaris	Round/oval yellowish areas, whitish keratotic plugs with a yellow peripheral keratotic ring
Prurigo nodularis	White starburst pattern, scales, crusts and erosions, follicular plugging, dotted/glomerular vessels
Lichen simplex chronicus	Erythematous background, scales and hair breakage: broom hair fibers
Lupus erythematosus (cutaneous)	Homogeneous red lines, red background
Lichen planus	Wickham striae, peripheral dotted/linear vessels, grey-blue dots and grey-blue background
Common urticaria	Network of linear vessels surrounding avascular areas
Sarcoidosis (cutaneous)	Orange-yellowish globules or areas and linear vessels
Granuloma anulare	Dotted and/or linear vessels with a white, red or yellow background
Necrobiosis lipoidica	Prominent network of linear arborizing vessels and a yellowish background
Morphea	Fibrotic beams and linear vessels
Systemic sclerosis	Dilated and giant capillaries, loss of capillaries, hemorrhages and avascular areas
Systemic lupus erythematosus	Twisted unenlarged capillaries and extravasates
Lichen sclerosus	White/yellowish structureless areas, yellowish keratotic plugs, scales and comedo-like openings
Darier's disease	Pseudocomedones, erythema, dotted/linear vessels
Dermatitis herpetiformis	Erythematous and violaceous dots, erythematous and brown dots
Pemphigus	Extravasations, yellow hemorrhagic crusts, linear serpentine vessels and glomerular vessels, whitish halo
Grover's disease	Star-like (stellate) pattern, linear/irregular vessels and dotted vessels
Frontal fibrosing alopecia	Reduced follicular ostia, perifollicular scales, perifollicular erythema and branching capillaries
Discoid lupus erythematosus	Arborizing red lines, hyperkeratotic perifollicular white scales and an absence of follicular ostia
Lichen planopilaris	Simple red loops, arborizing red lines, honeycomb pigment pattern, white dots and scales, absence of follicular ostia
Central centrifugal cicatricial alopecia	Honeycomb pigmented network, peripilar white/gray halo, white patches and dots, scales
Folliculitis decalvans	Simple and twisted red loops, arborizing red lines, white dots and scales and an absence of follicular ostia
Alopecia mucinosa	Patulous follicular openings
Pseudopelade of brocq	Lack of follicular ostia without any other specific findings
Erythematotelangiectatic rosacea	Polygonal vessels
Papulopustular rosacea	Follicular plugs, follicular pustules and polygonal vessels

Supplementary table 1. Search strategy

Search 03-10-2016

Search topic	Pubmed	Cochrane Library	Embase	Web of Science
Search A: (video) dermoscopy	MeSH terms: #1 dermatoscopy #2 dermatoscopies #3 dermoscopy # 4 dermoscopies # 5 (#1 OR #2 OR #3 OR #4) In free text (title or abstract): #6 dermatoscop* #7 dermoscop* #8 videodermoscop* #9 videodermatoscop* #10 dermoscopic imag* #11 skin surface microscop* #12 inflammoscop* #13 epiluminescen* microscop* #14 epiluminescence dermatoscop* #15 epiluminescence dermoscop* #16 in vivo cutaneous microscop* #17 magnified oil immersion diascop* #18 teledermoscop* #19 teledermatoscop* #20 (#6 OR #7 ... OR #17 OR #18) #21 (#5 OR #19) Citations: 3113	Mesh terms: #1 dermoscopy (exp) In free text (title, abstract or keywords): #2 dermatoscop* #3 dermoscop* #4 videodermoscop* #5 videodermatoscop* #6 dermoscopic imag* #7 skin surface microscop* #8 inflammoscop* #9 epiluminescen* microscop* #10 epiluminescence dermatoscop* #11 epiluminescence dermoscop* #12 in vivo cutaneous microscop* #13 magnified oil immersion diascop* #14 teledermoscop* #15 teledermatoscop* #16 (#2 OR #3 ... OR #14 OR #15) #17 (#1 OR #16) Citations: 182	Emtree terms: #1 epiluminescence microscopy In free text (title or abstract): #2 dermatoscop* #3 dermoscop* #4 videodermoscop* #5 videodermatoscop* #6 dermoscopic imag* #7 skin surface microscop* #8 inflammoscop* #9 epiluminescen* microscop* #10 epiluminescence dermatoscop* #11 epiluminescence dermoscop* #12 in vivo cutaneous microscop* #13 magnified oil immersion diascop* #14 teledermoscop* #15 teledermatoscop* #16 (#2 OR #3 ... OR #14 OR #15) #17 (#1 OR #16) Citations: 6210	In free text (Topic): #1 dermatoscop* #2 dermoscop* #3 videodermoscop* #4 videodermatoscop* #5 dermoscopic imag* #6 skin surface microscop* #7 inflammoscop* #8 epiluminescen* microscop* #9 epiluminescence dermatoscop* #10 epiluminescence dermoscop* #11 in vivo cutaneous microscop* #12 magnified oil immersion diascop* #13 teledermoscop* #14 teledermatoscop* #15 (#1 OR #2 ... OR #13 OR #14) Citations: 7487

Search topic	Pubmed	Cochrane Library	Embase	Web of Science
Search B: inflammatory skin diseases	MeSH terms: #1 skin and connective tissue diseases In free text (title or abstract): #2 skin #3 cutaneous #4 dermatos* #5 (inflammatory AND skin) #6 papulosquamous #7 psoriasis #8 pityriasis #9 lichen* #10 eczema* #11 dermatitis #12 urticaria #13 erythema* #14 scleroderma #15 morphea #16 lupus #17 vesiculobullous #18 bullous #19 pemphig* #20 bullosa #21 (infectious AND skin) #22 (viral AND skin) #23 herpes #24 molluscum contagiosum #25 (bacterial AND skin) #26 staphylococ* #27 impetigo #28 dermatomycos* #29 tinea #30 (infestations AND skin) #31 mite #32 larva #33 leishmaniasis #34 scabies #35 rosacea #36 acne #37 mycosis fungoides #38 (#2 OR #3 … OR #36 OR #37) #39 (#1 OR #38) Citations: 1.657.997	MeSH terms: #1 skin and connective tissue diseases In free text (title, abstract or keywords): #2 skin #3 cutaneous #4 dermatos* #5 (inflammatory AND skin) #6 papulosquamous #7 psoriasis #8 pityriasis #9 lichen* #10 eczema* #11 dermatitis #12 urticaria #13 erythema* #14 scleroderma #15 morphea #16 lupus #17 vesiculobullous #18 bullous #19 pemphig* #20 bullosa #21 (infectious AND skin) #22 (viral AND skin) #23 herpes #24 molluscum contagiosum #25 (bacterial AND skin) #26 staphylococ* #27 impetigo #28 dermatomycos* #29 tinea #30 (infestations AND skin) #31 mite #32 larva #33 leishmaniasis #34 scabies #35 rosacea #36 acne #37 mycosis fungoides #38 (#2 OR #3 … OR #36 OR #37) #39 (#1 OR #38) Citations: 73.549	Emtree terms: #1 exp skin disease In free text (title or abstract): #2 skin #3 cutaneous #4 dermatos* #5 (inflammatory AND skin) #6 papulosquamous #7 psoriasis #8 pityriasis #9 lichen* #10 eczema* #11 dermatitis #12 urticaria #13 erythema* #14 scleroderma #15 morphea #16 lupus #17 vesiculobullous #18 bullous #19 pemphig* #20 bullosa #21 (infectious AND skin) #22 (viral AND skin) #23 herpes #24 molluscum contagiosum #25 (bacterial AND skin) #26 staphylococ* #27 impetigo #28 dermatomycos* #29 tinea #30 (infestations AND skin) #31 mite #32 larva #33 leishmaniasis #34 scabies #35 rosacea #36 acne #37 mycosis fungoides #38 (#2 OR #3 … OR #36 OR #37) #39 (#1 OR #38) Citations: 1.109.243	In free text (Topic): #1 skin #2 cutaneous #3 dermatos* #4 (inflammatory AND skin) #5 papulosquamous #6 psoriasis #7 pityriasis #8 lichen* #9 eczema* #10 dermatitis #11 urticaria #12 erythema* #13 scleroderma #14 morphea #15 lupus #16 vesiculobullous #17 bullous #18 pemphig* #19 bullosa #20 (infectious AND skin) #21 (viral AND skin) #22 herpes #23 molluscum contagiosum #24 (bacterial AND skin) #25 staphylococ* #26 impetigo #27 dermatomycos* #28 tinea #29 (infestations AND skin) #30 mite #31 larva #32 leishmaniasis #33 scabies #34 rosacea #35 acne #36 mycosis fungoides #37 (#1 OR #2 … OR #35 OR #36) Citations: 1.120.441

Combined searches	Search A AND search B Limits: English, German, Dutch, humans Citations: 2608	Search A AND search B Limits: English, German, Dutch, humans Citations: 170	Search A AND search B Limits: English, German, Dutch, humans Citations: 4836	Search A AND search B Limits: English, German, Dutch Citations: 6364

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5

Summary and discussion

Summary and discussion

Morphological characterisation of the skin plays an important role in establishing the diagnosis of a skin lesion and for monitoring treatment responses. In case a clinical diagnosis is challenging, histopathological evaluation of a skin biopsy is the gold standard. However, a skin biopsy is an invasive diagnostic procedure, which can result in scarring and inflammation. In addition, it hinders the ability to monitor dynamic processes over time and is prone to sampling errors. Sampling errors can occur due to sampling of an inappropriate site within a heterogeneous lesion. Moreover, it may also occur when the most aggressive or active part within a punch biopsy is missed during histopathological examination. Subsequently, sampling error can lead to under-, mis- or non-specific diagnoses and inadequate treatment. In recent years, non-invasive imaging techniques have been developed to evaluate the morphology of the skin in more detail. Among these techniques, reflectance confocal microscopy (RCM) and videodermoscopy (VD) offer the possibility to examine the skin at a high resolution. By using these techniques, the diagnostic accuracy can be improved without the need for invasive skin biopsies. Overall, the objective of this thesis was to adjust current protocols in order to optimise the diagnostic process and to explore the morphology of the skin using advanced imaging techniques.

Aim 1: To investigate the sampling error in superficial basal cell carcinoma and its clinical effect

Basal cell carcinoma is the most common malignancy in the Caucasian population, emphasizing the importance of its diagnosis and treatment.[1] Histological subtyping of BCC is essential, as sBCC can be treated with non-invasive treatments while aggressive BCCs (mnBCC and iBCC) require a larger surgical margin compared to nBCC. European guidelines recommend a punch biopsy in clinically suspicious BCC.[2] However, in 11% to 39.1% of the cases, a punch biopsy failed to identify an aggressive BCC subtype correctly.[3-5] This may be due to sampling error of a punch biopsy. In **Chapter 2.1** the sampling error within primary punch biopsies, that were diagnosed as sBCC, is addressed. The accuracy of the current histological examination process (evaluation of one level only) was compared to a more extensive step-section method (evaluation of 5 levels within a punch biopsy). Assessment of 5 levels resulted in 22.4% detection of other BCC subtypes (nBCC, mnBCC en iBCC). This study showed that histological examination of only one level within a punch biopsy leads to underdiagnosis of other BCC subtypes. However, the procedure of sectioning, staining and evaluation of 4 additional levels is more labour intensive, time consuming and costly compared to the current method. Therefore, a protocol which is less intense, but still yields a high detection of more aggressive BCC subtypes, is preferable. Alternative protocols can consist of evaluation of 2 addition levels (sections at 400 μm and 800 μm deeper then the initial H&E stained section) or one additional level (one section at 800 μm deeper then the initial H&E stained section), yielding detection rates of other BCC subtypes of respectively 20.7% and 19.8%. Furthermore, in the Radboudumc commonly 4 mm punch

biopsies are obtained from clinically difficult to diagnose inflammatory skin diseases. These biopsies are, thereafter, cut in the middle and evaluated at 2 levels. This protocol provides a larger sample size and is less time consuming for the pathologist compared to the examination of 5 levels. However, these 2 levels are both sectioned near the cut in the middle and are, therefore, located very close together. This does not offer a broad overview of the punch biopsy. For that reason, this method is not an appropriate alternative for histological investigation of a punch biopsy suspicious for BCC. In addition, BCCs most frequently occur in the facial area where a larger punch biopsy is not favoured due to the risk of a larger scar. In the present study, clinical recurrences and treatment failures to non-invasive treatments were only seen in a small number of the biopsied lesions, most probably due to a short follow-up time. The follow up period in our study was less than a year, whereas sBCC recurrences are reported up to 3-4 years post-treatment.[6-8] Therefore, we plan to extend our follow up period in order to investigate whether the detection of a more aggressive BCC subtype is associated with a higher number of recurrences.

MAL-PDT is considered to be a non-surgical treatment option for sBCC with good cosmetic outcome.[9] The current European MAL-PDT protocol consists of 2 treatment sessions one week apart and to be repeated at 3 months if required. This protocol requires at least 2 hospital visits, which is costly and unpractical for patients. In **chapter 2.2** a pilot study on the clinical efficacy of two MAL-PDT illumination schemes on the same day in sBCC is described. It was shown that MAL-PDT, using 2 illumination sessions on the same day, shows promising results in the treatment of sBCC. Interestingly, 5 clinical treatment failures and recurrences were noted, of which 3 appeared to be mixed types BCCs in the excision specimen. This is caused by sampling error or histological underdiagnosis of the primary punch biopsy. Two of the 3 mixed type BCCs were identified using a more extensive step-sectioning method, as described earlier. However, additional step sectioning is not able to detect mixed type BCCs as a result of sampling error of the punch biopsy location within the lesion. The usage of non-invasive diagnostic tools, such as the RCM, can reduce the risk of sampling error as it offers the possibility to visualise the entire lesion and distinguish different BCC subtypes.[10,11]

Aim 2: To investigate the applicability of in vivo reflectance confocal microscopy in non-melanoma skin cancer

Non-melanoma skin cancer, predominantly comprising BCC and SCC, is the most common type of malignancy in the fair-skinned population. Moreover, its incidence and thereby health care costs, are rising.[1] This emphasizes the importance of the appropriate diagnosis and treatment. Histological confirmation to establish the diagnosis is considered the gold standard. However, as stated above, punch biopsies obtained from skin lesions and histological examination of these biopsies are prone to sampling errors. To reduce the risk of sampling error and to make the

diagnosis more patient friendly and efficient, non-invasive diagnostic techniques can be used. In **Chapter 3** we investigated the applicability of *in vivo* RCM to diagnose NMSC non-invasively.

Early recognition of SCC is important, as it attributes to the majority of NMSC-related metastases and deaths.[12] However, clinical differentiation between invasive SCC, SCC *in situ*, its precursor lesions and variants can be difficult. **Chapter 3.1** describes the diagnostic and monitoring RCM features for entities within the continuum of AK and SCC. An extensive literature search revealed 25 eligible studies on AK (n=15), AC (n=1), EoQ (n=1), BD (n=7), SCC (n=8) and KA (n=2). Most studies were case series, followed by case-control studies, cohort studies and one case report. Overlapping RCM features for all these entities were scale, hyperkeratosis and atypical keratinocytes arranged in an atypical honeycomb pattern (mild atypia) or architectural disarray (severe atypia) in the stratum granulosum and stratum spinosum. Actinic keratosis and AC showed similar features including parakeratosis, poorly defined and irregular keratinocyte cell boundaries, exocytosis, dilated blood vessels and dermal solar elastosis. Bowens disease and EoQ also displayed (epi)dermal dendritic cells, whereas KA demonstrated round nucleated cells. Squamous cell carcinoma showed more extensive atypia in the spinous-granular layer, severe architectural disarray in the stratum granulosum and increased diameter of blood vessels compared to AK. Furthermore, nest-like structures can be found in the dermis in SCC. When using RCM as a monitoring tool after treatment, a decrease in epidermal atypia was observed in AK and AC. Overall, the range of sensitivity and specificity of RCM for the diagnosis of these entities was 79-100% and 78-100%. Unfortunately, a limited depth penetration in hyperkeratotic lesions imposes a major restriction for RCM evaluation. Careful curettage of the hyperkeratotic scale or pretreatment with keratinolytic agents can evade this problem.[13] Moreover, the majority of the studies within this systematic review reached a moderate methodological quality (using the Strengthening the Reporting of Observational studies in Epidemiology criteria (STROBE)). This was mainly due to the lack of description of potential sources of bias, statistical methods and descriptive data. A randomised controlled trial of high methodological quality, comparing RCM with punch biopsy or surgical excision for clinical suspected lesions in this particular area, can contribute to determining the diagnostic accuracy.

In daily clinical practice, AKs are frequently diagnosed. However, their clinical appearance can be similar to SCCs. Therefore, it is important to distinguish AKs from invasive SCCs. In **Chapter 3.2** we report the use of RCM in differentiating between these two entities. The presence of architectural disarray in the stratum granulosum in combination with architectural disarray in the stratum spinosum and/or tumour nests in the dermis were the main RCM features to distinguish SCC from AK. This study also showed a poor to no interobserver agreement between an experienced and inexperienced RCM user. This illustrates the importance to learn to interpret the RCM images for AK and SCC specific features. Pellacani *et al.* showed a significant correlation in grading RCM and histopathological keratinocyte atypia in AK, by experienced blinded RCM

observers.[14]A study on the learning curve of new RCM users in detecting specific RCM features will give more information on the time needed to invest in training in order to develop adequate education programmes. In addition, using the software available on the Vivascope systems (most widely used RCM device), it is possible to send the RCM images to experts in other centres for analysis and diagnosis. This contributes in the learning curve of beginning RCM users and increasing the number of centres that offer RCM as a non-invasive diagnostic method.

Up until now, multiple studies have addressed the role of RCM in diagnosing BCC.[10,11,15-22] It is postulated that RCM is more patient friendly due to its non-invasive character and more cost-effective compared to the gold standard using histology. In **Chapter 3.3** protocol for a prospective multicenter RCT is described which evaluates the non-inferiority of RCM to histopathology using 3 mm punch biopsies in diagnosing BCC and its subtype. Furthermore, the quality of life, costs and quality adjusted life years will be evaluated using several questionnaires. A total of 329 patients with clinically suspicious BCC lesions and eligible for RCM imaging will be included. Initially, 3 centers in the Netherlands consented in joining this study (Radboud university medical center, Nijmegen; Canisius-Wilhelmina hospital, Nijmegen; Rijnstate hospital, Arnhem). Thereafter, a fourth center joined the study (The Netherlands Cancer Institute, Amsterdam). Patients will be randomised into either the RCM or punch biopsy group. In case a BCC is diagnosed using RCM or punch biopsy, surgical excision will follow. This RCT started in January 2016 and is expect to finish at the end of 2018.

Aim 3: To investigate the applicability of (video)dermoscopy in inflammatory skin diseases

Inflammatory lesions can be difficult to diagnose due to their heterogeneity, with risk of sampling an inappropriate site with relatively few morphological changes to establish a specific diagnosis. Using (video)dermoscopy, a large surface area can be examined under a high magnification, non-invasively. Videodermoscopy offers the ability to use a magnification up to 1000x, allowing visualisation of skin details that are invisible to the naked eye. Therefore, (video)dermoscopy can improve the clinical diagnosis without the need for invasive skin biopsies.

In **Chapter 4.1** current literature was systematically reviewed on the value of (video)dermoscopy in the diagnosis and monitoring of common inflammatory skin diseases. After an extensive literature search, 82 publications were included in the following categories: spongiotic dermatitis, psoriasiform dermatitis, nodular and diffuse dermatitis, palisading granulomatous dermatitis, sclerosing dermatitits, bullous dermatitis, alopecia and miscellaneous inflammatory and reactive disorders. Of the included studies, there was one RCT of moderate methodological quality. The remaining articles were cross-sectional studies (n=35), case reports (n=25), case series (n=15), cohort studies (n=4) and case-control studies (n=2) of moderate to low methodological quality. Each diagnosis showed distinctive (video)dermoscopic features, but also depicted features that

are similar to other ISD. Compared to dermoscopy, VD was able to detect specific vascular patterns in skin lesions and in the nail hyponychium of psoriasis patients that were not visible using dermoscopy. Furthermore, VD can contribute to standardisation of dermoscopic imaging by calibrating external factors resulting in more reproducible images. Subsequently, this can enhance the clinical judgment of skin lesions. VD can also aid in establishing a diagnosis more objectively, with the use of software programs that have a standardised pattern algorithm built in.[23,24] Currently, the exact value of VD over conventional dermoscopy remains unclear.

General discussion and future perspectives

This thesis shows various methods to reduce sampling errors. When using histopathology for the diagnosis of sBCCs, additional sectioning of the diagnostic punch biopsy, will diminish the risk of underdiagnosis due to sampling error. However, development of a more timesaving and cost-effective histopathological procedure is required prior to implementation. Furthermore, the use of non-invasive diagnostic tools, such as RCM and VD, offer the possibility to establish a diagnosis without the need to obtain invasive biopsies, while potentially reducing the risk of sampling error.

In our opinion, the major clinical application of RCM lies in the field of dermato-oncology for both melanoma and NMSC. In the area of melanoma, Pellacani *et al.* showed that RCM is an useful technique when implemented in the standard care for melanoma in the university hospital of Modena and Reggio Emilia, Italy. It showed a significant reduction in the number of benign lesions excised when RCM was added as a diagnostic tool compared to the conventional medical care. Moreover, a reduction of the overall costs with 27% was observed.[25] In the field of BCC, a RCT has recently been published that showed a higher treatment convenience in patients in the RCM group (diagnosing and subtyping BCC using RCM, followed by direct surgery) compared to group receiving standard care (histopathological evaluation of a punch biopsy prior to excision).[26] The results of this study, combined with the future results of our ongoing RCT on the cost-effectiveness, will aid in incorporating RCM in the Dutch BCC guidelines and its implementation in the daily clinical practice.

In this thesis, the use of *in vivo* RCM has been studied in NMSC in order to reduce the risk of sampling error. However, *ex vivo* RCM can also be a technique to reduce this risk. *Ex vivo* RCM enables visualisation of the skin layers in the horizontal view or vertical view, depending on how the tissue is fixated in the device. Using *ex vivo* RCM, an entire punch biopsy or excised lesion can be imaged for the presence of a BCC and its subtype without the need for extensive tissue preparation, staining and sectioning. In order to resolve the limitation in laser penetration depth, both sides of the punch biopsy or excised tumour can be imaged. In addition, the biopsies or excised tissues can be cut in thinner pieces before imaging. Furthermore, different fluorophores

can be used to provide contrast in *ex vivo* RCM images in order to aid in the BCC diagnosis and subtyping.[27] Therefore, *ex vivo* RCM can be an alternative to additional step-sectioning of a punch biopsy, when it comes to reducing the sampling error within a punch biopsy. Other clinical applications of *in vivo* and *ex vivo* RCM include BCC margin determination pre- and peroperatively.[27-29]

In future, non-invasive diagnostic techniques will play a major role in dermatology. Though, more steps have to be taken to determine the value of the current available techniques, like RCM and VD, in the dermatological care. We have aimed to encourage others to use and further investigate the applicability of non-invasive imaging tools which will lead to implementation of these techniques in the daily clinical practice, resulting in more efficiency, more patient's comfort and reduction in health care costs.

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6

Nederlandse samenvatting

Nederlandse samenvatting

Het beschrijven van de morfologie van de huid speelt een belangrijke rol in de dermatologische diagnostiek. Tevens is het van belang om het effect van een behandeling te evalueren. Wanneer het niet mogelijk is om een diagnose te stellen op basis van het klinisch beeld, wordt veelal een punch biopt afgenomen. Een punch biopt wordt als de 'gouden standaard' gezien voor aanvullend onderzoek. Echter, een punch biopt is een invasieve diagnostische methode en kan daarmee leiden tot ontstekingen en littekenvorming van de huid. Daarnaast belemmert het afnemen van een huidbiopt de mogelijkheid om histopathologische veranderingen in de huid op dezelfde locatie te vervolgen in de loop van de tijd. Een veel voorkomend probleem is dat een punch biopt niet altijd representatief is voor de gehele laesie, waardoor een sampling error kan optreden. Een andere type sampling error ontstaat wanneer het meest agressieve of actieve deel van een punch biopt wordt gemist bij het histopathologisch onderzoek. Een sampling error kan resulteren in een aspecifieke diagnose of onder- of misdiagnose, hetgeen weer kan leiden tot inadequate behandelingen. Vanwege deze nadelen zijn in de afgelopen jaren nieuwe, niet-invasieve beeldvormende technieken ontwikkeld. Deze technieken maken het mogelijk om de morfologie van de huid in detail te evalueren zonder dat er een punch biopt noodzakelijk is. Van de beschikbare beeldvormende technieken bieden de *in vivo* reflectie confocale microscoop (RCM) en de videodermatoscoop (VD) de mogelijkheid om te huid te onderzoeken met een hoge resolutie. Het doel van de onderzoeken, die staan beschreven in dit proefschrift, was om de huidige protocollen van het diagnostisch proces te optimaliseren en de morfologie van de huid te onderzoeken met behulp van geavanceerde beeldvormende technieken.

Doel 1: Het onderzoeken van de sampling error in superficiële basaalcelcarcinomen en het klinisch effect hiervan

Het basaalcelcarcinoom is de meest voorkomende maligniteit in de Caucasische populatie.[1] De histologische subtypering van het BCC is van belang, omdat de behandeling per subtype kan verschillen. Het superficiële BCC (sBCC) kan worden behandeld met niet-invasieve therapieën, terwijl agressieve BCCs (micronodulaire BCC (mnBCC) en infiltratieve BCC (iBCC)) met een grotere marge geëxcideerd worden dan laagrisico BCCs (nodulaire BCC (nBCC) en sBCC). De Europese richtlijn geeft de aanbeveling om een punch biopt af te nemen bij klinisch suspecte BCCs.[2] Echter, in verschillende studies werd in 11% tot 39,1% van de gevallen bij excisie een agressievere BCC subtype gevonden, welke initieel gemist werd bij het punch biopt.[3-5] Dit kan het resultaat zijn geweest van een sampling error van het punch biopt. In **hoofdstuk 2.1** wordt de sampling error binnen een 3 mm punch biopt onderzocht. Alle onderzochte punch biopten hadden initieel de diagnose sBCC, gebaseerd op histopathologisch onderzoek van het punch biopt op 1 niveau. We vergeleken de nauwkeurigheid van het huidig histopathologisch proces (beoordeling van 1 niveau binnen een punch biopt) met een uitgebreid protocol (beoordeling van 5 niveaus). Histopathologisch onderzoek van 5 niveaus resulteerde in detectie van andere

BCC subtypes in 22,4%. Echter, het extra doorsnijden, kleuren en beoordelen van coupes op 4 additionele niveaus is tijdrovend, arbeidsintensief en duur in vergelijking met de huidige histopathologische methode. Daarom heeft een protocol dat minder uitgebreid is, maar alsnog een hoog aantal agressievere BCC subtypes detecteert, de voorkeur. Alternatieve protocollen zijn de evaluatie van 2 additionele niveaus (400 μm en 800 μm dieper dan de initiële H&E gekleurde coupes) of 1 additioneel niveau (800 μm dieper dan de initiële H&E gekleurde coupes). Dit levert een detectie van respectievelijk 20,7% en 19,8% op van andere BCC subtypes. Tevens kan een ander bestaand protocol als alternatief overwogen worden. In het Radboud universitair medisch centrum (Radboudumc) wordt doorgaans bij inflammatoire huidaandoeningen, die klinisch moeilijk te diagnosticeren zijn, een 4 mm punch biopt afgenomen. Deze biopten worden vervolgens centraal doorgesneden en op 2 niveaus bekeken. Het voordeel is dat in dit protocol een groter biopt wordt onderzocht en dat het voor pathologen minder tijdrovend is vergeleken met het protocol waarbij 5 niveaus wordt geëvalueerd. Echter, de 2 niveaus die bij de 4 mm punch biopten worden onderzocht, liggen zeer dicht bij elkaar. Hierdoor wordt geen goed overzicht verkregen van het gehele punch biopt. Om deze reden lijkt deze methode niet geschikt. Daarnaast ontstaan BCCs vaak in het gelaat, waar het afnemen van een grotere punch biopt niet gewenst is vanwege het risico op een groter litteken. Opvallend in deze studie was, dat maar een klein aantal klinische recidieven en therapie falen werd gevonden. De follow up periode in dit onderzoek was korter dan een jaar, terwijl recidieven tot 3-4 jaar na behandeling nog kunnen ontstaan.[6-8] Ons voornemen is om de follow up periode te verlengen om te onderzoeken of de detectie van een agressievere BCC subtype is geassocieerd met een hoger aantal recidieven.

Een niet-chirurgische behandeling voor sBCC, met goede cosmetische resultaten, is methylaminolevulinaat fotodynamische therapie (MAL-PDT).[9] De behandeling bestaat uit 2 behandelsessies met een tussenpoos van 1 week. Eventueel kan dit, indien nodig, na 3 maanden herhaald worden. Echter, dit protocol vereist minstens 2 ziekenhuis bezoeken. Dit is onpraktisch voor patiënten en kostbaar. In **hoofdstuk 2.2** wordt een pilot studie beschreven waarin de klinische effectiviteit van 2 MAL-PDT behandelsessies op 1 dag voor de behandeling van sBCC werd onderzocht. De studie laat veelbelovende resultaten zien. Echter, bij 5 patiënten sloeg deze therapie onvoldoende aan. Bij verder onderzoek bleken 3 van deze 5 sBCCs toch een gemengd type BCC te betreffen. Dit kan of het gevolg zijn geweest van een sampling error of van histologische onderdiagnose van het punch biopt. Twee van de 3 gemengde BCCs werden geïdentificeerd met behulp van het intensievere protocol (evaluatie van 5 niveaus), zoals eerder beschreven. Echter, dit protocol is niet in staat om gemengde BCCs te detecteren die het gevolg zijn van een sampling error waarbij het minst agressieve deel van de laesie is gebiopteerd. In dergelijke gevallen kunnen niet-invasieve beeldvormende technieken, zoals RCM, het risico op sampling error verkleinen. RCM biedt de mogelijkheid om de gehele tumor in beeld te brengen en verschillende BCC subtypen te onderscheiden.[10,11]

Doel 2: Het onderzoeken van de toepasbaarheid van in vivo reflectie confocale microscopie bij niet-melanoom huidkanker

Niet-melanoom huidkanker (NMSC), voornamelijk bestaande uit het BCC en plaveiselcelcarcinoom (PCC), is het meest voorkomende type kanker in de blanke populatie.[1] Pathologisch onderzoek met punch biopten wordt gezien als de 'gouden standaard' om deze typen huidkanker te diagnosticeren. Echter, dit onderzoek is gevoelig voor sampling errors. Niet-invasieve diagnostische technieken, waarmee de gehele tumor kan worden onderzocht, kunnen het risico op sampling error verkleinen. Daarnaast zijn deze technieken patiënt vriendelijker vanwege het niet-invasieve karakter en de mogelijkheid om direct bij het eerste consult de diagnose te kunnen stellen. In **hoofdstuk 3** wordt beschreven of RCM geschikt is om NMSC te diagnosticeren.

Vroegtijdige detectie van PCC is van belang vanwege het risico op metastasering en de daarmee samenhangende mortaliteit.[12] Echter, in de dagelijkse praktijk kan het klinisch onderscheid tussen invasief SCC, SCC *in situ* en de voorlopers en varianten van PCC soms lastig zijn. In **hoofdstuk 3.1** worden zowel de diagnostische als monitoring kenmerken van RCM beschreven voor aandoeningen binnen het continuüm van actinische keratose (AK) en PCC. Een uitgebreid literatuuronderzoek leverde 25 studies op die in het systematic review werden geïncludeerd; AK (n=15), actinische cheilitis (AC) (n=1), erytroplasia van Queyrat (EoQ) (n=1), Morbus Bowen (BD) (n=7), SCC (n=8) en keratoacanthoom (KA) (n=2). Het merendeel van de studies waren case-series, gevolgd door case-control studies, cohort studies en een case-report. Overlappende RCM kenmerken voor alle aandoeningen binnen dit continuüm waren schilfering, hyperkeratose en atypische keratinocyten gerangschikt in een atypische honingraatpatroon (milde atypie) of een onregelmatige architectuur (ernstige atypie) in het stratum granulosum en stratum spinosum. Actinische keratose en AC lieten soortgelijke kenmerken zien met daarbij ook parakeratose, slecht afgrensbare en onregelmatige keratinocyten celgrenzen, exocytose, gedilateerde bloedvaten en dermale solaire elastose. Morbus Bowen en EoQ vertoonden ook (epi)dermale dendritische cellen, terwijl in KA ronde nucleaire cellen te zien waren. Plaveiselcelcarcinomen vertoonden meer uitgebreide keratinocyten atypie in de strata spinosum en granulosum, onregelmatige architectuur in het stratum granulosum en bloedvaten met een grotere diameter ten opzichte van AK. Tevens kunnen bij PCC nestachtige structuren worden gevonden in de dermis. In AK en AC werd middels RCM een vermindering van de epidermale atypie waargenomen na een periode van behandeling. In het algemeen was de diagnostische sensitiviteit en specificiteit van RCM tussen de 79-100% en 78-100%. Een limitatie van RCM is de beperkte penetratiediepte van de laser, met name in hyperkeratotische laesies. Curettage van de hyperkeratotische schilfering of voorbehandeling met keratinolytische middelen is dan aangewezen.[13] Verder zijn het merendeel van de geïncludeerd studies in deze systematische review van matige methodologische kwaliteit (volgens de STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) criteria). Deze matige kwaliteit was voornamelijk het gevolg van gebrek aan omschrijving van potentiële bronnen van bias, uitleg over statistische methodes en descriptieve data. Een

gerandomiseerde gecontroleerde studie (RCT) van hoge methodologische kwaliteit, waarbij RCM met een punch biopt of chirurgische excisie wordt vergeleken op dit gebied, zou kunnen bijdragen aan de diagnostische nauwkeurigheid van de RCM.

In de dagelijkse praktijk presenteren patiënten zich vaak met AKs. Echter, het klinisch beeld van AK kan veel overeenkomsten vertonen met PCC. In **hoofdstuk 3.2** hebben we daarom RCM kenmerken beschreven die onderscheid kunnen maken tussen AK en PCC. De aanwezigheid van een onregelmatige architectuur in het stratum granulosum gecombineerd met een onregelmatige architectuur in het stratum spinosum en/of dermaal gelokaliseerde tumornesten zijn de belangrijkste RCM kenmerken om onderscheid te maken tussen AK en PCC. Deze studie laat tevens zien dat er een slechte tot geen interobserver overeenkomst is tussen een ervaren en onervaren RCM gebruiker. Dit geeft aan hoe lastig het is voor een onervaren RCM gebruiker om de specifieke RCM kenmerken voor deze aandoeningen te interpreteren. Pellacani *et al.* rapporteerden een significante correlatie tussen het classificeren van keratinocyten atypie in AK middels RCM en histopathologie door ervaren geblindeerde RCM gebruikers.[14] Een studie naar de leercurve van RCM gebruikers in het herkennen van specifieke RCM kenmerken zal meer informatie kunnen geven over hoeveel tijd geïnvesteerd moet worden aan training om zo adequate scholingsprogramma's te ontwikkelen. Bovendien is het mogelijk om met de Vivascope software (meest gebruikte RCM apparaat) RCM beelden naar experts in andere centra te versturen voor analyse. Dit draagt bij aan de leercurve van een beginnende RCM gebruiker en hierdoor kan het aantal centra toenemen dat niet-invasieve RCM diagnostiek aanbiedt.

Meerdere studies hebben de diagnostische rol van RCM bij BCCs bestudeerd.[10,11,15-22] RCM zou patiëntvriendelijker zijn vanwege het niet-invasieve karakter en de directe diagnosestelling, maar ook meer kosteneffectief zijn vergeleken met de huidige standaard methode voor diagnostiek middels histopathologisch onderzoek. In **hoofdstuk 3.3** wordt het protocol beschreven voor een prospectief multicenter RCT waarmee onderzocht wordt of RCM minstens zo effectief is in het diagnosticeren van een BCC en het subtype, als de huidige diagnostische standaard, het histopathologisch onderzoek van een punch biopt. Tevens worden de kwaliteit van leven, aangepaste levensjaren en de kosten onderzocht met behulp van vragenlijsten. In totaal zullen 329 patiënten met een klinisch suspecte BCC worden geïncludeerd. Aanvankelijk participeerde 3 centra in Nederland (Radboud universiteit medisch centrum, Nijmegen; Canisius-Wilhelmina Ziekenhuis, Nijmegen; Rijnstate, Arnhem). Later nam ook het Antoni van Leeuwenhoek ziekenhuis in Amsterdam deel aan de studie. Patiënten worden gerandomiseerd voor RCM of voor een punch biopt. Indien een BCC wordt gediagnosticeerd, zal deze chirurgisch worden geëxcideerd. De studie startte in januari 2016 en zal naar verwachting lopen tot eind 2018.

Doel 3: De toepasbaarheid van (video)dermatoscopie in inflammatoire huidaandoeningen

Inflammatoire aandoeningen zijn soms lastig te diagnosticeren vanwege hun heterogeniteit. Het risico bestaat dat een punch biopt wordt afgenomen van het deel van de laesie met relatief weinig morfologische veranderingen, waardoor het niet mogelijk is om een specifieke diagnose te stellen. Met behulp van (video)dermatoscopie kan een groot huidoppervlakte worden onderzocht onder hoge vergroting. Videodermatoscopie biedt de mogelijkheid om een het beeld tot 1000x te vergroten, waardoor details van de huid zichtbaar worden die niet met het blote oog kunnen worden waargenomen. Mogelijkerwijs zou (video)dermatoscopie de klinische diagnostiek verbeteren zonder de noodzaak van een invasief biopt.

In **hoofdstuk 4.1** wordt de rol van (video)dermatoscopie beschreven in het diagnosticeren en monitoren van algemene inflammatoire huidaandoeningen (ISD). In een uitgebreide literatuuronderzoek werden 82 publicaties geïncludeerd in de volgende categorieën: spongiotische dermatitis,psoriatiforme, perivasculaire dermatitis, nodulaire en diffuse dermatitis, palissaderende granulomateuze dermatitis, bulleuze dermatitis, alopecia en verscheidene inflammatoire en reactieve huidaandoeningen. Van alle geïncludeerd studies, was er één RCT van matige methodologische kwaliteit. Het resterend deel van de studies waren cross-sectionele studies (n=35), case reports (n=25), case series (n=15), cohort studies (n=4) en case-control studies (n=2) van matig tot laag methodologisch kwaliteit. Elke diagnose had onderscheidende (video)dermatoscopische kenmerken, maar had ook gemeenschappelijke kenmerken met andere inflammatoire huidaandoeningen. Met VD was het mogelijk om bij psoriasis patiënten specifieke vaatpatronen te detecteren in huidlaesies en de hyponychium van de nagel. Deze waren niet zichtbaar met de gewone dermatoscoop. Verder kan het gebruik van VD bijdragen aan het standaardiseren van dermatoscopische beelden omdat het kalibratie van externe factoren mogelijk maakt. Dit resulteert in reproduceerbare beelden, hetgeen een verbetering van het klinische oordeel van een laesie kan geven. Tevens zou met behulp van software programma's, met een ingebouwd gestandaardiseerd algoritme voor patronen, behulpzaam kunnen zijn bij het stellen van de klinische diagnose.[23,24] Echter, de meerwaarde van VD boven conventionele dermatoscopie is momenteel nog niet helemaal duidelijk.

Algemene discussie en toekomstperspectieven

De onderzoeken in dit proefschrift beschrijven verschillende methoden om het risico op sampling error te verminderen. Indien histopathologisch onderzoek als diagnostisch middel wordt gebruikt, zou het extra doorsnijden van het diagnostisch biopt dit risico kunnen verkleinen. Dit kan bijvoorbeeld het geval zijn als de primaire diagnose sBCC is, waarbij de meeste behandelingsmogelijkheden niet-chirurgisch van aard zijn. Hierbij zou een sampling error de reden kunnen zijn waardoor een behandeling niet aanslaat en waarna alsnog een chirurgische behandeling moet plaatsvinden.

Echter, verdere ontwikkeling van een meer tijdbesparend en kosteneffectief histopathologisch onderzoeksprotocol wordt geadviseerd. Daarnaast kan het gebruik van niet-invasieve diagnostische technieken, zoals RCM en VD, het mogelijk maken om een diagnose te stellen zonder dat invasieve biopten noodzakelijk zijn en waarmee het risico op sampling error vermindert.

Naar onze mening ligt de belangrijkste klinische toepassing van RCM op het gebied van dermato-oncologie, voor zowel melanomen als NMSC. Op het gebied van melanomen, toonde Pellacani *et al.* aan dat RCM een bruikbare techniek is wanneer het wordt geïmplementeerd in de dagelijkse patiëntenzorg voor melanomen. Door toepassing van RCM werd een significante reductie van het aantal geëxcideerde benigne huidlaesies ten opzichte van één melanoom aangetoond. Tevens werd een daling in de kosten gezien van 27%.[25] Op het gebied van NMSC, werd recent een RCT gepubliceerd die een hoger patiëntentevredenheid aantoonde in de RCM groep (diagnose en subtypering middels RCM, direct gevolgd door chirurgische excisie) vergeleken met de groep die de standaardzorg (histopathologische evaluatie van een punch biopt, gevolgd door excisie) ontving.[26] De uitkomsten van deze studie, gecombineerd met de resultaten van onze lopende studie over de kosteneffectiviteit, kunnen bijdragen aan het opnemen van RCM als een diagnostisch middel in de Nederlandse BCC richtlijn en de implementatie ervan in de dagelijkse dermatologische praktijk.

In dit proefschrift wordt de toepassing van *in vivo* RCM beschreven om het risico op sampling error te reduceren. Echter, *ex vivo* RCM zou hiervoor ook een geschikte methode kunnen zijn. Het is mogelijk om met *ex vivo* RCM de huidlagen in het horizontale of verticale vlak te visualiseren, afhankelijk van hoe het weefsel in het apparaat wordt gefixeerd. Middels *ex vivo* RCM kan een punch biopt of excisiepreparaat in zijn geheel onderzocht worden op de aanwezigheid van BCC nesten en subtypering zonder noodzaak tot uitgebreide weefselbewerking voor histopathologisch onderzoek. Beide zijden van het punch biopt of excisiepreparaat kunnen met *ex vivo* RCM onderzocht worden, of het weefsel kan worden doorgesneden. Op deze wijze is de beperkte penetratiediepte van RCM geen limitatie. *Ex vivo* RCM kan om deze reden tevens als alternatief dienen voor het uitgebreid histopathologisch protocol dat in dit proefschrift is beschreven. Verder kunnen bij *ex vivo* onderzoeken met RCM verschillende kleuringen gebruikt worden die kunnen bijdragen aan de verdere diagnostiek.[27] Andere klinische toepassingen van RCM omvatten *in vivo* en *ex vivo* bepaling van de vrije marge voor en tijdens de chirurgische excisie van een BCC.[27-29]

In de toekomst zullen niet-invasieve diagnostische technieken een grote rol spelen in de dermatologie. Echter, er zullen meer stappen ondernomen moeten worden om de rol van de huidig beschikbare technieken, zoals RCM en VD, te bepalen binnen de dermatologische zorg. Middels dit proefschrift willen wij andere onderzoeksgroepen stimuleren om de toepassingen van niet-invasieve beeldvormende technieken verder te onderzoeken en te ontwikkelen, hetgeen zal leiden tot de implementatie van deze technieken in de dagelijkse praktijk, resulterend in een efficiëntere patiëntenzorg, een hogere mate van patiënttevredenheid en een reductie van de zorgkosten.

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7

List of publications
Curriculum Vitae
Dankwoord
Toegift paranimfen
Portfolio
Research data stewardship and accessibility
List of abbreviations

List of publications

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**These authors contributed equally*

Curriculum vitae

Kim Nguyen werd geboren op 10 oktober 1988 te Oss. Hier groeide zij op in een Vietnamees gezin, samen met haar twee zusjes en jongere broertje. Nadat zij in 2007 aan het Maasland College haar tweetalig VWO diploma cum laude behaalde, begon zij aan de studie Geneeskunde aan de Radboud Universiteit, te Nijmegen. In het laatste jaar van deze studie verruilde zij Nederland tijdelijk voor Tanzania, waar zij een coschap tropengeneeskunde deed. Bij terugkomst, bestudeerde ze de reflectie confocale microscopie gedurende haar wetenschappelijke stage op de afdeling Dermatologie van het Radboudumc, te Nijmegen, onder leiding van dr. Gerritsen en dr. Peppelman. Na het behalen van haar artsexamen in 2014 ging zij aan de slag als basisarts in het Allergologie Centrum Arnhem/Velp. De aantrekkingskracht van de onderzoekswereld bleek echter te groot, waarop zij in juli 2015 startte met haar promotie onderzoek op het gebied van niet-invasieve technieken binnen de klinische dermatologie. De resultaten van haar onderzoek staan beschreven in verschillende wetenschappelijke publicaties en in dit proefschrift. Tevens heeft zij de onderzoeksresultaten gepresenteerd op verschillende nationale en internationale congressen, hetgeen tijdens de jaarlijkse bijeenkomst van de Nederlandse Vereniging voor Experimentele Dermatologie 2015 bekroond werd met de prijs voor beste presentatie. Tijdens haar promotie onderzoek was zij tevens werkzaam als ANIOS op de afdeling Dermatologie van het Radboudumc. Momenteel werkt zij als ANIOS Dermatologie in het Canisius-Wilhelmina Ziekenhuis, te Nijmegen.

Dankwoord

"Everything will be okay in the end. If it's not okay, it is not the end." (John Lennon)

Na twee mooie jaren is het dan zo ver, mijn proefschrift is af! Ik heb er met veel plezier aan gewerkt en had dit niet kunnen doen zonder de hulp en steun van velen. Graag wil ik iedereen bedanken voor hun geleverde bijdrage, getoonde interesse en bemoedigende woorden!
In het bijzonder wil ik nog graag de volgende personen bedanken:

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Prof. dr. dr. P.C.M. van de Kerkhof, beste professor; bedankt voor de mogelijkheid om dit promotietraject op uw afdeling te mogen hebben doorlopen en uw enthousiasme voor mijn onderzoek.

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RCM-team, lieve Esther en Lisa; bedankt voor de mooie basis die jullie hebben gelegd voor het RCM-onderzoek, waarop ik met mijn eigen visie mocht voortborduren. Ik wens jullie heel veel succes met jullie verdere carrières.

Dr. W.A.M. Blokx en G.J. Knuiman, beste Willeke en Jimmy; ik wil jullie bedanken voor de prettige samenwerking. Met behulp van jullie kennis, visie en kritische blik hebben we een aantal mooie artikelen op papier kunnen zetten. Nu jij nog Jimmy ;)

Inge, Lieke (paranimf), Tessa, Juul, Selma en Jorre: van offers brengen aan de Boeddha (in de hoop dat onze artikelen werden geaccepteerd) tot de (soms meerdere) softijs-breaks en de DJ-momenten op vrijdagmiddag, het is nooit saai met jullie in de bieb. Ik voel me bevoorrecht om in zó een fijne club te mogen werken. Helaas kan ik jullie niet allemaal paranimf maken, maar één voor één zijn jullie er voor mij geweest en dat betekent enorm veel voor me! Ik wens jullie allemaal veel succes met jullie onderzoeken en verdere carrières.

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Loes Vos: bedankt voor jouw inzet als medische studente met betrekking tot het onderzoek. We hebben samen een mooi resultaat weten neer te zetten!

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Rinke, Manon en Leonard: wat gaat er boven een stukje hardlopen om te ontstressen? Niets! Sinds de oprichting van ons Derma-hardloopclubje hebben we al een heleboel kilometers erop zitten (halve marathon Stevensloop, Zevenheuvelenloop). Maar het is vooral altijd gezellig!

Lieve papa, mama, Mai (paranimf), Long, Vivan, tante Thuy en Xinh; bedankt dat jullie er altijd voor mij zijn en voor alles wat jullie voor mij doen en gedaan hebben. Dat is mij alles waard! Ik hou van jullie.

Thưa ba má cùng với em Mai, em Long, em Vivan, dì Thụy, dì Xinh, tất cả các cô, bác, anh và chị. Kim rất là biết ơn tất cả mọi người đã giúp đỡ và dành những tình cảm tốt đẹp cho Kim để có ngày hôm nay. Kim yêu mọi người lắm.

Kim

Toegift paranimfen

Naast haar werk is Kim een liefhebber van Marvel films en geniet zij van borrelen met vrienden en collega's. Als geen ander kan zij mensen overhalen om toch nog één dansje mee te gaan doen. Op sportief gebied blijft ze zichzelf uitdagen met nieuwe sporten zoals hockey en surfen (vooral blijven oefenen), halve marathons en is het ''Radboudrondje'' een wekelijkse routine. Kim is daarnaast een wereldreiziger die tijdens het bezoek aan het ene werelddeel alweer plannen maakt voor een volgend continent. Ondanks een altijd veel te zware backpack weet zij met haar doorzettingsvermogen elke bergtop te bereiken. Toch is er nog ruimte voor verdere ontplooiing, met name op culinair gebied. Waar maaltijden bij haar ooit begonnen met een voorspelbare samenstelling van rijst en kip, weet zij haar vrienden nu te verrassen met culinaire variaties op haar voorkeursgroente; de paprika.

Voor ons is Kim een gedreven collega, vriendin en zus, met brede interesses, een gezonde eigenwijsheid, en veel loyaliteit. Met haar kundigheid, durf, en graag gebruikte sarcasme, zijn wij overtuigd dat ze dit proefschrift met verve zal verdedigen. We zijn trots op haar!

De paranimfen

Institute for Health Sciences
Radboudumc

Name PhD candidate:	Kim Nguyen Department of Dermatology	**PhD period:**	1-7-2015 until 1-7-2017
		Supervisor:	Prof. dr. PCM van de Kerkhof
		Co-supervisor:	Dr. MJP Gerritsen and dr. M. Peppelman
Graduate school:	Radboud Institute for Health Sciences		
Theme:	Healthcare improvement science		

		Year(s)	ECTS
	TRAINING ACTIVITIES		
a)	**Courses & Workshops**		
	- Introduction day Radboudumc	2015	0.5
	- EPIC training	2015	0.4
	- RIHS Introduction course	2016	1.0
	- RIHS Scientific Integrity course	2017	1.0
	- BROK course	2017	1.75
b)	**Seminars & lectures**		
	- Seminars at Dermatology Department	2015-2017	2.4
c)	**(Inter)national Symposia & congresses**		
	- Annual meeting Dutch Society for Experimental Dermatology (NVED), Lunteren (oral presentation)	2015	0.5+0.25
	- World congress of Dermoscopy and Skin Imaging, Vienna	2015	0.75
	- Annual meeting Dutch Society for Experimental Dermatology (NVED), Lunteren (poster presentation)	2016	0.5+0.25
	- International Symposium of the International Confocal Working Group, Madrid (poster presentation)	2016	0.5+0.5
	- Annual meeting Dutch Society for Experimental Dermatology (NVED), Lunteren (oral presentation)	2017	0.5+0.25
d)	**Other**		
	- Research meetings and Journal clubs at Dermatology Department	2015-2017	2
	- Presentation for companies (Galderma)	2016	1
	TEACHING ACTIVITIES		
e)	**Lecturing**		
	- Teacher CKO6v	2015-2016	1.2
f)	**Supervision of internships/other**		
	- Student coaching internship Scientific research	2015	2
	- Student coaching internship Dermatology	2015-2016	2
TOTAL			**19.3 ECTS**

Research data stewardship and accessibility

The data management of the studies included in this thesis followed the guidelines for the handling of research data established by the Clinical Research Center Nijmegen (CRCN). The guidelines comply with the Dutch Personal Data Protection Act (*De Wet Bescherming Persoonsgegevens, Wbp*) and the Code of Proper Conduct (*Code Goed Gedrag - FEDERA*).

The data management was applied in the following way:

1. Case Report Forms (CRFs) for data collection were designed by the investigators and subsequently approved by the ethics committee Regio Arnhem-Nijmegen (*Commissie Mensgebonden Onderzoek, CMO*). Each CRF contains demographical information, code and research data of a single volunteer. CRFs were designed in a paper version. These CRFs were kept in a locked cabinet at the Dermatology department of Radboud university medical center.

2. The codelists were created in both a paper and electronic version. The paper version was kept in a locked cabinet at the Dermatology department of Radboud university medical center, in a location different from the one used for the paper CRFs. The electronic version was saved in a password-protected folder on a server of the Dermatology department.

3. At the end of the study and after publication, the paper CRFs will be sealed and kept in the locked cabinet at the Dermatology department of Radboud university medical center. The paper version of the codelists will be printed and the name of the principal investigator, signature and date will be placed on the documents. Afterwards, these documents will be scanned into an electronic version, which will be saved in a password-protected folder on a server of the Dermatology department. The electronic codelists will be locked.

4. At the end of the study and after publishing the data, the data and codelists on paper will be archived at the Dermatology department for a period of one year. Thereafter, they will be archived in an external paper archive. The locked electronic data sets and codelists will be saved on a password-protected folder on a server of the Dermatology department.

5. At any time during and after the studies, access to the CRFs and codelist was restricted to the investigators, the principal investigator, the research nurse and the study monitors.

List of abbreviations

5-FU	5- Fluorouracil cream
AC	actinic cheilitis
ACD	allergic contact dermatitis
AD	atopic dermatitis
AK	actinic keratosis
AM	alopecia mucinosa
BCC	basal cell carcinoma
BD	Bowen's disease
CCCA	central centrifugal cicatricial alopecia
CHE	chronic hand eczema
CLE	chronic lupus erythematosus
CM	cutaneous mastocytosis
CS	cutaneous sarcoidosis
CU	common urticaria
DC	dissecting cellulitis
DLE	discoid lupus erythematosus
EoQ	erythroplasia of Queyrat
ER	erythematoteleangiectatic rosacea
FD	folliculitis decalvans
FFA	frontal fibrosing alopecia
GA	granuloma annulare
GP	guttate psoriasis
GF	granuloma faciale
GP	guttate psoriasis
H&E	hematoxylin and eosin
iBCC	infiltrative basal cell carcinoma
ICD	irritant contact dermatitis
ISD	inflammatory skin lesions
KA	keratoacanthoma
KIN	keratinocyte intraepithelial neoplasia
LE	lupus erythematosus
LP	lichen planus
LPP	lichen planopilaris
LS	lichen sclerosus
LSC	lichen simplex chronicus
LV	lupus vulgaris
MAL-PDT	methyl aminolevulinate photodynamic therapy

mnBCC	micro-nodular basal cell carcinoma
nBCC	nodular basal cell carcinoma
NL	necrobiosis lipoidica
NMSC	non-melanoma skin cancer
NVC	nailfold videocapillaroscopy
PALGA	Pathologisch Anatomisch Landelijk Geautomatiseerd Archief (registry of histo-and cytopathology in the Netherlands)
PCA	primary cicatricial alopecia
PD	polarised dermoscopy
PLC	pityriasis lichenoides chronic
PN	prurigo nodularis
PNCD	polarized light noncontact dermoscopy
PP	plaque psoriasis
PR	pityriasis rosea
PRP	pityriasis rubra pilaris
Radboudumc	Radboud university medical center
RCM	reflectance confocal microscopy
SB	stratum basale
sBCC	superficial basal cell carcinoma
SC	stratum corneum
SCC	squamous cell carcinoma
SD	seborrhoeic dermatitis
SDI	Standards for Dermatological Imaging
SG	stratum granulosum
SLE	systemic lupus erythematosus
SNFC	stereomicroscope nailfold capillaroscopy
SS	stratum spinosum
SSc	systemic sclerosis
STROBE	Strengthening the Reporting of Observational studies in Epidemiology
TMEP	teleangiectasia macularis eruptiva perstans
UP	urticaria pigmentosa
VAS	Visual Analogue Scale score
VC	verrucous carcinoma
VCAP	videocapillaroscopy
VD	videodermoscopy