Optical coherence tomography for non-invasive diagnosis of basal cell carcinoma

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CHAPTER 1

General introduction

Basal cell carcinoma (BCC) is the most common form of skin cancer. In Caucasian populations, one in five to six people will develop a BCC during their lifetime.[1-3] As the incidence of BCC continues to rise, it is an important health problem worldwide, involving high healthcare costs.

According to current (inter)national guidelines, lesions clinically suspected for BCC require a punch biopsy to confirm diagnosis and histopathological subtype.[4, 5] A punch biopsy is an invasive procedure with several disadvantages, including pain and discomfort for the patient, scarring, delay in the diagnostic process and costs for the healthcare system. Due to the high volume of BCCs, there is an increasing focus on novel diagnostic methods.

In an ideal situation, a non-invasive diagnostic method can (partly) replace the need for a biopsy, so that diagnosis and treatment of BCC can be accomplished in one day. This so-called 'one-stop shop' approach could thus result in a reduction of the number of biopsies and is expected to be more efficient, patient friendly and cost-saving than regular care. Implementation of such a non-invasive diagnostic method in clinical practice could also lead to a reduction in the workload for dermatologists. This is mainly due to less (telephone) consultations, which are needed to discuss the result of a punch biopsy with the patient. Extra consultation time is then created, for which there is a great demand. Hence, to enable this 'one-stop shop' approach, there is an increasing interest in non-invasive diagnostic methods, such as optical coherence tomography (OCT), reflectance confocal microscopy and Raman spectroscopy. Since BCC is a type of skin cancer with an indolent growth pattern, a good prognosis and a negligible risk of metastasis, it is ideally suited for a non-invasive approach. Among the abovementioned methods, recent results indicate that OCT is a promising method for diagnosis of BCC and practical in use.

Therefore, the studies in this thesis focus on OCT for non-invasive diagnosis of BCC. Both BCC and OCT are described in more detail below, as well as the different questions which are addressed.

Basal cell carcinoma

Basal cell carcinoma (BCC) is the most prevalent form of cancer. It is a slowly growing tumour, which is believed to derive from the epidermis, specifically the basal cell layer and hair follicle epithelium.[6] Although BCC rarely results in death or spread beyond the original tumour site, it can cause significant morbidity due to destructive local spread. Since most lesions are located in the head or neck area and are typically treated surgically, functional and cosmetic morbidity can be substantial.

Over the past decades, the incidence rates for BCC are increasing worldwide. In the Netherlands, the lifetime risk of developing a BCC is 16-20%.[3] Risk factors for the development of BCC comprise environmental, phenotypic and genetic factors. The most important environmental risk factor is ultraviolet radiation (UVR), which includes intense intermittent UVR exposure during beach holidays and outdoor activities, indoor tanning and UV-B and PUVA therapy. Other environmental risk factors are chronic exposure to the carcinogen arsenic, chronic use of immunosuppressive drugs and ionizing radiation therapy in the past.[7]

Phenotypic risk factors that increase the risk of developing a BCC include increasing age, male sex, light hair, eye and skin colour, a personal and/or family history of skin cancer, frequent childhood sunburns and signs of actinic damage. Apart from this, genetic factors also play a role. In the majority of BCCs somatic mutations can be found in the tumour suppressor genes patched 1 (PTCH1) and tumour protein 53 (TP53).[7] Certain genetic syndromes are also associated with the development of BCCs. The most common of these is Nevoid Basal Cell Carcinoma (synonyms: Gorlin; Basal Cell Nevus) syndrome, in which patients can develop hundreds of BCCs and a variety of developmental abnormalities. All the abovementioned factors may interact and increase the risk of developing a BCC.

Clinical and histopathological presentation of different BCC subtypes

Although there are many histopathologic subtypes, a simplified classification by Rippey roughly groups all BCCs into three subtypes: superficial, nodular, and aggressive.[8] More than half of all BCCs are of the nodular subtype.[6] Clinically, these present as papules or nodules with a pearly, shiny appearance with rolled borders and arborizing vessels (Figure 1a-b). Nodular BCCs are predominantly found in the head or neck area.[9] When the tumour enlarges, a central ulcer (rodent ulcer) may appear. Histopathologically, nodular BCCs demonstrate nests of basaloid cells in the papillary or reticular dermis with well-defined contours. Palisading of the peripheral row of cells is usually obvious, as well as retraction from surrounding stroma. Large nests may demonstrate central necrosis.[6, 8] Superficial BCC is the second most common subtype. It is mainly found on the trunk and extremities and clinically presents as a well-circumscribed, erythematous macule, patch, thin papule, or thin plaque with mild to moderate scaling and superficial ulceration. Sometimes a superficial BCC may be difficult to distinguish from actinic keratosis, Bowen's disease or benign lichenoid keratosis. Upon histopathology, relatively small nests of basaloid cells extend from the epidermis and hair follicle epithelium. These nests tend to have a broad base of attachment to the epidermis. Peripheral palisading and retraction spaces from surrounding stroma are often prominent and nests typically have a well-defined peripheral contour.[6, 8, 9]

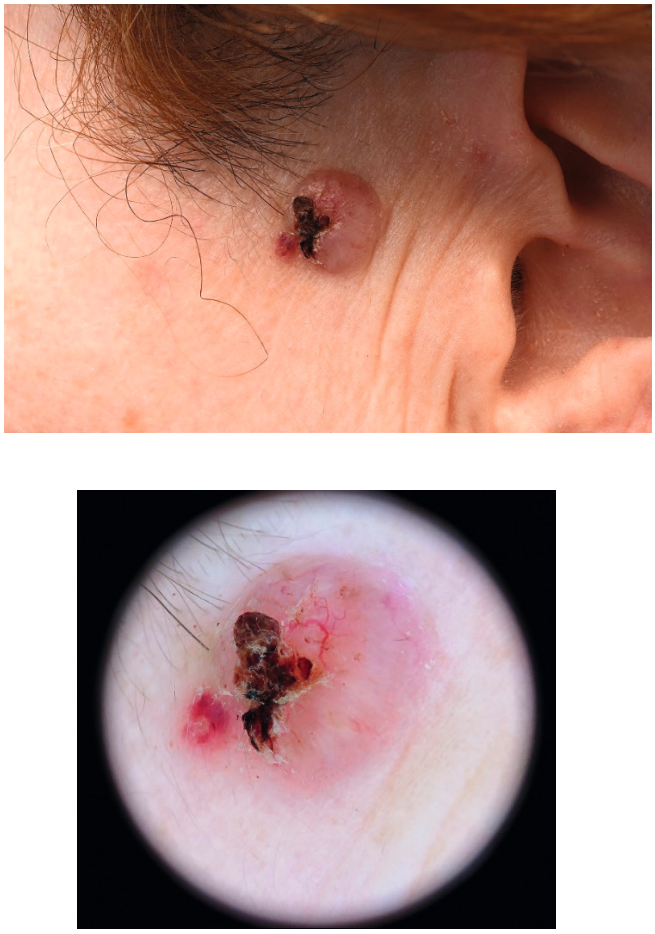

Figure 1 a-b. Clinical and dermoscopy image of a nodular basal cell carcinoma.

Aggressive BCC, which occurs less frequent, comprises the infiltrative, micronodular and basosquamous subtype. Clinically, aggressive BCC may be difficult to recognize. It may present as a pink-to-ivory-white, shiny, smooth, scar-like, indurated plaque or depression with poorly defined borders.[6, 9] Based on its clinical appearance, it is sometimes referred to as morpheaform BCC. Aggressive BCC, opposed to nodular and superficial BCC, tends to exhibit subclinical spread with the potential for extensive local destruction. Histopathologically, infiltrative subtypes demonstrate small and elongated basaloid cell nests. Peripheral palisading is rare or absent and the surrounding stroma is fibrous. The tumour usually invades the deeper dermis and may show invasion into the subcutaneous fat and muscle.

Micronodular BCC demonstrates rounded nests of basaloid cells similar to nodular BCC, but smaller and more dispersed. Basosquamous BCC is characterized by a mix of basaloid cells and squamous cells, representing a collision between two skin tumours with a more aggressive growth pattern.[6, 8]

Combinations of the abovementioned subtypes may be found within one tumour, which is then referred to as a BCC with mixed histopathology. These BCCs with mixed histology account for approximately 40% of all BCCs.[10, 11] Currently, the histopathological examination of a punch biopsy is the gold standard to discriminate BCC from alternative diagnoses and to determine the histopathologic subtype.[5] Accurate subtyping of BCC is important to decide on the optimal treatment strategy: superficial BCCs can be treated with topical creams or photodynamic therapy, in nodular BCC surgery is preferred, but margins can be small and for aggressive BCCs surgical excision with wide margins or Mohs'micrographic surgery are indicated.

Diagnostic methods

Clinical examination with the naked eye appears to be very sensitive for diagnosing BCC (90%). However, specificity is reported to be low (28.6-48.9%).[12, 13] Dermoscopy is a non-invasive technique for the diagnosis of skin lesions. A dermoscope is a handheld device which enables visualizing skin structures not visible to the naked eye. Several criteria for dermoscopic diagnosis of BCC have been described, including vascular structures, such as arborizing vessels, pigmented structures, such as blue-gray ovoid nests, and ulceration.[14-16] Addition of dermoscopy to clinical examination can increase specificity to 47.5-55.6%, compared to clinical examination alone.[12, 13, 17] However, the level of expertise with dermoscopy significantly affects diagnostic accuracy.[14] The abovementioned specificity estimates of clinical examination and dermoscopy are lower than rates reported elsewhere, since these studies included unclear lesions and lesions with a clinical suspicion of BCC, which not always tend to display distinct features on dermoscopy.[12, 13, 17] With regard to discrimination between superficial and non-superficial BCC subtypes, clinical examination shows a sensitivity of 89% and a specificity of 64%.[18] For dermoscopy, data on diagnostic accuracy for subtyping of BCC is not available.[14] Since clinical and dermoscopic examination are not optimal, most patients still undergo a punch biopsy to establish BCC diagnosis and to determine the histopathologic subtype.[5, 19]

Punch biopsy

A punch biopsy is a small surgical procedure that acquires tissue for histopathologic examination by taking a punch-size piece of skin (usually 3mm) from the body. It is a relatively low-risk procedure that is typically performed after local anaesthesia with lidocain 1% (1-2ml). A punch biopsy may be painful and is sometimes complicated

by bleeding, infection and scarring. When, afterwards, non-invasive treatment is initiated, the scar of the biopsy can still be visible. Besides the inconvenience of a biopsy, the time needed to process the punch biopsy, histological assessment and informing the patient usually takes one to two weeks, which causes treatment delay. After discussing the results with the patient in a new (telephone) consultation, treatment is initiated.

Optical coherence tomography

In recent years, non-invasive diagnostic methods have become available for diagnosis of skin cancer. Especially BCC is ideally suited for such an approach, as explained above. OCT is a non-invasive imaging method, which is based on light and optics. In ophthalmology, OCT is a routinely used and well-established diagnostic tool. OCT was first introduced in dermatology in 1997 by Welzel et al.[20]

To date, studies on various skin diseases have been conducted, of which skin cancer, in particular, non-melanoma skin cancer (NMSC), is the most comprehensively investigated topic. Below, a brief description of the technology behind OCT is provided.

Technical aspects

OCT is a non-invasive imaging method capable of generating real-time, in-vivo, cross-sectional images of skin. Imaging principles between OCT and ultrasound imaging are similar. Ultrasound uses sound waves, whereas OCT uses an eye-safe infrared (1305nm) laser light source to obtain an image. The use of light instead of sound results in a higher resolution than ultrasonography. The driver for advances in OCT technology for imaging skin has been the clinical interest in imaging skin cancer, in particular BCC. Tumour nests are visible in OCT images of BCC, however, other features can be easily mistaken for tumour nests in OCT images, such as hair follicles, cysts and sebaceous glands. Therefore, high image resolution to successfully distinguish between these features was desired. Initial studies used OCT systems, which provided images of insufficient detail to achieve adequate sensitivity and specificity for NMSC diagnosis.[21, 22] Nevertheless, these studies demonstrated that clinically useful image features could be visualized and further improvements in resolution would likely yield useful results. This encouraged further development of higher resolution OCT systems.

The Vivosight OCT device (Figure 2 and 3) is the first practical and commercially available OCT system, which was launched in 2010 by Michelson Diagnostics. It uses a multi-beam technology: four beams of light, each focusing on a different depth, give the user not only a higher resolution but also a deeper penetration depth (1.0-1.5 mm) compared to a single beam system. With the Vivosight OCT device, a lateral (horizontal) resolution of <7.5 µm and an axial (vertical) resolution of <5µm can be achieved.

Figure 2. Vivosight OCT system (left) and its application in clinical practice (right).

OCT utilizes the property of coherence of laser light to detect backscattered light from tissue. OCT-emitted light is divided into two beams: a reference beam and a probe beam (Figure 3). The reference beam is directed to and reflected by a mirror system. The light in the probe beam is focused onto the skin. Backscattered light from the subsurface tissue is collected and made to interfere with the reference beam. Only backscattered photons that have retained their coherence by not being multiply scattered within the tissue will constructively interfere and generate a large signal response at the photo detector. The collected interference signal at the photo detector is transmitted to a computer to generate the image. Furthermore, the interferometer provides the depth information at which these photons were backscattered, thus enabling a 2-D image of the scattering centres within the tissue to be constructed. OCT provides cross-sectional images of the superficial skin resembling histopathological sections. Therefore, OCT is sometimes referred to as an 'optical biopsy', as it aims to provide histopathologic information non-invasively.[23-26]

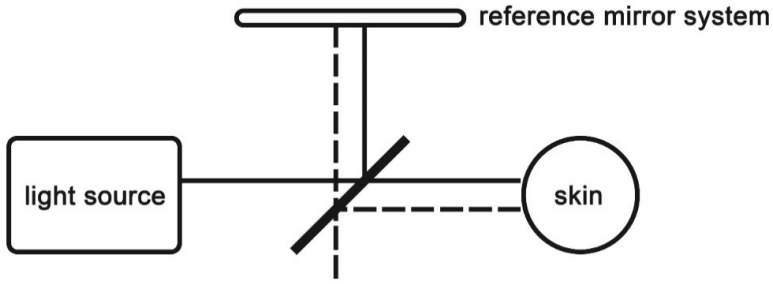

Figure 3. Typically, an OCT system is based on a Michelson interferometer. A fraction of the light source is directed to the skin and the remaining part to the reference mirror system (solid line). The backscattered light (dashed line) from both beams interferes on a photodetector.

Practical aspects

The handheld probe captures a 6 mm x 6 mm scan within only 15 seconds. This scan comprises 120 individual cross-sectional (vertical) OCT images. As mentioned before, these vertical images allow to assess the skin in the same direction as a histopathological section. For clinicians and dermatologist who are already used to histopathological assessment, the scans might therefore be easier to interpret. As each OCT investigation takes only few minutes, and the device can easily be moved, it is a practical tool. Although the resolution of OCT is not high enough to show individual cells, it is suitable for pattern recognition in tissue. Subsurface structures, including the epidermis, dermal-epidermal junction (DEJ), dermis, hair follicles, blood vessels and sebaceaous glands, can be recognized by different shades in the black and white spectrum. Contrast is provided by refractive index differences between cells and surrounding tissue.

Appearance of healthy skin on OCT

The appearance of healthy skin on OCT is shown in Figure 4. The entrance signal can be visualized as a narrow bright (hyperreflective) band caused by the shift from one medium (air) to another (skin). In some skin areas, such as the skin of the palms and soles of the hands and feet, the stratum corneum can be identified just below the entrance signal. The epidermis is usually the first distinguishable layer on the OCT scan, appearing as a dark (hyporeflective) homogenous layer with a well-defined border towards the papillary dermis. Skin appendages appear as hyporeflective discontinuations of the epidermis. The dermis has a brighter (hyperreflective) appearance, mainly due to collagen content, with hyporeflective cavities corresponding to skin appendages and vessels. The DEJ is seen as a clear transition in contrast between the epidermis and dermis.[27-29]

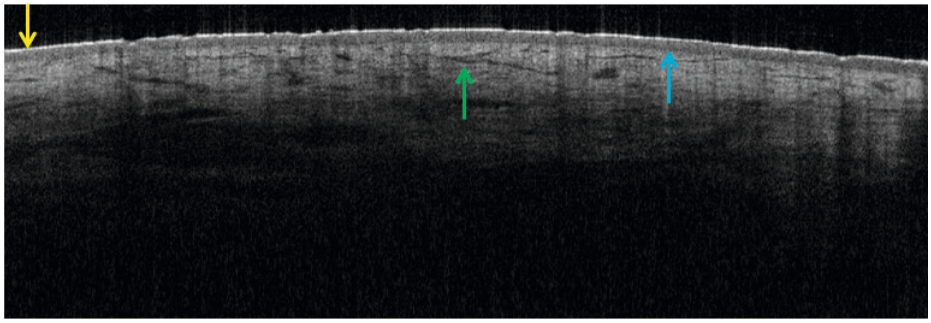

Figure 4. OCT image of healthy skin from the lower leg. The thin hyperreflective band (yellow arrow) represents the entrance signal. The DEJ is well-defined (blue arrow). Vessels are visualized as hyporeflective structures in the dermis with tapering ends (green arrow).

Appearance of BCC on OCT

Morphological features of BCC can be recognized on OCT images and include the following: rounded dark (hyporeflective) structures in the upper dermis, surrounded by a hyperreflective halo, sometimes surrounded by a hyporeflective border and changes or disruption of the DEJ.[28] The rounded dark structures resemble the basaloid cell nests seen upon histopathology, the hyperreflective halo surrounding the rounded structures corresponds to the surrounding tumour stroma and a hyporeflective border at the periphery resembles the peripheral palisading at the margins of basaloid cell nests.[29] In some larger basaloid cell nests, necrosis can be visualized as well-circumscribed hyporeflective to areflective areas.[30] Protrusions into the upper dermis with a dark rim, as shown in Figure 5a, are visible in superficial BCC, representing basaloid cell nests with a firm connection to the epidermis, disrupting the DEJ.[31] Nodular BCCs can be identified by fully encompassing signal-poor ovoid structures located in the dermis (Figure 5b). Smaller signal-poor ovoid structures with bright peritumoural stroma, also described as 'shoal of fish' or 'bunch of grapes' appearance, indicate an aggressive BCC subtype (Figure 5c).[12]

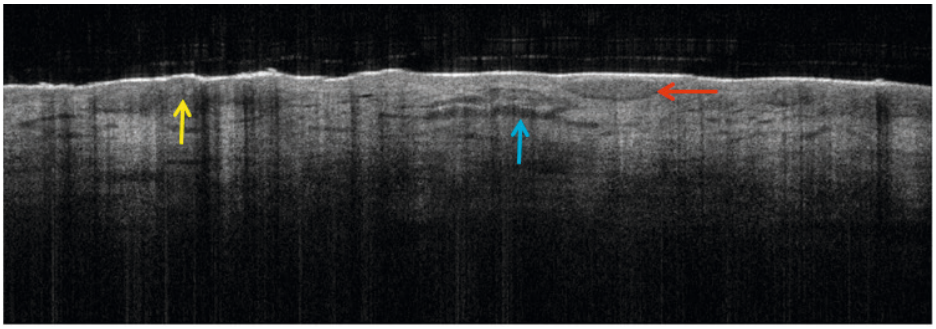

Figure 5a. OCT image of a superficial BCC. Red arrow points towards a protrusion into the upper dermis with a dark rim. Vessels in the upper dermis are dilated (blue arrow) and the DEJ is disrupted (yellow arrow).

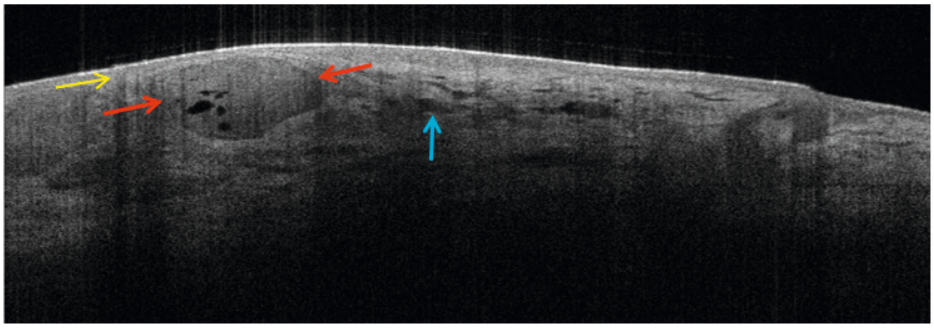

Figure 5b. OCT image of a nodular BCC. Red arrows point towards a signal-poor ovoid structure with a dark rim and bright peritumoural stroma. Inside the nest, black areas are observed, representing signs of liquefactive necrosis. Vessels in the upper dermis are dilated and directed towards the tumour nests (blue arrow). The epidermis above the nest is atrophic, the DEJ is disrupted (yellow arrow).

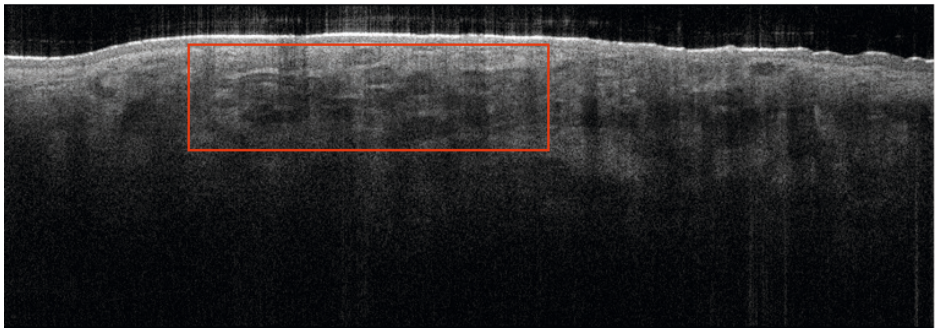

Figure 5c. OCT image of an aggressive BCC. Red rectangle represents an area where the morphologic feature 'shoal of fish' is visible.

Diagnostic accuracy of optical coherence tomography

Previous studies have shown that OCT allows for good discrimination of BCC from non-BCC lesions. Diagnostic accuracy has been evaluated in several studies, demonstrating quite large ranges in sensitivity (58-95.7%) and specificity (43-96%).[12, 13, 17, 32] In a recent prospective observational study conducted at our department, we found a specificity of 76.8% with a sensitivity of 95.2% for diagnosis of BCC.[17] With regard to BCC subtyping, OCT has the ability to distinguish between superficial BCC and other BCC subtypes in patients with a clinical suspicion of superficial BCC.[31, 33] Furthermore, OCT is investigated for the management of BCCs, namely as a tool for treatment monitoring as well as for the pre-surgical margin assessment of tumour resection margins.[33]

OCT seems a promising method for non-invasive diagnosis of BCC. OCT diagnosis, if made with high confidence, can obviate the need for biopsy, thereby enabling a so-called one-stop-shop approach. Currently, guidelines recommend to obtain a punch biopsy of clinically suspected lesions for histological verification of BCC diagnosis and subtype. The optimal treatment depends on the histopathological subtype: topical treatment (e.g. imiquimod) can be initiated for superficial BCC, whereas nodular and aggressive BCCs are usually treated with surgical excision.[5, 19] Replacing a punch biopsy with OCT will prevent the delay caused by awaiting histopathological results and treatment options can be discussed with the patient within the same consultation. For this approach, it is necessary that OCT diagnosis can be made with high confidence in the majority of patients and that the diagnostic accuracy of a high confidence OCT diagnosis is substantial. Prior to wide scale implementation of OCT in clinical practice, proper evaluation whether use of OCT is non-inferior, safe and cost-effective compared to regular care punch biopsy is warranted.

Treatment

Different treatment options are available for BCC. Treatment depends on the subtype, localization and whether it is a primary or recurrent BCC. Furthermore, age and cosmetic results are considered when deciding on the treatment strategy.

Surgical excision

Currently, surgical excision is still the gold standard treatment for BCC regardless of the histopathological subtype, since it has the highest clearance rate.[4, 34, 35] Since the risk of developing one or more subsequent BCCs in the first 5 years following diagnosis of the first BCC is approximately 30%, this leads to multiple excisions and scar formation. Surgical excision is generally performed in a hospital setting, where the tumour is excised under local anaesthesia. For superficial and nodular BCC, a 3mm clinical safety margin is accepted in the Netherlands, whereas aggressive BCC subtypes require excision with a 5mm clinical safety

margin.[5] Following excision, the tissue specimen is examined by the pathologist to assess whether the tumour is completely excised. One of the advantages of surgical excision is that histopathological examination following excision provides information on the tumour characteristics and whether the tumour was completely removed. Although recurrence rates 5 years after surgical excision are very low (ranging between 2-8%), there are several disadvantages of this treatment.[34, 36] There is the possibility of a complication (postoperative infection, bleeding, pain) and surgical excision leads to development of a scar. Furthermore, suture removal requires an extra hospital visit or visit to the general practitioner and the stress associated with a surgical procedure should also not be underestimated, especially in the elderly population.

Mohs' micrographic surgery

Mohs' micrographic surgery (MMS) is a single-day, outpatient procedure in which evaluation of 100% of the resection margins leads to complete tumour removal and preservation of healthy tissue.[37] MMS is performed with an increasing frequency, especially in the head and neck area, due to the high cure rates combined with the tissue sparing capacity.[38] Before starting the procedure, tumour margins are set on the basis of clinical and dermoscopic examination. Subsequently, the tumour is excised with a minimal safety margin and processed with frozen sections.[37] Histopathological assessment of the frozen sections takes place immediately and if remaining tumour is identified, only that specific part is removed, and the procedure is repeated.[39] Due to the time needed to make the frozen sections and the possibility of multiple consecutive stages, MMS is a labor-intensive and time-consuming procedure. Consequently, there are limitations in the number of patients that can be treated per day and the clinical condition of the patient may be a contraindication for this treatment.[40]

Imiquimod

Due to the disadvantages of surgical excision, non-invasive treatment options are interesting as alternative treatment, especially in patients with multiple BCCs. Currently, several non-invasive treatments are available for the treatment of superficial BCC, including imiquimod cream, 5-fluorouracil cream and photodynamic therapy. In a large randomised controlled trial, the effectiveness of these three treatment options were compared for the treatment of superficial BCC.[41] Five years after treatment, imiquimod cream was associated with a significantly lower risk of tumour recurrence compared to 5-fluorouracil cream and photodynamic therapy. Imiquimod cream is therefore considered as the most effective non-invasive treatment, with a 5-year clearance rate of 82.5%.[42] Hence, only a detailed description of this non-invasive treatment will be provided. Imiquimod is a topical immune response modifier. The effects of imiquimod are mediated through agonistic activity towards toll-like receptors 7 and 8, which are located on antigen-

presenting cells.[43] Stimulation of these receptors by imiquimod leads to activation of the central transcription factor, nuclear factor kappa B (NF-kB). This activation leads to the production and release of several pro-inflammatory cytokines and chemokines (e.g. interferon gamma (INF-γ), interferon alpha (IFN-α) and tumour necrosis factor alpha (TNF- α) and interleukines). These cytokines and chemokines stimulate the innate and acquired immune system, resulting in activity against tumour cells.[44, 45] Besides its mode of action via the immune system, imiquimod also binds to the Fas receptor, thereby enhancing susceptibility of tumour cells to apoptotic stimuli.[45, 46]

For treatment of superficial BCC, imiquimod 5% cream (Aldara®, Meda Pharmaceuticals) is applied once daily, five days in a row during six consecutive weeks. Side effects include erythema, erosions, crusting, oedema, vesicles and occasionaly, flu-like symptoms. Usually, side effects do not require treatment and resolve when imiquimod application is stopped.

Aims of this thesis

The primary aim of this thesis is to evaluate optical coherence tomography as a non-invasive diagnostic method for diagnosis and subtyping of basal cell carcinoma. **Chapter 2** includes studies that aim at providing evidence as to whether OCT guided diagnosis and treatment of patients with clinical suspicion of BCC is non-inferior to regular care (always punch biopsy), leads to cost reduction and is preferable to biopsy for patients. In **Chapter 3**, the possibilities for application of OCT in Mohs surgery and specific populations are explored. **Chapter 4** consists of studies that seek to find out how BCC diagnosis with OCT can be improved.

Chapter 2. (Cost-)effectiveness of optical coherence tomography for non-invasive diagnosis of basal cell carcinoma and patient preferences

Chapter 2.1 describes the results of a prospective non-inferiority randomised multi-centre trial with a follow-up period of 12 months. The trial had the aim to explore whether an OCT-guided diagnosis and treatment of patients with clinical suspicion of BCC is non-inferior to regular care and whether this alternative strategy is cost-effective. Patients were randomised to either regular care or OCT-guided diagnosis and treatment. In patients assigned to regular care, the choice for treatment was always guided by the histological result of a punch biopsy. In the OCT group, the treatment choice was based on the OCT diagnosis, if this diagnosis could be made with high confidence. If there was still doubt after examination with OCT, patients still received a punch biopsy. The primary outcome was the probability of remaining free from a recurrent or residual (pre-)malignant lesion 12 months after treatment. Secondary outcomes were the proportion of patients in whom biopsy could be avoided, the frequency of misclassifications, diagnostic accuracy of high

confidence OCT diagnosis and cost-effectiveness of an OCT-guided diagnostic strategy compared to punch biopsy from a healthcare perspective.

In **Chapter 2.2,** we describe the results of a discrete choice experiment, which we performed in order to examine patient preferences for OCT or punch biopsy as diagnostic strategy for BCC. Prior to implementation of new technologies in clinical practice, it is relevant to obtain insight into patient preferences, since this may indicate whether an innovation, in this case a non-invasive diagnostic method, will be accepted by patients in clinical practice.[47, 48]

Chapter 3. Possibilities for application of optical coherence tomography in Mohs surgery and specific populations

A systematic review provides recommendations for the use of OCT to delineate BCC prior to MMS.[33] Two case reports and five case series included in this systematic review describe promising results for the use of OCT to delineate in-vivo BCCs.[33] These few studies with a small number of patients provide no estimates of sensitivity and specificity. Hence, we conducted a case-control study, which is described in **Chapter 3.1**, to estimate the sensitivity and specificity of OCT for the in-vivo assessment of MMS margins for primary BCC.

In **Chapter 3.2** we describe a patient with a periocular BCC, which was diagnosed with OCT. There were no clinical and dermoscopic signs of BCC, but with OCT it was possible to establish a diagnosis in this vulnerable skin area.

Certain subgroups of patients, including patients with a very high clinical suspicion for a low-risk BCC or patients with multiple BCCs, undergo direct surgical excision without prior histopathological verification of BCC diagnosis.[4, 19] The aim of **Chapter 3.3** is to investigate whether in this subgroup of patients, OCT has additional diagnostic value and can help to reduce the risk of misclassification of non-BCC lesions as BCC.

Chapter 4. Optimization of the diagnostic accuracy of optical coherence tomography for diagnosis of basal cell carcinoma

OCT does not provide a resolution with which it is possible to visualize individual cells, but it is suitable for pattern recognition in tissue similar to e.g. ultrasound. Hence, morphological features of BCC can be identified on an OCT scan. In the past years, numerous features have been established.[12, 28, 31, 49, 50]

It remains unknown which features are most discriminative for BCC diagnosis. Therefore, **Chapter 4.1** evaluates which OCT features can best discriminate between BCC and non-BCC lesions and between BCC subtypes and whether

use of a combination of a small set of the most discriminative features results in adequate diagnostic performance.

In **Chapter 4.2**, we evaluate whether topical application of glycerol, a so-called optical clearing agent, can increase penetration depth and improve the image quality and visibility of characteristic BCC features on OCT images.

For optimal implementation of OCT in clinical practice, several problems need to be addressed. A study by Olsen et al. showed that the diagnostic accuracy of OCT varies greatly among assessors, partially due to differences in experience.[32] Therefore, back up from OCT experts for novice assessors might be valuable, but such experts are not yet readily available in all dermatology departments. Consequently, a clinical scenario is imaginable in which OCT experts at a distance are consulted for re-assessment of OCT scans. As these experts will have to interpret OCT scans without visual information on the suspected lesion, the question rises to what extent they can optimize the diagnostic process.

In **Chapter 4.3** we aim to assess the diagnostic accuracy of high confidence OCT diagnosis of a novice assessor, who obtained and interpreted OCT scans in combination with direct visual inspection of the lesion. A second aim is to evaluate whether diagnostic performance could be improved by back-up of an OCT expert at a distance who has no visual information on the suspected lesion. The frequency and nature of discrepancies between both OCT assessors will be further explored.

Finally, the results as described in this thesis are summarized and discussed in **Chapter 5**. Future perspectives are provided.

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CHAPTER 2

(Cost-)effectiveness of optical coherence tomography for non-invasive diagnosis of basal cell carcinoma and patient preferences

CHAPTER 2.1

Optical coherence tomography versus punch biopsy for diagnosis of basal cell carcinoma: a multicentre randomised non-inferiority trial and cost-effectiveness analysis

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ABSTRACT

Background: Punch biopsy is the gold standard for diagnosis and subtyping of basal cell carcinoma (BCC). The aim of this study was to assess if use of optical coherence tomography (OCT), a non-invasive imaging tool, may obviate the need for biopsy.

Methods: In a multi-centre randomised non-inferiority trial, patients (18 years or older) with an indication for biopsy of a suspected BCC outside the H-zone of the face were randomly assigned (1:1) to OCT or punch biopsy, via a web-based randomisation system. Stratification factors were participating centre and the grade of clinical BCC suspicion (high versus low). The primary outcome was the proportion of patients free from a recurrent/residual (pre-)malignant lesion 12 months after treatment. The incremental cost-effectiveness ratio (ICER) was expressed as the incremental costs per additional patient free from a recurrent or residual (pre-)malignant skin lesion 12 months after treatment. Modified intention-to-treat (mITT) and per-protocol analyses were conducted. This trial is now closed and registered with ClinicalTrials.gov number, NCT03848078.

Findings: Between February 25, 2019, and September 2, 2020, 598 patients were randomised with the primary outcome available in 553 patients. After median follow-up of 12.7 months (IQR 11.2-14.1) in the OCT group and 12.6 months (IQR 10.8-14.3) in the regular care group, 94.4% (253/268) patients in the OCT group were free from a recurrence/residue compared to 93.3% (266/285) patients following regular care. According to mITT analysis, the absolute difference (OCT versus regular care) was +1.07% (95% CI: -2.93 to 5.06) with the lower limit of the 95% CI not exceeding the predefined non-inferiority margin (-10%). Total mean costs for the OCT strategy were €689 versus €758 for regular care.

Interpretation: OCT-guided diagnosis and treatment of BCC is non-inferior and cost-effective compared to regular care with punch biopsy. Implementation of OCT for diagnosis of BCC reduces the number of consultations, invasive procedures and it is cost-saving.

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INTRODUCTION

In Caucasian populations, one in five people will develop a basal cell carcinoma (BCC).[1, 2] For diagnosis of lesions with a clinical suspicion of BCC, guidelines recommend a punch biopsy to guide the decision on optimal treatment.[3, 4] Histopathological diagnosis is important to distinguish between BCC and non-BCC lesions and to determine the histopathological subtype. In case of superficial BCC, topical therapy may be prescribed, whereas in non-superficial BCC, the width of resection margins or an indication for Mohs' micrographic surgery is based on the subtype. Besides the inconvenience of a biopsy, awaiting histopathological examination causes treatment delay. Recently, optical coherence tomography (OCT) has emerged as a promising non-invasive tool for BCC diagnosis, generating real-time in-vivo cross-sectional images of tissue microarchitecture with a depth of 1.0-1.5 mm.[5] OCT is based on light interferometry: the interference of two optical beams reflected by tissue produces distinguishable shades in the black and white spectrum, which allows the identification of morphological BCC characteristics.[6]

OCT might obviate the need for biopsy if an OCT diagnosis of BCC and subtype can be made with high confidence.[7-9] A treatment plan can be made instantly and only in case of doubt, a biopsy is taken for diagnosis. Following this strategy, it has been reported that a punch biopsy could be omitted in 30-40% of patients, with limited risk of misclassification.[7-10] There is a small risk that non-BCC lesions are misdiagnosed as BCC or that nodular or aggressive BCC subtypes are underdiagnosed as superficial BCC by OCT.

To date, it remains unclear to what extent misclassifications would result in a higher risk of treatment failure. We therefore initiated a randomised controlled trial to rule out that OCT-guided diagnosis and treatment results in an unacceptable increase in treatment failures when compared to regular care. The primary objective of this study was to evaluate whether OCT-guided diagnosis and treatment of clinically suspected BCC is non-inferior to regular care, where diagnosis and treatment is based on histopathological examination of a biopsy. Secondary objectives were to evaluate the diagnostic accuracy of high confidence OCT diagnosis for discrimination of BCC from other diagnoses and to distinguish superficial from non-superficial BCC when it is used in combination with clinical and dermoscopic examination. Furthermore, we investigated the cost-effectiveness of an OCT-guided diagnostic strategy compared to punch biopsy from a healthcare perspective.

METHODS

Study design and participants

For this multicentre, prospective randomised non-inferiority trial consecutive patients were included who visited the dermatology departments of one academic and two general Dutch hospitals. Eligible for participation were adult patients (18 years or older) with an indication for biopsy of a lesion with BCC in the differential diagnosis based on clinical and dermoscopic examination, including lesions in which BCC diagnosis was considered, but where another benign or (pre-) malignant diagnosis was also possible as well as lesions with a high suspicion for BCC, but where doubt remained about BCC subtype. The grade of clinical and dermoscopic BCC suspicion was based on the treating physician's judgement prior to randomisation. Excluded were patients in whom the diagnosis of BCC was so evident, that the lesion could be treated directly without prior biopsy, patients with lesions located in the 'H-zone' of the face or with locally advanced BCC and patients who were incompetent to sign informed consent. All patients provided written informed consent before randomisation. The trial was performed according to the principles of the Declaration of Helsinki, and the protocol and two amendments were approved by the Medical Ethical Committee of MUMC+ (METC 18-043). The study protocol and statistical analysis plan are available upon request (Appendix).

Randomisation and masking

Patients were enrolled by their treating physician and were randomly assigned (1:1) to one of two diagnostic strategies. In the OCT group, diagnosis and treatment was based on OCT only if the diagnosis of BCC and subtype could be made with high confidence. If there remained doubt about the correct diagnosis, a 3 mm punch biopsy was still obtained and histopathological diagnosis was used. In the regular care group, diagnosis and treatment was always based on a biopsy. Randomisation was stratified by participating centre and by the grade of clinical BCC suspicion (high versus low). Randomisation schemes were made with an online computer-generated list using block sizes of 4, 6 and 8. The randomly assigned treatment allocations were revealed to the investigator using an online system (Castor Electronic Data Capture System).

The investigator who assessed all OCT scans set the indication for treatment together with the supervising dermatologist. They were not masked to treatment arm but blinded for the biopsy results in case OCT diagnosis was made with high confidence. Due to the nature of the procedure, patients could not be masked to group assignment. Evaluation of the treated site at 12 months was done by the patients' own dermatologist who was blinded for the study arm. The dermato-pathologists of the centre where the patient was recruited, who were responsible

for histopathological examination, were blinded to the OCT results. Analysis of the data was performed by a statistician who was unaware of the coding for randomised groups.

Procedures

In the OCT group, one investigator (FA) made OCT scans of all lesions. The area that seemed most aggressive based on clinical and dermoscopic examination was marked as the biopsy area and centred in the OCT scan. OCT scans were made with a Vivosight Multi-beam Swept-Source Frequency Domain OCT (Michelson Diagnostics, Maidstone, Kent, UK; resolution <7.5 mm lateral, <5 mm axial, depth of focus 1.0 mm, scan area 6 x 6 mm^2). All OCT images were coded and saved anonymously. The investigator evaluated the OCT scan and decided whether the lesion was a BCC or not, based on established morphological BCC features.[6] Her training consisted of a study of the literature on OCT in dermatology, attendance of an OCT convention and assessment of more than 500 scans, within a period of four months.[11]

The level of confidence in BCC diagnosis was documented using a 5-point Likert-scale (Appendix, p1), scored with 0 to 4, where score 4 indicated high confidence in the OCT diagnosis and BCC subtype. In case of confidence score 4, the BCC subtype was further subclassified as superficial, nodular or aggressive and the treatment strategy was discussed during the same visit. If non-invasive treatment was indicated and preferred, it was immediately prescribed and if surgery was indicated, the procedure was scheduled. In patients with lower confidence scores (0-3), a 3 mm punch biopsy was still obtained, and the histopathological result was awaited to determine diagnosis and treatment. For safety reasons, a punch biopsy was also taken in the patients with high confidence OCT diagnosis, and one experienced dermatologist per centre checked the results and intervened only if treatment based on OCT would seriously compromise patient safety. For the decision on treatment a standardized treatment protocol was used: patients with a diagnosis of superficial BCC were offered the choice between imiquimod 5% cream or surgical excision; patients with a diagnosis of nodular or aggressive BCC were treated with surgical excision or Mohs' micrographic surgery. For alternative diagnoses, treatment was based on the guideline for that specific diagnosis. Alternative treatments were allowed if there were valid reasons to choose for another therapy.

The cost-effectiveness analysis followed the Dutch guidelines for cost-calculations in healthcare and was performed from a healthcare perspective with a time horizon of 12 months.[12] The reason for using the healthcare perspective was that productivity loss and out-of-pocket costs such as travel costs or use of services outside health care were expected to be minimal.

For the cost-analysis, a distinction was made between the diagnostic, treatment and post-treatment phase. Resource use related to the diagnostic phase consisted of an outpatient visit, a clinical photograph, an OCT scan, a punch biopsy and a telephone consultation. A punch biopsy was always included in the economic evaluation for the regular care group. For the OCT group, costs of a biopsy were only included if BCC diagnosis could not be made with high confidence in order to reflect real world clinical practice and avoid trial-induced costs. If OCT diagnosis was certain, no telephone consultation was needed because diagnosis and treatment were immediately discussed. For both the OCT and regular care group, extra visits or telephone consultations related to questions about therapy were registered.

Cost prices were obtained from the hospital financial department or the Dutch manual for costing research.[12] The cost price for biopsy (€126.70) is the sum of the cost prices for performing a biopsy (€51.58) and the cost price for histopathological examination (€75.12). These cost prices include personnel and material costs as well as general hospital overhead of 38%. Since cost prices are not yet available for OCT examination, OCT costs were calculated based on the methodology described in the Dutch manual for costing.[12] These calculations include equipment costs (€75.000), annual maintenance costs and depreciation period (total €12.875), the annual number of procedures and personnel costs. The average annual number of procedures in the participating centres was 627, which was the number of skin lesions suspected for BCC, where OCT could have been used. Personnel costs were €0,50 per minute for a physician and €2,07 per minute for a dermatologist, multiplied by the average time needed for OCT assessment which was 4.33 minutes. General hospital overhead of 38% were additionally allocated to the direct costs since it is an in-hospital procedure.[12]

Treatment costs covered costs of personnel, therapeutic agent (cream), material and surgical procedure (conventional excision or Mohs micrographic surgery). Post-treatment costs included outpatient control visits at 4 months in case of non-invasive treatment, a visit for suture removal in case of surgery, additional control visits related to adverse events (AE) and any extra telephone consultations.

All resource use was collected from the hospital information systems of the participating hospitals. Since all data with regard to the costs and effectiveness was collected within a one year time horizon, no discounting was applied. All costs were indexed to 2019.

Outcomes

The primary outcome was the proportion of patients remaining free from recurrent or residual (pre-)malignant lesion at 12 months after treatment. We considered a

follow-up period of 12 months long enough to capture the majority of recurrences, since most recurrences following non-invasive treatment appear within the first year.[13] Follow-up visits were scheduled at 12 months after the end of treatment with a time window of 9-18 months (due to the COVID-19 pandemic). After non-invasive treatment, an extra consultation took place at 3-4 months after treatment to evaluate whether there was complete tumour clearance. A dermatologist, who did not know to which randomization group the patient was assigned, evaluated the treated site at 12 months after treatment. Clinically suspected recurrence had to be verified by histopathological examination.

The outcome for the cost-effectiveness analysis was the incremental cost-effectiveness ratio (ICER), expressed as the incremental costs per additional patient free from a recurrent or residual (pre-)malignant skin lesion 12 months after treatment. This ratio is calculated as the difference in costs divided by the difference in effectiveness, i.e. recurrence-free rate, at 12 months follow-up. Secondary outcomes were the proportion of patients in whom punch biopsy could be avoided (OCT diagnosis with confidence level 4), the diagnostic accuracy of high confidence OCT diagnosis, the frequency of misclassifications and the area under the receiver operating characteristic (ROC) curve as measure of overall diagnostic performance of OCT. The histopathologic result from the punch biopsy was used as the gold standard.

The secondary outcome measure for the economic evaluation was costs per QALY, based on the recommendations of the Dutch manual for costing.[12] QALYs were calculated by using scores on the EQ-5D-5L questionnaire, a generic health related quality of life questionnaire that includes five dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression.[14] The Dutch tariff for the EQ-5D-5L was used to value the health states as experienced by patients.[15]

Statistical analysis

For the sample size calculation, it was assumed that the proportion of patients free from a recurrent or residual (pre-)malignant lesion 12 months after treatment in the regular care group would be 85%. To be 90% sure that the lower limit of a two-sided 95% confidence interval will exclude a difference in favour of the regular care group of more than 10% (non-inferiority margin), 538 (2*269) patients were required. Accounting for a 10% loss to follow-up, 598 patients needed to be included. Patients were not assessable for inclusion when they declined to participate. One-sided p-values of 2.5% (corresponding with two-sided p=values of 5%) were considered to indicate statistical significance.

Non-inferiority of OCT compared to regular care was evaluated by calculating the absolute difference in proportions of patients free from a recurrent or residual (pre-) malignant lesion at 12 months after treatment with a two-sided 95% confidence

interval. Both modified intention-to-treat (mITT) and per protocol (PP) analyses were performed. The protocol planned ITT analysis became a mITT analysis, since only patients who were randomised and for whom the primary outcome was available could be included in the analysis. Excluded from the PP population were patients with a (pre-)malignant lesion who did not start treatment. One lesion per patient was included to ensure independence of observations.

Diagnostic performance in patients with a high confidence OCT diagnosis was expressed as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) with corresponding 95% confidence intervals. Receiver operating characteristic (ROC) curves were constructed to visualize the sensitivity and specificity at alternative thresholds for a positive test result and the area under the curve with 95% confidence interval was calculated as measure for overall diagnostic performance of OCT. The cost-effectiveness analysis was performed according to the modified intention-to-treat (mITT) principle. Since cost data are generally skewed, a bootstrap analysis (1000 samples) was performed to generate 95% confidence intervals (CIs) around the difference in mean costs and to quantify the uncertainty surrounding the cost-effectiveness ratio. The bootstrap method estimates the sampling distribution of a statistic through a large number of simulations, based on sampling with replacement.[16] Results of the bootstrap analysis are presented in cost-effectiveness planes and acceptability curves. A cost-effectiveness plane is a graphical presentation of four quadrants in which additional costs and health outcome effects of two interventions are compared. The southeast (SE) quadrant represents the position where the intervention (OCT) is dominant, and thus more effective and less costly than the alternative (regular care). The northwest (NW) quadrant represents the position where the intervention is inferior and is both more costly and less effective than the alternative. The northeast (NE) quadrant, with higher costs and more effects, and the southwest (SW) quadrant, with cost savings and less effects, represent the situation where the intervention may be cost-effective compared with the alternative. The acceptability curve shows the probability of OCT-guided diagnosis and treatment being more cost-effective compared to regular care biopsy for a range of possible threshold values. In case of a non-inferiority trial, a threshold value for the southwest quadrant indicates the amount of money a decision maker is willing to accept for an additional recurrent or residual (pre-)malignant lesion.

The bootstrap analysis was performed using Microsoft Excel 2016. To test the robustness of the cost-effectiveness results, four univariate sensitivity analyses were conducted: first, a PP analysis was performed in which patients who did not start treatment for a (pre-)malignant skin lesion were excluded. Second, we performed a sensitivity analysis in which OCT costs were calculated based on personnel costs of a dermatologist instead of a physician, which means OCT

costs are €40.69 instead of €31.35. In the third sensitivity analysis we doubled the OCT costs from €31.35 to €62.70 to account for an unexpected rise in costs. In the fourth, we set the percentage of biopsies that could be omitted at 40%, since previous studies reported that an OCT diagnosis of BCC could be made with high confidence (and therefore biopsies can be omitted) in 30-40%of patients.[8-10] For this sensitivity analysis, we assumed that diagnostic accuracy and thereby the risk of misclassifications and associated risk of recurrent BCC did not change, although these values can be correlated to level of confidence in diagnoses of an OCT assessor.

To estimate the costs per QALY, a cost-utility analysis was performed using a regression based correction method to correct for baseline differences in utility scores.[17] SPSS (version 25) and STATA (version 14, StataCorp LLC, College Station, TX) were used for statistical analyses. This trial is registered with ClinicalTrials.gov number, NCT03848078.

Role of the funding source
The sponsor of the study had a role in the study design, but not in data collection, data analysis and data interpretation, or writing of the report. All authors had access to all the data reported in the study. The corresponding author had full access to all the data and the final responsibility to submit for publication.

RESULTS

From February 25, 2019, to September 2, 2020, 604 patients were assessed for eligibility (Figure 1). A total of 598 patients were randomised in three participating centres (Maastricht University Medical Centre+ (344), Catharina Hospital (176) and Zuyderland Medical Centre (78)). Half of the patients (299) were randomised and diagnosed according to regular care and the other half (299) to OCT-guided diagnosis and treatment. According to histopathology, the prevalence of BCC was 225 (75.3%) of 299 in the OCT group and 215 (71.9%) of 299 in the regular care group. Table 1 shows that the distribution of baseline characteristics was comparable between randomised groups. Data on race/ethnicity was not collected. In the OCT group, a high confidence diagnosis of BCC and BCC subtype could be made in 196 patients (65.6%). The remaining 103 patients (34.4%) still required a biopsy to establish a diagnosis. Histologically superficial BCCs received non-invasive treatment in 36 (45%) of 80 patients in the OCT group, compared to 37 (51%) of 73 in the regular care group (Table 2).

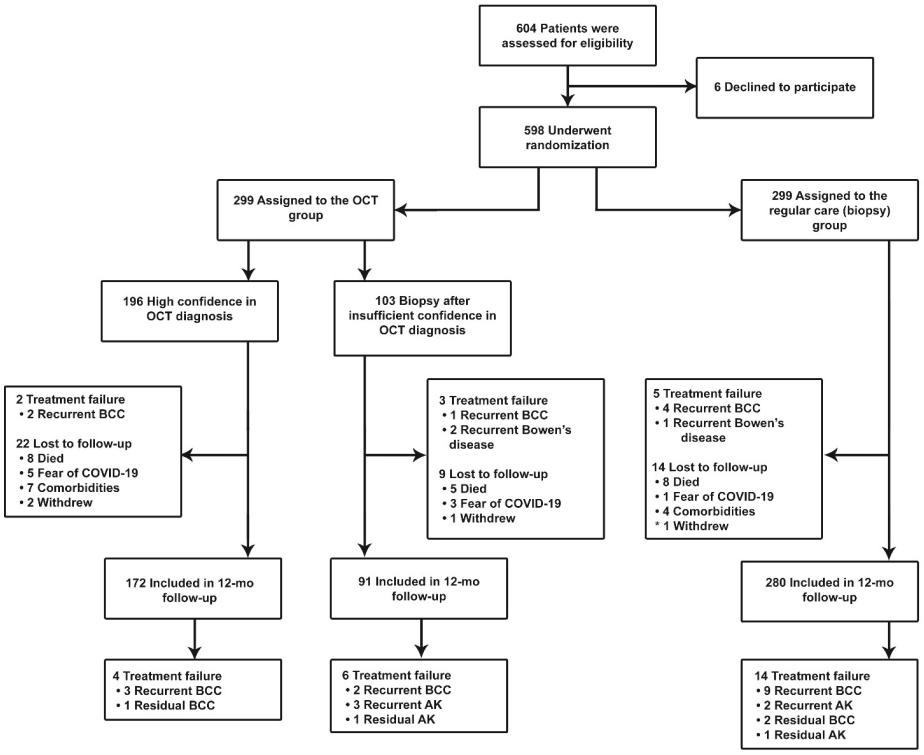


Figure 1. Screening, randomisation, and follow-up.

Information on the primary endpoint (patient free from a recurrent or residual (pre)-malignant lesion) was available for 553 of 598 randomised patients. Median follow-up was 12.7 months (IQR 11.2-14.1) for the OCT group and 12.6 months (IQR 10.8-14.3) for the regular care group. Forty-five patients (7.5%) did not attend the planned 12-months follow-up for various reasons (Figure 1). Loss-to-follow-up, partly attributable to COVID-19 related issues, was more common in the OCT group (10.4%) than in the regular care group (4.7%), which is probably due to chance. In the majority of patients with clinical suspicion of a residual or recurrent (pre-)malignant lesion, histopathological verification was obtained with exception of eight patients who considered a biopsy too burdensome.

Table 1. Baseline characteristics of 598 patients.

Characteristic	OCT group (N=299)	Regular care group (N=299)
Median age (range) - years	72 (22-94)	73 (21-92)
Sex - no. (%)		
Male	164 (54.8)	162 (54.2)
Female	135 (45.2)	137 (45.8)
Localization – no. (%)		
Head/neck	94 (31.4)	97 (32.4)
Upper anterior chest	37 (12.4)	33 (11.0)
Trunk	89 (29.8)	87 (29.1)
Extremities	79 (26.4)	82 (27.4)
Histologic diagnoses, n (%)		
BCC	225 (75.3)	215 (71.9)
No BCC	74 (24.7)	w84 (28.1)
BCC subtypes, n (%)		
Superficial	80 (35.6)	73 (34.0)
Nodular	113 (50.2)	106 (49.3)
Aggressive (morpheaform/micronodular)	32 (14.2)	36 (16.7)
Other diagnoses (non-BCC), n (%)		
Benign lesion[1]	34 (11.4)	37 (12.4)
Actinic keratosis	24 (8.0)	23 (7.7)
Bowen's disease	9 (3.0)	18 (6.0)
SCC	5 (1.7)	4 (1.3)
Superficial spreading malignant melanoma	1 (0.3)	0
Atypical fibroxanthoma	1 (0.3)	0
Primary cutaneous follicle center lymphoma	0	1 (0.3)
Sebaceous carcinoma	0	1 (0.3)

Abbreviations: BCC, basal cell carcinoma; SCC, squamous cell carcinoma.
[1]Including: sebaceous gland hyperplasia and/or adenoma, dermatofibroma, dermal nevus, seborrhoic keratosis, scar, benign lichenoid keratosis, folliculitis, neurofibroma, trichofolliculoma, venous stasis dermatitis, sclerosing dermatitis, excoriation, dilated hair follicle, angioma, chronic inflammation, eczema, apocrine hidrocystoma, epidermoid cyst, blue nevus, halo nevus, solar elastosis, solar lentigo, verruca vulgaris, lichen planopilaris, lichenoid dermatitis, nodular prurigo, dermal mucinosis.

The modified intention-to-treat (mITT) analysis was based on 553 patients who were randomised and for whom data on the primary outcome was available (268 in the OCT group and 285 in the regular care group). One year after treatment, 253 (94.4%) patients were free from a recurrent/residual (pre-)malignant lesion in the OCT group, versus 266 (93.3%) in the regular care group (Figure 1). The absolute difference (OCT-regular care) was +1.07% (95% CI: -2.93 to 5.06, one-sided p=0.30). Among the patients with residual or recurrent (pre-)malignant lesions, 9 of 15 patients had a malignant lesion in the OCT group versus 15 of 19 in the regular care group.

Table 2. Comparison of the outcomes in the randomised groups. Numbers and percentages are presented per randomised group.

	OCT group % (x/n)	Regular care group % (x/n)	p-value
Diagnosis			
Based on OCT	65.6 (196/299)	0 (0/299)	--
Based on biopsy	34.4 (103/299)	100 (299/299)	--
Frequency of misclassifications*	18.4 (36/196)	--	--
Type of misclassification			
Histological non-BCC as BCC	2.0 (4/196)	--	--
Classified as sBCC (imiquimod)	*2*	--	--
Classified as other subtype (excision)	*2*		
Histological sBCC as other subtype	34.7 (25/72)	--	--
Treated with imiquimod	1		
Treated with excision	24	--	--
Histological non-sBCC as sBCC	5.8 (7/120)		
Treated with imiquimod	4		
Treated with excision	3		
Treatment with imiquimod			
sBCC**	45.0 (36/80)	50.7 (37/73)	0.49
nBCC	5.3 (6/113)	0.9 (1/106)	0.08
aBCC	--	--	--
Surgical treatment			
sBCC	55.0 (44/80)	42.5 (31/73)	0.13
nBCC[1]	92.0 (104/113)	96.2 (102/106)	0.21
aBCC[2]	100 (32/32)	94.4 (34/36)	0.28
AK[3]	0 (0/24)	4.3 (1/23)	0.49
Bowens' disease	44.4 (4/9)	44.4 (8/18)	1.00
SCC[4]	80.0 (4/5)	100 (4/4)	0.56
Other malignancies	100 (2/2)	100 (2/2)	--
Frequency of recurrence or residual (pre-)malignant lesion			
All lesions	5.6 (15/268)	6.7 (19/285)	0.61
Misclassified lesions	0 (0/36)	--	--

* Misclassification; diagnosis by OCT compared to punch biopsy diagnosis. ** Regular care: 3 sBCCs were treated with 5-FU, 1 did not begin imiquimod treatment, 1 switched from imiquimod to MAL-PDT. [1]OCT: 3 patients with nBCC did not begin treatment, regular care: 3 patients with nBCC did not begin treatment. [2]Regular care: 2 patients with aBCC did not begin treatment. [3]AK were treated with cryotherapy (OCT: 18; 2 did not begin treatment, regular care: 12; 5 did not begin treatment), 5-FU (OCT: 1; 1 did not begin treatment, regular care: 5), imiquimod (OCT: 2). [4]One patient with SCC in OCT group did not begin surgical treatment since it was radically removed with punch biopsy.

For the per-protocol (PP) analysis, 12 patients who did not start treatment were excluded. Five patients (2 in the OCT group and 3 in the regular care group) had residual BCC or actinic keratosis (AK) at 12 months follow-up and in 7 patients, the (pre-)malignant lesion was no longer visible at follow-up. PP analyses led to proportions free from a residual/recurrent (pre-) malignant lesion of 95.1% (250/263) in the OCT group and 94.2% (262/278) in the regular care group and an absolute difference of +0.81% (95% CI: -2.98 to 4.60), one-sided p=0.34). The numbers of patients with a malignant lesion were 8 of 13 patients in the OCT group and 13 of 16 patients in the regular care group. As the lower limit of the 95% CI does not

exceed the non-inferiority margin of -10%, OCT-guided diagnosis and treatment was non-inferior to regular care.

The AUC as measure for diagnostic performance of OCT was 95.2% (95% CI, 92.1-98.3) and the ROC curve is presented in the Appendix (p8). In this RCT, the ability of a high confidence OCT diagnosis to discriminate between BCC and non-BCC lesions and between superficial and more aggressive BCC subtypes is of primary interest. The results of comparison of high confidence diagnosis by OCT with histopathological diagnosis are presented in Table 3. From the 225 histologically verified BCCs in the OCT group, 192 BCCs were detected by high confidence OCT diagnosis corresponding with a sensitivity of 85.3% (95% CI: 82.9-86.5). The specificity was 94.6% (95% CI: 87.1-98.2), 70/74 histological non-BCC lesions were diagnosed as a non-BCC lesion by OCT (Table 3). Among the 192 BCCs that were identified by OCT, OCT correctly identified 47/72 histologically superficial BCCs (specificity=65.3%, 95% CI: 57.4-70.4) and 113 of 120 other subtypes (sensitivity=94.2%, 95% CI: 89.5-97.2). With OCT, presence of BCC was predicted in 196 lesions, of which 192 were histologically confirmed BCC, corresponding with a positive predictive value of 98.0% (95% CI: 95.1-99.3). Four lesions were non-BCC lesions according to histopathological diagnosis: actinic keratosis (2), Bowen's disease (1) and osteoma cutis (1). The two actinic keratosis were classified as superficial BCC by OCT and treated with imiquimod 5% cream, the other two lesions were classified as non-superficial BCC and were treated with surgical excision. The group of 192 BCCs that were correctly identified as BCC by OCT, histologically consisted of 72 superficial BCCs and 120 non-superficial BCCs. With OCT, 56 BCCs were classified as superficial BCC, but 7 of those were non-superficial BCC according to histopathology (Table 3). Four of these seven non-superficial BCC subtypes were treated with imiquimod 5% cream, and none of these four patients developed a recurrent BCC 12 months after treatment. Three of the 7 patients preferred surgical excision. A total of 140 lesions were classified as non-superficial BCC by OCT, but 25 of 140 lesions were superficial BCC on histology. Based on the OCT diagnosis, 24 of these 25 BCCs were treated with surgical excision. A non-superficial BCC was diagnosed in 13 of the 24 available excision specimens. One patient with a non-superficial BCC on OCT preferred imiquimod treatment because he had multiple BCCs. However, the BCC was resistant to treatment and histology of the residual tumour confirmed the presence of non-superficial BCC. In total, high confidence OCT diagnosis resulted in an incorrect diagnosis in 36 patients, but none of these patients had a residual or recurrent (pre-)malignant lesion (Table 2).

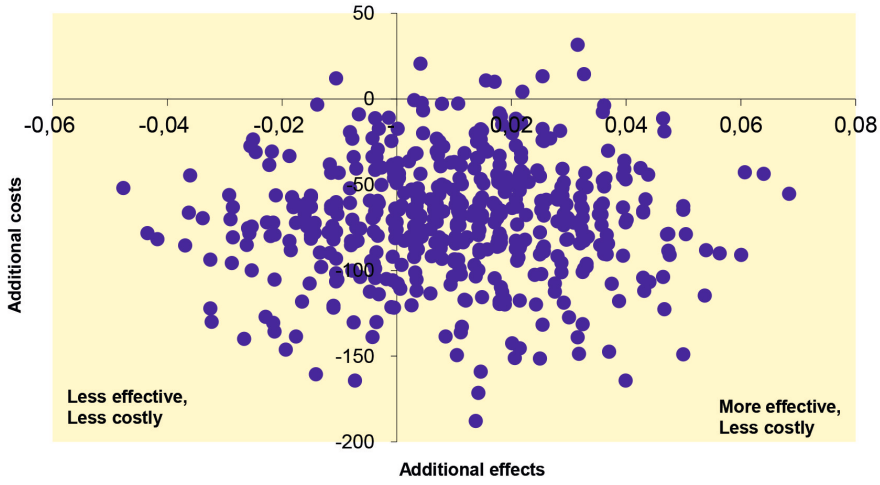
Table 3. Results for ability of high confidence OCT diagnosis (Likert score 4) to discriminate between: BCC and non-BCC lesions and superficial BCC and non-superficial BCC subtypes.

OCT	**Histopathology**			
Tumour	***BCC***		***No BCC***	***Total***
BCC	192		4	196
No BCC	33		70	103
Total	225		74	299
Subtype	***Superficial***	***Non-superficial***	***No BCC***	***Total***
Superficial	47	7	2	56
Non-superficial	25	113	2	140
Total	72	120	4	196

The Appendix (p3) shows results of the cost analysis for the OCT group and the regular care group. The total mean costs of the diagnostic phase were significantly lower for the OCT group: €233 versus €308 in the regular care group. There were no significant differences in treatment, post-treatment and total mean costs between the two groups.

The ratio for OCT-guided diagnosis and treatment compared to punch biopsy indicates that OCT is a cost-effective strategy, i.e. lower costs (€689 versus €758) and slightly higher effectiveness (0.94 versus 0.93) (Table 4). Bootstrap results show that the majority of CE ratios (67%) were located in the southeast quadrant where OCT is more effective and cost saving while 32% lie in the southwest quadrant (Figure 2a) which represents cost savings with less effectiveness. The acceptability curve (Figure 2b) for the southwest quadrant shows that for threshold values varying between €500 and €5000, the probability of OCT-guided diagnosis and treatment being more cost-effective compared with punch biopsy is higher than 80%. The Appendix (p4) presents the results of the sensitivity analyses. Including the PP data leads to similar results as the mITT analysis with 66% of all ratios falling in the SE quadrant. When OCT costs are calculated based on personnel costs of a dermatologist, total mean costs for the OCT-guided strategy are €698 compared to €758 for punch biopsy and 66% of all ratios fall in the SE quadrant (Appendix, p4-5). If OCT costs are doubled, total mean costs for the OCT-guided strategy are €720 compared to €758 for regular care and 60% of all ratios fall in the SE quadrant (Appendix, p5). When only 40% of biopsies can be omitted, total mean costs are €732 for the OCT-guided strategy compared to €758 for regular care and the majority of ratios (52%) still fall within the SE quadrant (Appendix, p6). The results of the cost-utility analysis show that mean costs were lower in the OCT arm (€688 versus €761 in the regular care arm) and mean QALYs were slightly higher (p7, Appendix), suggesting that an OCT-guided diagnostic strategy is cost-effective (cheaper and with higher QALYs).

a.



b.

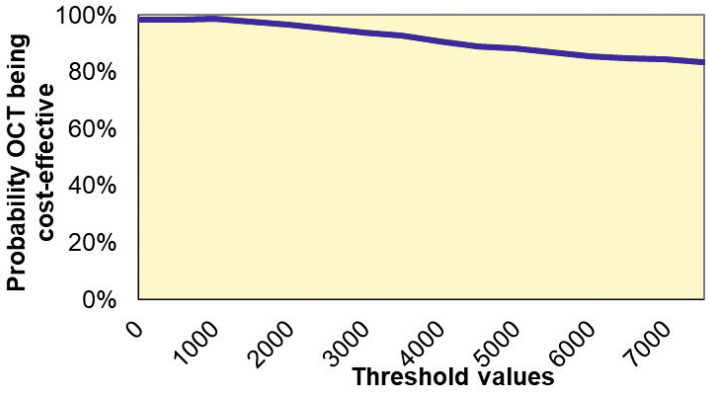


Figure 2a-b. Cost-effectiveness plane (a) and acceptability curve (b) for OCT-guided diagnosis and treatment vs. regular care (punch biopsy).

Table 4. Results of cost-effectiveness analysis.

Analysis	OCT mean costs (€)	Regular care mean costs (€)	OCT effectiveness	Regular care effectiveness	ICER	CE-plane
mITT analysis (65.6% of biopsies omitted) (n=553)	689	758	0.94	0.93	Dominant	SE (dominant): 67%, SW: 32%, NE: 1%, NW: 0%

DISCUSSION

This trial shows that OCT-guided diagnosis and treatment is non-inferior to regular care and does not compromise patient safety. In the OCT group 253 (94.4%) of patients were free from a recurrent or residual (pre-)malignant lesion compared to 266 (93.3%) in the regular care group. In the OCT group, a high confidence OCT diagnosis could replace a punch biopsy in 196/299 (65.6%) of patients. In this study, none of the misclassifications that occurred had severe clinical implications and none of the 15 recurrences in the OCT group were due to misclassification by OCT. The cost-effectiveness results indicate that OCT-guided diagnosis and treatment is a cost-effective strategy compared to regular care punch biopsy.

The largest risk of OCT-guided diagnosis is that a more aggressive malignancy (for example a melanoma) could be wrongfully diagnosed as BCC and treated non-invasively. In a study by Cheng et al. one amelanotic melanoma was misclassified as superficial BCC.[7] In our study population, one patient had a melanoma, which was clinically highly suspect for BCC, but correctly identified with OCT as a non-BCC lesion with an indication for biopsy.

Another risk is that misclassification of a non-superficial BCC as superficial BCC may result in the decision to treat such a lesion non-invasively, whereas excision is indicated. However, the 5-year sustained clearance in low-risk nodular BCC treated with imiquimod cream is still 81% with recurrences being detected early during follow-up.[18] For aggressive BCCs, treatment with imiquimod cream seems more harmful. Imiquimod treatment for aggressive BCCs was only investigated in a small study, in which the majority (8/13) did not respond to treatment with imiquimod cream.[19] At a low-risk location, resistant aggressive BCCs can be easily retreated with surgical excision with wide margins, but such retreatment is more complex in the H-zone. There is also a risk that superficial BCCs are misclassified as a more aggressive subtype, which results in a decision to treat the lesion with excision. Although surgery is a very effective treatment for superficial BCC, the choice for a non-invasive treatment would then be wrongfully withheld.[4]

In this trial, 25 of 72 BCCs diagnosed as superficial by punch biopsy, were classified as non-superficial BCC by OCT diagnosis and treated with surgery. However, in 13 of these 25 patients a non-superficial component was actually detected with histological examination of the excision specimen. This illustrates that OCT can also have an advantage over a 3 mm punch biopsy, as the entire lesion is visualized instead of only 3mm. It is known that biopsies, either punch or shave, do not always represent the entire lesion.[20, 21]

In the OCT arm 55% of the BCCs were excised versus 42.5% in the regular care arm and generally more excisions were done for superficial BCCs in the OCT arm. This

imbalance is partly because of over classification. If those 25 BCCs over classified on OCT as non-superficial would have been diagnosed as superficial, the number of superficial BCCs treated surgically could have been reduced to minimally 19/80 (Table 2) which may impact the overall costs. However, as some patients choose for invasive treatment of superficial BCC as was also the case in the regular care arm, it is difficult to predict this impact. A second explanation for the fact that more superficial BCCs received excision in the OCT arm could be the fact that neither the patient, nor the investigator and supervisor were blinded for the study arm when deciding on the appropriate treatment. In the choice between non-invasive treatment or surgical excision, the potential uncertainty of the OCT-scan might have influenced their preference for the certainty of excision.

OCT-guided diagnosis of BCC has potential advantages. From a patient's perspective, an OCT-guided strategy is an attractive option, because an invasive procedure can be omitted, and BCC treatment can be initiated immediately.

We demonstrated that OCT-guided diagnosis and treatment is a cost-effective strategy compared to regular care punch biopsy. The bootstrap analysis showed that the majority of the cost-effectiveness ratios lie within the quadrant where OCT strategy is considered a dominant cost-effective strategy, leading to more effects and less costs. For the ratios in the southwest quadrant (less effective but cost saving), the acceptability curve shows the probability that the OCT strategy is cost-effective for different monetary threshold values. In this case, the value indicates the amount of money society is willing to accept for an additional patient with a residual or recurrent (pre-)malignant skin lesion. However, since there is no threshold value for this, we considered a threshold value should at least include the costs of treatment of a recurrent tumour. Using the total treatment costs of a surgical excision, minimum cost savings should be around €500. At a threshold of €500, the probability of OCT-guided diagnosis strategy being cost-effective is 99%. The acceptability curve shows that even at much higher threshold values, the probability of OCT being cost-effective is around 80%.

In 196/299 (65.6%) of patients, OCT diagnosis was certain, and biopsy could be omitted. Savings of costs of a punch biopsy, histopathological examination and a post-biopsy (telephone) consultation to discuss results, resulted in lower costs for the total OCT-guided strategy, even though in 103/299 (34.4%) of patients both a biopsy and an OCT scan were obtained. Moreover, misclassification by OCT did not lead to higher treatment costs in the OCT group compared to regular care punch biopsy. Sensitivity analyses showed that the OCT-guided strategy was still cost-effective compared to regular care when a PP analysis was performed, when the personnel costs of a dermatologist instead of a research physician were used, when the OCT costs were doubled and when only 40% of biopsies

can be omitted (compared to 65.6% as achieved in this study). The cost-utility analysis showed similar results compared to the cost-effectiveness analysis: mean costs were observed to be lower in the OCT arm and mean QALYs were slightly higher, suggesting that an OCT-guided diagnostic strategy is cheaper and leading to slightly higher QALY's. Cost prices used are specific for the Dutch healthcare system and might differ per country, but data on resource use allow for determination of applicability per situation.

A limitation is that the results of this study strongly hinge on the OCT diagnoses made by a single, well-trained physician, who had evaluated 500 scans before the start of the study. A punch biopsy could be omitted in 65.6% of patients which is more than the 30-36% that could be achieved in previous studies.[8, 9] The level of diagnostic performance of an OCT assessor determines how often a biopsy can be omitted as well as the risk of misclassifications. Therefore, an important condition for successful implementation of OCT in clinical practice is sufficient training of OCT users.[22] To incorporate OCT in dermatologic practice, it is critical to set criteria for adequate performance and to quantify the time and training required to achieve such performance. In a former study, we have illustrated how cumulative sum analysis can be used to train novice assessors and to monitor the level of diagnostic performance over time.[22]

Also, this study excluded patients with large lesions or lesions located in the 'H-zone' of the face because it was not yet known whether OCT-guided diagnosis and treatment could compromise patient safety. BCC at this location has a higher risk of aggressive behaviour.[4] Furthermore, in the H-zone surface areas are often convex or concave, which may impact the quality of the OCT image. More studies are therefore required to determine whether OCT is suitable in this subpopulation. Finally, although in the Netherlands, the majority (63-90%) of lesions are diagnosed by biopsy, there remains substantial variation between centers.[23, 24] To increase the generalizability of results, this multi-center study was conducted in two general hospitals and one academic hospital. Generally, patients with lesions where the diagnosis of BCC is very evident are directly treated without biopsy and these lesions were excluded from this study. A recent study confirmed that in this subgroup of patients, the additional diagnostic value of OCT is limited.[25]

In conclusion, this trial shows that OCT-guided diagnosis and treatment is safe and non-inferior compared to regular care. In two thirds of patients a biopsy could be avoided, limiting treatment delay. Misclassifications did not have large clinical implications and did not lead to higher treatment costs in the OCT group compared to punch biopsy, but the risk of over- or undertreatment must always be carefully weighed against the advantage of treatment without delay and less invasive procedures.

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Panel: **Research in context**

Evidence before this study
We searched PubMed, Cochrane databases, reference lists, controlled-trials.com, clinicaltrial.gov and the NHS centre for reviews and dissemination on 6 March 2018. We used the terms 'optical coherence tomography or OCT', 'basal cell carcinoma or BCC', 'specificity' and 'sensitivity' for articles published in English, with no date limits. Inclusion criteria were: population of patients with a skin lesion suspected for BCC, histological assessment with a punch biopsy or excision used as gold standard and sensitivity and specificity estimates could be derived from the study. Five prospective cohort studies fulfilled these inclusion criteria and were judged based on the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) criteria. In none of these 'Low risk at bias' could be scored, mostly due to the non-transparency concerning patient flow and methods for estimation of sensitivity and specificity. The "reference standard" was judged as unclear in all studies, because the gold standard was not defined clearly and/or because it was not reported whether an independent experienced dermato-pathologist, judged the histopathological slides. Our review revealed that current literature shows promising results to justify dermatologists' interest in this technique.

In December 2018, a Cochrane systematic review on the use of OCT for diagnosing skin cancer concluded that conventional OCT may have a role for the diagnosis of BCC in clinically challenging lesions. The meta-analysis showed a higher sensitivity and specificity of OCT when compared to visual inspection and dermoscopy, but due to a small number of studies and varying methodological quality implications to guide clinical practice could not be drawn yet: appropriately designed prospective comparative studies were needed.

Added value of this study

To our knowledge, this is the only clinical trial that evaluates whether OCT-guided diagnosis and treatment of clinically suspected BCC is non-inferior in terms of clinical effectiveness, and cost-effectiveness compared to regular care punch biopsy.

Implications of all the available evidence

The evidence generated by our clinical trial justifies incorporation of OCT in (inter) national guidelines. Implementation of OCT requires the re-organization of current clinical practice where a punch biopsy with one week waiting time for the results can be replaced by a one-stop-shop approach in around two-thirds of suspected BCC cases. An important condition for successful implementation of OCT in clinical practice is sufficient training. Therefore, it is critical to set criteria for adequate diagnostic performance and quantification of time and training required to achieve good diagnostic performance.

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APPENDIX

Table 1. Classification of diagnosis according to level of confidence in BCC diagnosis and BCC subtype. BCC, basal cell carcinoma.

Level of confidence	
0.	This is not a BCC
1.	Suspicion on BCC is low
2.	Suspicion on BCC is high, but I consider another diagnosis
3.	Surely a BCC, but I would biopsy in order to determine the BCC subtype
4.	Surely a BCC and sure about the BCC subtype, I would omit the biopsy and start treatment

Table 2. Overview of the costs per unit.

Resource use	Unit	Cost (€)	Reference
University hospital	Outpatient visit	174.02	Dutch manual for costing [1]
General hospital	Outpatient visit	85.41	Dutch manual for costing
General practitioner	Consultation	35.23	Dutch manual for costing
Telephone consultation	Phone call	32.17	MUMC+
Medical photography	Photo	4.01	MUMC+
Biopsy[a]	Test	126.70	MUMC+
OCT Personnel (physician) Device costs Maintenance costs	Test Minute Device Maintenance	31.35 0.50 75000 3500	MUMC+ MUMC+ Vivosight (Michelson Diagnostics, Maidstone, Kent, UK)
Surgical excision[b]	Procedure	471.46	MUMC+
Mohs' Micrographic surgery	Procedure	1823.81	MUMC+
Treatment costs imiquimod 5% cream	Sachets (30)	145.65	Pharmacotherapeutic Compass
Treatment costs 5-fluorouracil cream	Grams (40)	40.02	Pharmacotherapeutic Compass
Treatment costs MAL-PDT [c]	Procedure	341.09	MUMC+ and Pharmacotherapeutic Compass
Cryotherapy	Session	15.67	MUMC+
Topical treatments in case of side effects			
Chlorhexidine cream	Grams (30)	7.43	Pharmacotherapeutic Compass
Fusidic acid 2% cream/ ointment	Grams (30)	7.35	Pharmacotherapeutic Compass

MAL-PDT, methyl aminolaevulinate photodynamic therapy; MUMC+, Maastricht University Medical Centre+.;
[a] Cost price is the sum of the cost prices for performing a biopsy (€51.58) and the cost price for histopathological examination by a pathologist (€75.12).
[b] Average cost price for surgical excision, which took place either at the outpatient clinic (general hospitals) or at the operating room (MUMC+). For lesions located in the face, 45 minutes were scheduled at the operating room, whereas 30 minutes were usually scheduled for lesions at other body locations. [b,c] Additional hospital overhead of 38% is included.

Table 3. Results of the cost analysis for the OCT group and the regular care group at 12-months follow-up after treatment.

	Randomization group - mean cost (€)				**Difference (95% CI)**
	OCT	Average resource use per patient	**Regular care**	Average resource use per patient	OCT-Regular care
Number of patients	299		299		
Total costs	685.23		750.18		-65 (-134-3)
Diagnostic phase	232.84		308.29		-75 (-89 - -62)
Baseline outpatient visit	137.54	1.01	140.45	1.02	
Medical photograph	4.01	1	4.01	1	
OCT[a]	31.35	1	-	-	
Biopsy	45.76	0.36	128.82	1.02 [b]	
Telephone consultation	13.99	0.43	35.07	1.09	
Treatment phase	333.59		341.21		-8 (-68-57)
Imiquimod 5% cream	21.92	0.15	19.48*	0.13	
Surgical excision outpatient clinic	219.86	0.61	208.08	0.56	
Mohs' Micrographic surgery	79.30	0.04	103.69	0.06	
5-fluorouracil cream	0.54	0.01	2.14	0.05	
MAL-PDT	1.14	0.003	1.14	0.003	
Cryotherapy	9.18	0.06	5.30	0.05	
Other topical creams	0.70	0.09	0.93	0.11	
Post-treatment phase	119.51		101.99		18 (-6-41)
Telephone consultation	10.86	0.34	11.62	0.36	
Outpatient follow-up visits	69.32	0.81	70.82	0.85	
Extra follow-up visits	38.83	0.30	19.64	0.14	
Fusidic acid cream	0.17	0.02	0.10	0.01	
Antibiotics	0.07	0.02	0.19	0.007	
Painkillers	0.09	0.007	-	-	
Clobetasol ointment	0.05	0.003	-	-	
Biopsy	0.42	0.003	-	-	

MAL-PDT, methylaminolaevulinate-photodynamic therapy; CI, confidence interval; [a] Control visits related to both adverse events as well as questions about diagnosis or therapy. *One patient switched from imiquimod 5% cream to MAL-PDT. [b] Average resource use can be more than 1, because both in the OCT group and in the regular care group 4 patients underwent another biopsy due to an inconclusive diagnosis.

Table 4. Results of cost-effectiveness sensitivity analyses.

Sensitivity analyses	OCT mean costs (€)	Regular care mean costs (€)	OCT effectiveness	Regular care effectiveness	ICER	CE-plane
PP analysis (n=541)	694	764	0.95	0.94	Dominant	SE (dominant): 66%, SW: 31%, NE: 2%, NW: 1%
OCT by dermatologist (n=553)	698	758	0.94	0.93	Dominant	SE (dominant): 66%, SW: 29%, NE: 4%, NW: 2%
Doubled OCT costs	720	758	0.94	0.93	Dominant	SE (dominant): 60%, SW: 26%, NE: 11%, NW: 3%
40% of biopsies omitted (n=553)	732	758	0.94	0.93	Dominant	SE (dominant): 52%, SW: 23%, NE: 20%, NW: 6%

Table 5. Results of cost-utility analysis.

Analysis	OCT mean costs (€)	Regular care mean costs (€)	OCT mean QALYs	Regular care mean QALYs	Incremental cost per QALY (€)	CE plane
mITT (n=547)	688	761	0.89	0.88	5197	SE (dominant): 85%, SW: 12%, NE: 2%, NW: 0%

*Mean costs differ slightly because of bootstrap simulation.

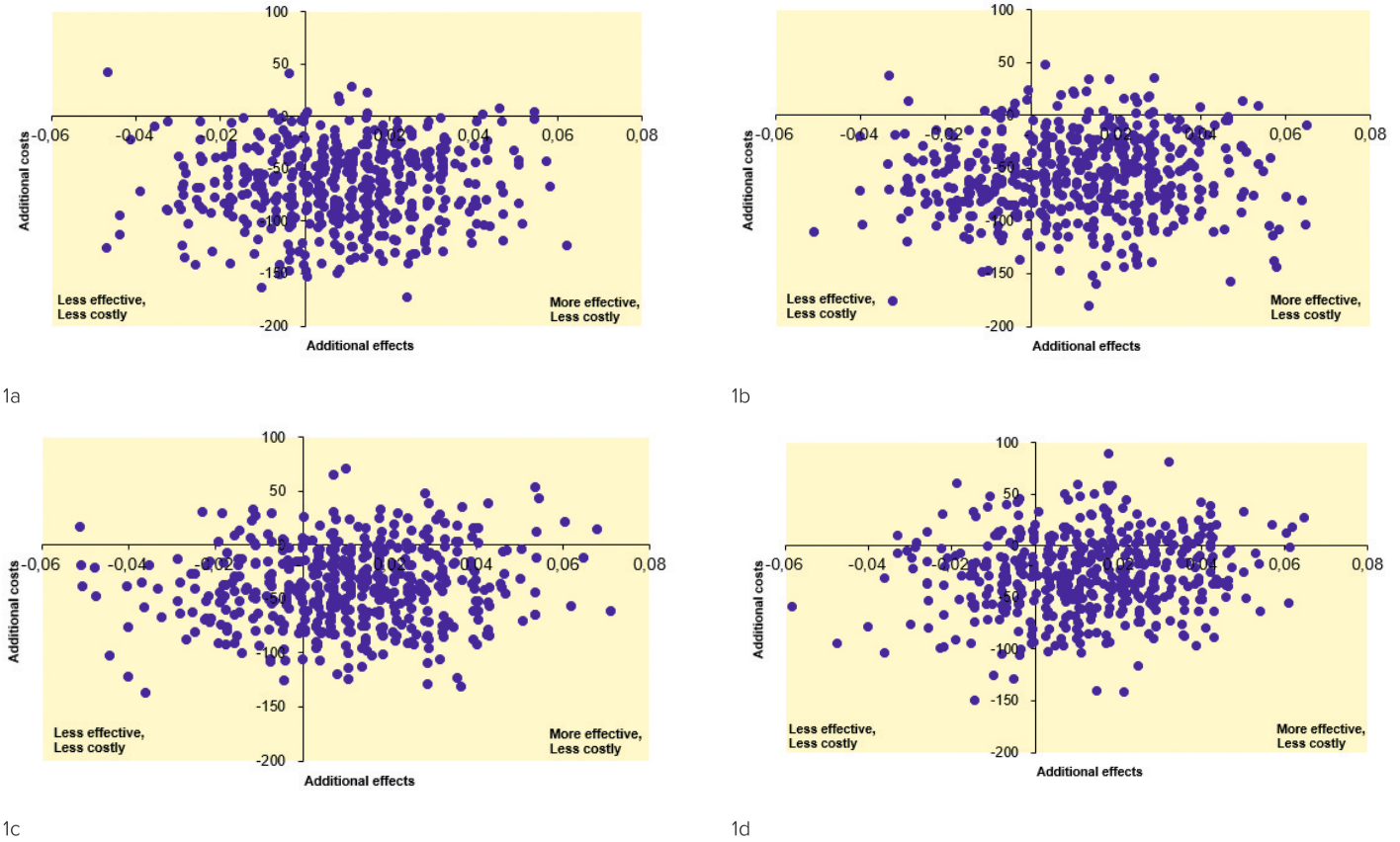

Figure 1 a-d. Cost-effectiveness planes for OCT vs. Regular care. The bootstrapped difference in costs and effectiveness (cost-effectiveness ratios) cover 66% (PP analysis, 1a), 66% (OCT by dermatologist, 1b), 60% (doubled OCT costs, 1c) and 52% (40% of biopsies omitted, 1d) of the quadrant where OCT is considered more effective and less costly compared to regular care.

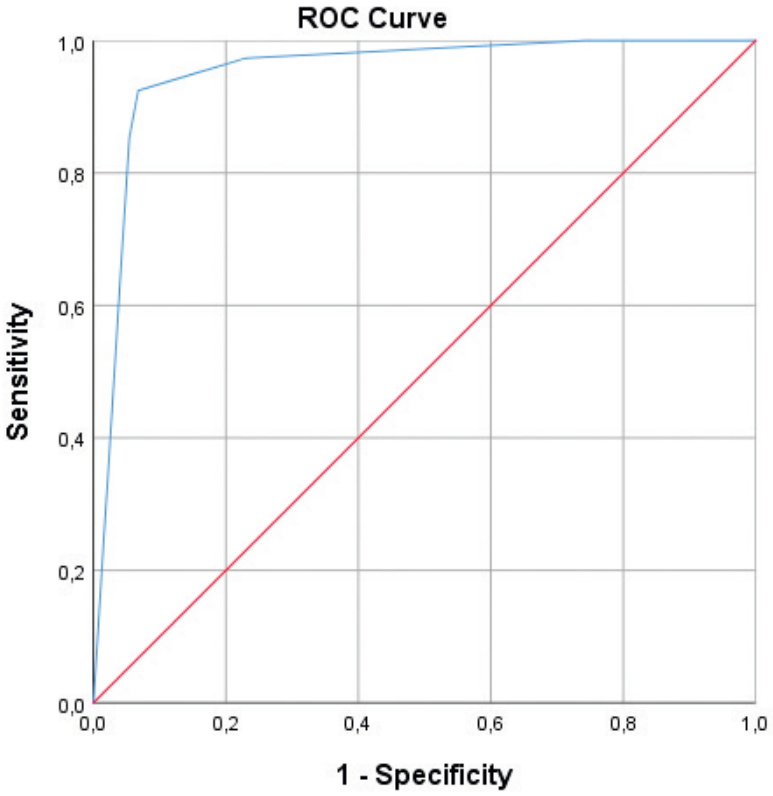

Figure 2. ROC curve for OCT. Abbreviations: OCT, optical coherence tomography; ROC, receiver operating characteristic.

Site	Principal investigator responsible	Number of participants
Maastricht University Medical Centre+	K. Mosterd	344
Catharina hospital Eindhoven	T. Brinkhuizen	176
Zuyderland Medical Centre Heerlen	J.P.H.M. Kessels	78

List of participants for each participating centre and principal investigator responsible.

CHAPTER 2.2

Patient preference for optical coherence tomography versus punch biopsy for diagnosis of basal cell carcinoma: a labelled discrete choice experiment

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ABSTRACT

Non-invasive technologies, such as optical coherence tomography (OCT), are increasingly available for diagnosis of basal cell carcinoma (BCC) and might partly replace punch biopsies.

We conducted a discrete choice experiment (DCE), alongside a multi-centre randomised non-inferiority trial, to examine patient preferences for an OCT-guided diagnostic strategy compared to biopsy. A multinomial logit regression model was used to analyse the data. In total, 344 patients filled out the questionnaire. A higher sensitivity and lower false positive rate showed a significant impact on the preference for OCT while a higher level of confidence of the physician in the diagnosis, a longer waiting time, a lower false positive rate and short-lasting severe pain influenced the choice for punch biopsy. Provided that the best levels for sensitivity and false positive rate are achieved, the preference of patients for OCT as initial diagnostic strategy increases, showing the potential of this innovation for clinical practice.

INTRODUCTION

Basal cell carcinoma (BCC) is the most prevalent form of skin cancer with a rapidly rising incidence worldwide, causing a major burden on healthcare systems.[1, 2] Histopathological examination of a punch biopsy is the current gold standard which is used to diagnose BCC and to determine the histopathological subtype.[3, 4] A biopsy is a minor surgical procedure which may be painful and carries a small risk of complications such as bleeding, infection and scarring. Moreover, awaiting histopathological examination causes treatment delay which may cause stress for patients. In recent years, optical coherence tomography (OCT) emerged as a promising non-invasive imaging tool for BCC diagnosis, generating real-time in-vivo cross-sectional images of tissue microarchitecture with a depth of 1.5-2 mm.[5] Quite large ranges in sensitivity (58-95.7%) and specificity (43-96%) have been reported for OCT when used to diagnose BCC.[6-9] The idea is that non-invasive diagnostic techniques, such as OCT, may make it possible to obviate a biopsy in part (30-40%) of the patients for whom the OCT diagnosis of BCC can be made with high confidence, consequently resulting in more efficient patient care.[7, 10, 11] However, prior to implementation of new technologies in clinical practice, it is relevant to obtain insight into patient preferences, since this may indicate whether an innovation, in this case a non-invasive diagnostic tool, will be accepted by patients in clinical practice.[12, 13] Conjoint analysis methods, particularly discrete choice experiments (DCEs), are increasingly being used to quantify preferences. Recent applications have focused on patient preferences for BCC treatment.[14, 15] So far, preferences regarding different diagnostic strategies, are under addressed. We conducted a DCE in order to investigate the importance placed by patients on different characteristics associated with OCT and biopsy, to be able to determine patient preferences for either OCT or biopsy as a diagnostic strategy.

MATERIALS AND METHODS

The DCE was conducted alongside a multi-centre randomised non-inferiority trial comparing OCT with punch biopsy for diagnosis and subtyping of BCC. Adult patients (18 years or older) with an indication for biopsy of a lesion clinically suspected for BCC were included in three Dutch hospitals. Patients were randomised between two groups: in the OCT group, diagnosis and treatment were based on OCT, but only if the OCT diagnosis could be made with high confidence. In these patients, a biopsy was still taken for safety reasons, but the investigator who evaluated the OCT scans remained blinded to the histopathological result and could directly initiate treatment. Patients in whom the OCT diagnosis could not be made with high confidence, the histopathological result was used to determine diagnosis and treatment. In the regular care group, diagnosis and treatment was always based on a biopsy. All patients received the questionnaire two months after the diagnostic procedure. Written informed consent was obtained from all patients prior to inclusion.

Sample size

Given the lack of a definite method for calculating a sample size regarding DCEs, we used the rule of thumb as proposed by Johnson and Orme which showed that a minimum of 166 participants is required.[16]

Discrete choice experiment

A DCE elicits preferences by asking individuals to indicate their choice between two or more options. The underlying assumption of a DCE is that an intervention or treatment can be described by different characteristics, or attributes, with different levels. Hypothetical choice sets are created based on attributes and their levels. A labelled design was deemed necessary as the levels of the different attributes were specific for either OCT or biopsy (Figure 1). Moreover, the choice between a non-invasive and invasive diagnostic strategy makes the use of a labelled design more realistic and a better reflection of the choices patients face in clinical practice, instead of strategy ‘A’ or ‘B’.

Attribute	Optical Coherence Tomography (OCT)	Punch biopsy
Waiting time for results	No waiting timing, immediate results	14 days
Confidence of doctor in diagnosis	30% (30 out of 100 people) (in 70 out of 100 people the doctor is not confident about BCC diagnosis and a punch biopsy is taken anyhow)	90% (90 out of 100 people)
In how many patients where the doctor is confident about BCC diagnosis, the BCC diagnosis is correct	94% (94 out of 100 people)	97% (97 out of 100 people)
In how many patients where the physician is confident about BCC diagnosis, the BCC diagnosis is incorrect	12% (12 out of 100 people)	6% (6 out of 100 people)
Chance of side effects	No side effects	3% (3 out of 100 people)
Short lasting pain associated with procedure	No pain	Moderate pain
Which situation would you prefer? (Tick one box only)	OCT ☐	Punch biopsy ☐

Figure 1. Example of choice set.

Identifying attributes and levels

An initial selection of attributes was based on literature review and expert opinion.[6, 7, 10, 17] Three attributes were associated with diagnostic accuracy of OCT and biopsy (sensitivity, false positive rates and physicians' confidence in diagnosis), two with side effects (bleeding and infection, painfulness of procedure) and one with time to diagnosis. The importance of the selected attributes was confirmed by patients in a focus group. This group consisted of ten adult patients with an indication for biopsy, who underwent an OCT scan prior to biopsy and therefore experienced both strategies. Alternative specific levels were defined for all attributes (Figure 1). The selected alternative specific levels include values derived from literature, expert opinion and hypothetical values. The hypothetical values are levels that are not yet described in literature, but that experts think might be achieved in the future.

Attributes and levels

A literature study was conducted, focusing on diagnostic accuracy of punch biopsy and OCT for diagnosis and subtyping of BCC.[6-10, 17] We chose to present sensitivity, false positive rate and physician's confidence in BCC diagnosis, since we hypothesized that these attributes will most likely influence a patient's choice for either OCT or biopsy. For example, if BCC diagnosis can be made with OCT,

and the skin lesion turns out not to be a BCC (false positive result), this may have led to an unnecessary treatment, and consequently a patient might in the future not prefer OCT. Presenting both attributes sensitivity and specificity was thought to be too difficult for patients, and therefore we chose to only present sensitivity. Sensitivity reflects the ability of a test (OCT) to correctly identify patients with BCC, a concept which we thought was easier understood by patients than specificity, which reflects the ability of OCT to correctly identify patients without BCC. Sensitivity values were derived from literature (Table 1). A false positive rate of 9% was calculated based on the study of Sinx et al.[7] According to expert opinion, both a higher and lower false positive rate were considered achievable and therefore hypothetical values of 6% and 12% were added. Regarding diagnostic accuracy of biopsy, Kadouch et al. reported a false negative rate of 6.1% and a false positive rate of 21.4%.[17] The false positive rate was considered unrealistically high, since the possibility exists that in that study BCCs were already completely removed with a biopsy, leading to a higher false positive rate upon surgical excision.[17] Based on expert opinion, false negative and false positive rates are comparable for biopsy. Therefore, a false positive rate of 6% for biopsy was considered fit and hypothetical values of 3% and 9% were added.

Confidence in diagnosis was added as an attribute to give a realistic representation of clinical practice, where OCT will only partly substitute a biopsy in cases where BCC diagnosis can be made with high confidence. Recent studies report that in 30-40% of patients, BCC diagnosis can be made with high confidence.[7, 10] A hypothetical confidence rate of 50% was added. Based on expert opinion, the dermatopathologist is 95% confident in the established diagnosis based on a punch biopsy. Alternative specific levels of 90% and 100% were added. Six dermatologists of different Dutch hospitals reported that the average time needed to acquire a biopsy result was between 7 and 14 days, and therefore levels were set at 7, 10 and 14 days. For OCT no levels were defined, since results are discussed immediately.[10] For painfulness of a biopsy, levels were categorized: a little bit of pain, moderate and severe pain. No levels were defined for OCT, since it is non-invasive and not painful. Side effects associated with biopsy were defined as severe bleeding requiring a suture or an infection of the biopsy wound. In the focus group a suture was required in one case (10%). The percentage of other side effects (infection, bleeding afterwards) was estimated even lower and therefore 10%, 6% and 3% were chosen as hypothetical values. Table 1 shows the chosen attributes with their corresponding levels.

Experimental design

An efficient design was produced using Ngene software version 1.2.1 with priors set to zero given that there was no prior information available. In total, 18 choice sets were generated and blocked into 2 versions of each 9 choice sets. A 10th choice set

was added to check for rationality of patient choices by including unambiguously higher levels of diagnostic accuracy for biopsy. Patients were randomly assigned to complete either choice sets 1 to 10 (Questionnaire I) or 11 to 20 (Questionnaire II). For each choice set, respondents were asked to choose their preferred strategy. The survey was pilot tested in five persons with different levels of education, after which it was converted into the final version. A multinomial logit regression analysis was conducted after 100 respondents had completed the survey, to check for functionality of the model.

DCE questionnaire

The DCE consisted of four parts: 1) a detailed explanation, 2) an example choice set, 3) information about punch biopsy, OCT, attributes and their associated levels, 4) ten labelled choice sets. All participants were asked to report on their experience with completing the DCE. Both percentages and natural frequencies of the levels were described.

Table 1. Attributes and levels used in the discrete choice experiment.

Attributes	OCT; alternative specific level*	Punch biopsy; alternative specific level*	References
Waiting time for results (days) The time patients need to wait before the results can be discussed	Not applicable	7, 10, 14	Expert opinion of six Dutch dermatologists
Confidence in diagnosis (%) How confident is the physician about presence of diagnosis of BCC	30, 40, 50	90, 95, 100	OCT: (7, 10)
Sensitivity (%) The chance that a skin lesion is correctly identified as BCC	70, 81, 94	91, 94, 97	OCT: (6-8, 10) Punch biopsy: (17)
False positive (%) The chance that a skin lesion is incorrectly identified as BCC	6, 9, 12	3, 6, 9	OCT: (7) Punch biopsy: (17)
Side effects (%) Defined as severe bleeding requiring a suture or infection	Not applicable	3, 6, 10	Expert opinion
Painfulness Pain associated with diagnostic procedure	Not applicable	A little, moderate, severe	(21)

**The numbers represent the levels of each attribute expressed in percentages.

Statistical analysis

Discrete choice data were analysed using a multinomial logit model with the Nlogit software (Nlogit version 6). Patient characteristics were analysed in SPSS (Version 24).

The utility function can be described as:

$V(OCT/Biopsy) = \beta_0 + \beta_1 * sensitivity\_oct + \beta_2 * sensitivity\_biopsy + \beta_3 * false\ positive\ rate\_oct + \beta_4 * false\ positive\ rate\_biopsy + \beta_5 * confidence\ in\ diagnosis\_oct + \beta_6 * confidence\ in\ diagnosis\_biopsy + \beta_7 * waiting\ time\ for\ results\_biopsy + \beta_8 * painfulness\_biopsy + \beta_9 * side\ effects\_biopsy + \varepsilon$

V = represents the relative utility that a respondent derives from choosing OCT or biopsy.
β_0 = the alternative specific constant, reflecting a preference for the label OCT or biopsy.
β_1- β_9 = the alternative specific coefficients of each attribute.
ε = unobserved component of the utility function or error term.

All attributes were continuous except for the attribute painfulness which was dummy coded. Theoretical validity of responses was checked by testing the direction and significance of the model attributes. A statistically significant coefficient (*p*-value <0.05) in the regression model indicates that respondents consider this an important attribute when showing their preference for a strategy with OCT or biopsy while the sign of the coefficient reflects whether the level of an attribute has a positive or negative impact on the preference. A priori we expected respondents to prefer increased sensitivity and confidence in diagnosis, decreased false positive rate and a decrease in painfulness, side effects and waiting time for results. Subgroup analyses were performed to test whether patients show different preferences depending on a medical history of non-melanoma skin cancer (NMSC) and thus experience with a biopsy. Additional subgroup analyses were conducted for patients in the 1) regular care group, where only a biopsy was obtained 2) OCT group, where a high confidence OCT diagnosis of BCC could be made and treatment was started immediately (but a biopsy was obtained for safety reasons) and 3) OCT group, where there was insufficient confidence in OCT diagnosis and a biopsy was obtained to establish diagnosis and treatment. Since the underlying scale of the attributes differs (continuous or dummy coded) the coefficients cannot be directly compared. Hence, to calculate the relative importance, for each attribute, the regression coefficient was multiplied with the difference between the highest and lowest level for each attribute range divided by the sum of all differences.[18]

RESULTS

Sample characteristics

Between May 2019 and September 2020, patients were recruited from three Dutch Hospitals: Maastricht University Medical Centre+, Catharina Hospital, Eindhoven and Zuyderland Medical Centre, Heerlen. In total, 344 patients completed the DCE, of which 24% reported difficulties when completing the questionnaire. Baseline characteristics are shown in Table 2.

Rationality test

In total, 258/344 (75%) had a preference for biopsy in the 10th choice set. The remaining 86/344 (25%) of respondents had a preference for OCT, failing the rationality test.

Table 2. Patient characteristics of 344 respondents.

	N=344 n, (%)
Gender	
Male	186 (54)
Female	158 (46)
Age in years	
Median	72 (21-92)
Randomisation group	
OCT	177 (51.5)
Regular care	167 (48.5)
Version	
1	169 (49)
2	175 (51)
History of (non-) melanoma skin cancer	
Positive	227 (66)
Negative	117 (34)
Location	
Head or neck area	120 (35)
Coeur	39 (11)
Extremities	88 (26)
Thorax	97 (28)
Difficulties in completing questionnaire	
Not completed	47 (14)
Very easy	27 (8)
Easy	73 (21)
Moderate	115 (33)
Difficult	68 (20)
Very difficult	14 (4)

DCE results

The results (Table 3) show that for OCT, sensitivity had a significant and positive impact, whereas false positive rate had a significant and negative impact on a respondents' choice. For biopsy, physicians' confidence in diagnosis and waiting time for results had a significant and positive impact, whereas severe short-lasting

pain and false positive rate had a significant and negative impact on the choice for biopsy. The relative-importance results show that for OCT, sensitivity is the most important attribute, contributing 74% to the respondents' choice, followed by false positive rate (26%). For punch biopsy, severe short-lasting pain is the most important attribute, contributing 34% to the respondents' choice, followed by confidence in diagnosis (23%), false positive rate (22%) and waiting time for results (21%).

Table 3. Results of the multinomial logit model for the whole sample.

Attributes	Coefficient	95% Confidence Interval		Relative Importance
OCT				
Confidence in diagnosis OCT	0.00866	-0.00053	0.01785	
Sensitivity OCT	0.02583***	0.01754	0.03413	0.74
False positive rate OCT	-0.03702**	-0.06884	-0.00520	0.26
Punch biopsy				
Waiting time for results[1]	0.03483***	0.00889	0.06077	0.21
Confidence in diagnosis punch biopsy	0.02672***	0.00701	0.04642	0.23
Sensitivity punch biopsy	-0.00571	-0.04073	0.02930	
False positive rate punch biopsy	-0.04244***	-0.07351	-0.01138	0.22
Side effects[1]	-0.00960	-0.03622	0.01702	
Moderate, short lasting pain[1]	0.09901	-0.13594	0.33396	
Severe, short lasting pain[1]	-0.40236***	-0.58692	-0.21779	0.34
No. of observations = 2906, No. of respondents = 344, Log likelihood= -1956.65				

[1]Only applicable for punch biopsy. ***, significance at 1% level, **, significance at 5% level. The relative importance of the attributes can be calculated by multiplying the coefficient of an attribute with the range used for the attribute levels or using the difference in coefficients between the best and worst level of the same attribute (in case of dummy coding). The resulting part-worth utility of each attribute was divided by the sum of all part-worth utilities which gives the relative importance per attribute.[22]

Simulation analysis

In 55% of the choice sets, a biopsy strategy is preferred and in 45% OCT strategy. However, when the highest levels are applied for OCT (sensitivity of 94%, confidence in diagnosis of 50% and false positive rate of 6%), the share numbers shift, and OCT is preferred in 58% of choice sets.

Subgroup analysis

Of all 344 respondents, 117 patients had no medical history of NMSC and 227 patients did have a medical history of NMSC. The results (Table 4, supplementary material) show that for both subgroups, the attribute sensitivity had a significant and positive impact on a respondents' choice for OCT. In the subgroup of patients

with a medical history of NMSC, the OCT strategy is preferred in 46% of choice sets and in 54% of the choices the biopsy strategy. For patients with no medical history of NMSC, OCT is preferred in 43% of all choice sets and biopsy in 57%. When looking at the different subgroups within the trial, respondents in the regular care group preferred OCT in 41% of all choice sets and biopsy in 59%. In the 'high confidence' OCT group, OCT was preferred in 52% and biopsy in 48% of choice sets. In the OCT group where biopsy was used to establish a diagnosis, OCT was preferred in 41% of choice sets and biopsy in 59%.

DISCUSSION

This is the first study to evaluate patient preferences for OCT and punch biopsy for diagnosis of BCC. The results show that sensitivity had a significant and positive impact on a respondents' choice for OCT, whereas false positive rate had a significant and negative impact. For punch biopsy, physicians' confidence in diagnosis and waiting time for results had a significant and positive impact on the choice for biopsy, whereas severe short-lasting pain and false positive rate had a significant and negative impact. Unexpectedly, a longer waiting time contributed positively to a patients' choice for biopsy, which might be explained by the fact that patients are willing to wait longer for the results in case they prefer biopsy.

Surprisingly, the attribute sensitivity did not significantly impact the preference for biopsy. A possible explanation might be that the levels associated with these attributes are always very high for biopsy, with less variation in associated levels (91-97% for sensitivity, 90-100% for confidence in diagnosis) compared to OCT, in which there is greater variation in the levels of these attributes.

In both patients with and without a medical history of NMSC, sensitivity had a significant and positive impact on the preference for OCT. The relative importance results show that sensitivity was the most important attribute influencing the choice for OCT. The simulation analysis showed that using the best levels for OCT, the choice shares increase for OCT from 45% up to 58%. In the current DCE, the levels of the attributes sensitivity, confidence in diagnosis and false positive rate of OCT were based upon available literature.[6-8, 10] Part of the levels for the attributes sensitivity (94%), physicians' confidence in diagnosis (30%) and false positive rate for OCT (9%) were based on a recently conducted prospective observational study at our department.[7] In a currently ongoing multi-centre randomised trial, we expect to reach similar levels.

As the DCE was performed after treatment completion and patients therefore had experience with OCT and biopsy (when randomised to the OCT group) or only biopsy (when randomised to regular care), experience with the diagnostic strategy impacted their preference, which was confirmed by subgroup analyses. This follows current evidence in healthcare, reporting that respondents usually ascribe more value to the things they have experienced (i.e., status quo bias).[19]

In the rationality test, 25% of patients had a preference for OCT, despite better levels for the biopsy strategy. However, conducting a rationality test in a labelled DCE is difficult, since other attributes, such as painfulness and side effects, also influence a respondents' choice and it is thus questionable whether a preference for OCT should be considered as an irrational choice.

So far, previous DCEs that have been conducted in the field of dermatology, in particular BCC, focused on different treatment strategies for BCC instead of diagnostics.[14, 15, 20] In the two recent studies, treatment costs were one of the included attributes.[14, 20] We did not include costs as an attribute, as diagnosis and treatment for BCC is covered by health insurance in the Netherlands.[14]

The DCE provides useful information, however completion difficulties in this elderly population (median age 72, up to 92 years) are not to be underestimated. Many patients (24%) in this study, as well as in a study by Tinelli et al. (22.4%), reported difficulties when completing the questionnaire.[14] Understanding concepts such as 'sensitivity' and 'false positive rate' might be difficult for patients, since it requires insight into the consequences of a wrongly diagnosed skin lesion, despite of which strategy is used. The attribute 'confidence in diagnosis' was added in order to most accurately reflect clinical practice, in which OCT only partly substitutes a biopsy in cases where the physician has high confidence in BCC diagnosis. In cases where the physician is not confident about BCC diagnosis, or another diagnosis is suspected, a biopsy is required to establish a diagnosis, a concept which might be difficult for patients to understand. Tinelli et al. proposed the idea that DCE questionnaires will benefit from providing respondents with help and support from research staff.[14] However, when help is provided by research staff, this does not guarantee independence of choice, since research staff might unwittingly influence the choices participants make. Providing elderly patients with visual tools might aid understanding and also guarantee independence of choice.

In conclusion, sensitivity had a significant and positive impact on a respondents' choice for OCT, whereas false positive rate had a significant and negative impact. For biopsy, severe short-lasting pain and false positive rate had a significant and negative impact, whereas physicians' confidence in diagnosis and waiting time for results had a significant and positive impact. If the best levels regarding sensitivity

and false positive rate are used, then the preference of patients for OCT as initial diagnostic strategy increases, showing the potential of this innovation for clinical practice.

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SUPPLEMENTARY MATERIAL

Table 4. Results of the multinomial logit model according to history of non-melanoma skin cancer.

Attributes	Coefficient	95% Confidence Interval		Relative Importance
Respondents with a history of non-melanoma skin cancer *No. of observations = 1910, No. of respondents = 227,* *Log likelihood= -1295.20*				
OCT				
Confidence in diagnosis OCT	0.00787	-0.00333	0.01907	
Sensitivity OCT	0.02383***	0.01354	0.03412	1.00
False positive rate OCT	-0.03270	-0.07203	0.00662	
Punch biopsy				
Waiting time for results[1]	0.03431**	0.00243	0.06619	0.27
Confidence in diagnosis punch biopsy	0.03052**	0.00614	0.05491	0.34
Sensitivity punch biopsy	-0.00736	-0.05020	0.03549	
False positive rate punch biopsy	-0.03800	-0.07609	0.00010	
Side effects[1]	-0.01002	-0.04303	0.02300	
Moderate, short lasting pain[1]	0.13791	-0.15316	0.42898	
Severe, short lasting pain[1]	-0.34380***	-0.57022	-0.11738	0.39
Respondents without a history of non-melanoma skin cancer *No. of observations = 996, No. of respondents = 117,* *Log likelihood= -658.78*				
OCT				
Confidence in diagnosis OCT	0.00965	-0.00655	0.02584	
Sensitivity OCT	0.02957***	0.01552	0.04362	1.00
False positive rate OCT	-0.04315	-0.09769	0.01139	
Punch biopsy				
Waiting time for results[1]	0.03463	-0.01035	0.07962	
Confidence in diagnosis punch biopsy	0.01759	-0.01628	0.05146	
Sensitivity punch biopsy	-0.00294	-0.06409	0.05820	
False positive rate punch biopsy	-0.05120	-0.10522	0.00283	
Side effects[1]	-0.01079	-0.05622	0.03463	
Moderate, short lasting pain[1]	0.01300	-0.38824	0.41424	
Severe, short lasting pain[1]	-0.52430***	-0.84577	-0.20282	1.00

[1]Only applicable for punch biopsy. ***, significance at 1% level, **, significance at 5% level.

CHAPTER 3

Possibilities for application of optical coherence tomography in Mohs surgery and specific populations

CHAPTER 3.1

Diagnostic accuracy of optical coherence tomography in the assessment of in-vivo primary basal cell carcinoma resection margins prior to Mohs' Micrographic Surgery

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ABSTRACT

Background: The evidence for the additional value of optical coherence tomography (OCT) for the assessment of resection margins of primary basal cell carcinoma (BCC) prior to Mohs' Micrographic Surgery (MMS) is scarce.

Objectives: To evaluate sensitivity and specificity of OCT for the in-vivo assessment of MMS resection margins for BCC using histopathology as gold standard. Secondary, we evaluated inter-observer agreement.

Methods: This multicenter, case-control study included patients with a biopsy proven primary BCC and an indication for MMS. OCT scans of BCCs were obtained prior to MMS. A random sample of quadrants of these OCT scans was evaluated independently by two observers. This random sample consisted of BCC quadrants, which according to histopathological examination still contained tumour tissue in resection margins (cases) as well as BCC quadrants that were free from tumour tissue in resection margins (controls).

Results: In 58 out of 92 quadrants with positive resection margins, tumour was visible on the OCT image, corresponding with a sensitivity of 63.0% (95% CI: 55.1-70.6). In 54 out of 102 quadrants with negative resection margins, no tumour tissue was visible on the OCT image corresponding with a specificity of 52.9% (95% CI: 45.8-59.7).

Conclusions: This study shows poor diagnostic performance of OCT for the assessment of in-vivo primary BCC resection margins prior to MMS.

INTRODUCTION

Basal cell carcinoma (BCC) is the most common form of cancer of which the incidence is still increasing.[1,2] Mohs' micrographic surgery (MMS) is a single-day, outpatient procedure in which evaluation of 100% of the resection margins leads to complete tumour removal and preservation of healthy tissue.[3] Because of the high cure rates combined with the tissue saving capacity, treatment with MMS is performed with an increasing frequency, especially in the head and neck area.[4] Before starting the procedure, tumour margins are set on the basis of clinical and dermoscopic examination. Subsequently, the tumour is excised with a minimal safety margin and processed with frozen sections.[3] If remaining tumour is identified by direct histopathological assessment of the frozen sections, only that specific part is removed, and the procedure is repeated.[3] Due to the time needed to make the frozen sections and the possibility of multiple consecutive stages, MMS is a labor-intensive and time-consuming procedure. Consequently, there are limitations in the number of patients that can be treated per day and also the clinical condition of the patient may be a contraindication for this treatment.[5,6] Optical coherence tomography (OCT) is a promising non-invasive imaging modality, which has been successfully used to diagnose BCC.[5–7] OCT uses the reflection of an optical beam to acquire real-time cross-sectional images of the skin with a < 7.5 µm lateral and < 5 µm axial optical resolution and a penetration depth of approximately 1 – 1.5 mm. Based on optical reflections, the epidermis, dermis, and skin appendages can be distinguished.[5,8,9]

A systematic review provides recommendations for the use of OCT in delineating BCC prior to MMS.[10] Two case reports and five case series included in this systematic review describe the use of OCT for delineating in vivo BCCs.[10] These few in-vivo studies with a small number of patients provide no estimates of sensitivity and specificity. Hence, the objective of this case-control study is to estimate the sensitivity and specificity of OCT for the in-vivo assessment of MMS margins for primary BCC. Secondary objectives are to evaluate inter-observer agreement.

MATERIALS AND METHODS

Included were patients aged 18 years or older with a histopathologically proven primary BCC and an indication for MMS following the Dutch BCC guidelines[11], who visited the dermatology outpatient clinic of Maastricht University Medical Centre+ (MUMC+, Netherlands) or Mohs Klinieken in Hoorn (Netherlands), between September 2017 and January 2018. Patients unable to sign informed consent or with a BCC of which it was not possible to obtain or assess an OCT-image were excluded. MMS tumour removal was performed using a bowl-shaped excision with 45° angles.[12]

The study was approved by the local ethics committee (METC 16-04-197) and was conducted according to the Declaration of Helsinki. Written informed consent was obtained from all patients prior to inclusion.

In this diagnostic case-control study, conventional OCT images were acquired prior to MMS. The en-face mode was used, where a 'multislice' area of 6x6 mm^2 was scanned, comprising 120 individual vertical OCT images. Subsequently, cases and controls were randomly selected. Cases were defined as OCT images of quadrants of BCCs, which according to histopathological examination contained tumour tissue in the margins after resection. Controls were defined as OCT images of quadrants of BCCs that were free from any residual tumour after resection. Histopathological examination was used as gold standard test. Removed tissue was prepared at the pathology laboratory and assessed for tumour free margins by both a dermatologist and dermatopathologist with over 10 years of experience, who were both unaware of the OCT diagnosis.

Sensitivity of OCT was estimated as the proportion of histopathology-positive quadrants wherein tumour tissue was visible on the OCT images (OCT-positive result). Specificity was estimated as the proportion of histopathology-negative quadrants wherein tumour tissue was not visible on the OCT images (OCT-negative result). Estimation of an expected sensitivity and specificity of OCT of 50% with an absolute margin of error of no more than 10% requires a sample size of 97 histopathology-positive quadrants and 97 histopathology-negative quadrants. To account for a drop-out rate of 10%, 216 (2*108) quadrants had to be included for assessment. The sample size was based on an expected sensitivity and specificity of OCT of 50%, because proportions close to 50% result in the largest sample sizes and therefore the sample size is also adequate if sensitivity and specificity would prove to be larger than 50%.

OCT imaging and analysis

Based on clinical and dermoscopic examination, tumour margins were marked with a dashed line and the coordinate system with a continuous line, both with a surgical pen. A POSCA PC-1MV pen with white ink was used to draw a safety distance of 2 mm around the margin (Figure 1). The POSCA PC-1MV pen is visible both on skin and on OCT images, where the ink reflects the light beam leading to a hyperreflective line with a shadow underneath. The skin directly underneath the hyperreflective line can therefore not be assessed for the presence of tumour on the OCT image. The outer border of the white line corresponded to the resection margin (Figure 2). After marking, a clinical photograph was made and each quadrant was scanned with the OCT device. If the quadrant border oversized the scanning head, extra overlapping scans were made per quadrant, in order to visualize 100% of the resection margins. All images were acquired by the same researcher per center using a commercially available OCT device (Vivosight Multi-beam Swept-Source Frequency Domain OCT, Michelson Diagnostics, specifications: class 1 eye safe, resolution <7.5µm lateral, <5µm axial). The enface mode was used to scan all BCCs, which scans a 'multislice area' of 6x6mm^2, comprising 120 individual cross-sectional OCT images. One image of healthy skin and one image of the center of the tumour were obtained as a reference. Subsequently, the resection margin and the tissue on the inner and outer part of the resection margin were scanned, corresponding to tumour tissue and clinically healthy tissue, respectively. The white line marking the resection margin was always located in the middle of the probe and therefore in the middle of the OCT image. Images were obtained clockwise: from axis A to axis B to the end of the coordinate system (Figure 3). The OCT probe was repositioned for each image in order to obtain an OCT image where tumour tissue was always visible on the left side and clinical healthy tissue was always visible on the right side of the OCT image.

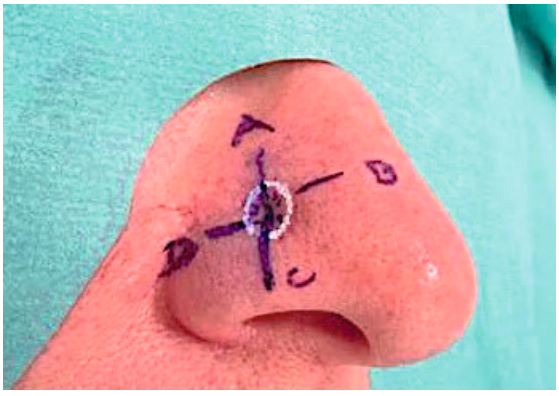

Figure 1. Clinical delineation and coordinate system of a basal cell carcinoma prior to excision. The purple dashed line is the clinical edge of the basal cell carcinoma. The white line is the primary resection margin with 2 mm safety margin from the clinical edge.

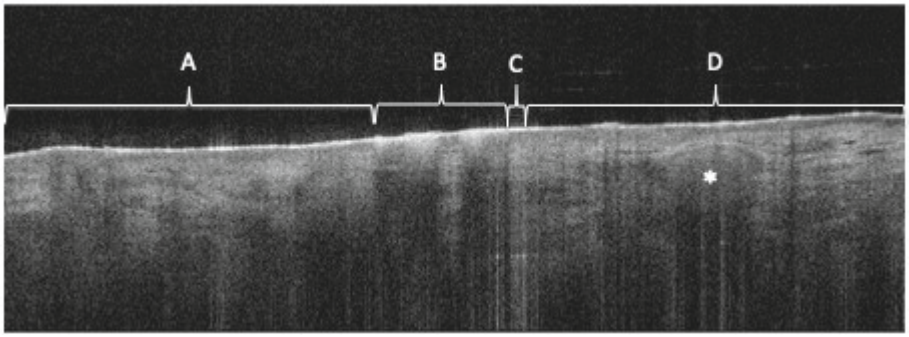

Figure 2. OCT-image of a basal cell carcinoma scanned with the enface mode. **A**, Tumour tissue and 2 mm safety margin. **B**, White line drawn with POSCA PC-1MV pen visible as a white hyperreflective line, casting a shadow underneath. **C**, Primary resection margin directly next to the white line. **D**, Tissue that was assessed. In this part, a BCC nest (asterisk) can be visualized.

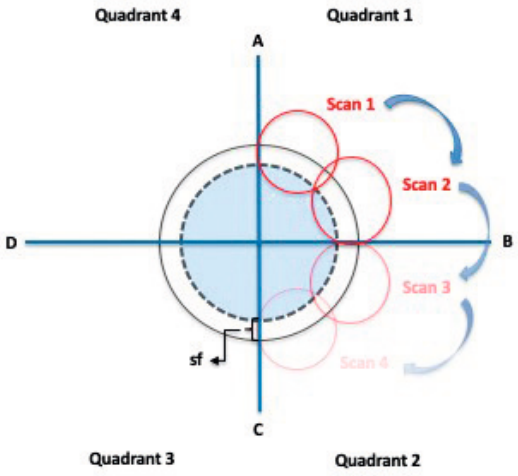

Figure 3. Schematic view of the scanning method starting and ending at axis A. The blue circle with the dashed line is the clinical edge of the basal cell carcinoma and the black circle corresponds to the primary resection margin with a safety distance of 2 mm (sf) from the clinical edge.

Two researchers independently evaluated all OCT images. Researcher one (FA) had one and a half year of experience and daily acquires and assesses OCT images of BCCs. Researcher two (EK) was a medical student in her last semester, who was trained with over 500 OCT images of BCCs, a number of scans exceeding the number required to obtain an adequate competence in distinguishing BCC from non-BCC.[13] Researchers assessed if BCC characteristics were visible on the right side of the hyperreflective line of the OCT image, based on previously defined criteria by Hussain et al.[14] Level of confidence in BCC diagnosis was recorded on

a five-point Likert scale (0=certainly not a BCC; 1= suspicion of BCC presence is low; 2= suspicion of BCC is high; 3= surely BCC but uncertain about subtype and 4= surely BCC and certain about BCC subtype). Features complicating OCT assessment such as presence of a crust, ulceration, keratosis, hair, noise level, large amount of sebaceous glands, vessels and incomplete image were recorded. Possible causes for an incomplete image were convex or concave areas. Since the OCT device is equipped with a flat headpiece, scanning those areas may result in an OCT image of which the lateral sides of the OCT image are not assessable for the presence of tumour.

The two researchers documented their diagnosis (OCT-positive versus OCT-negative result) and level of confidence in their diagnosis independently from each other to enable evaluation of inter-observer agreement. Confidence scores 2-4 were considered as OCT-positive whereas confidence scores 0-1 were considered as an OCT-negative result.

An ultimate diagnosis on whether tumour tissue was visible on OCT (OCT-positive versus OCT-negative result) was reached by consensus.

Statistical analysis
For the description of baseline characteristics of the study population, nominal and categorical variables are presented as numbers and percentages, respectively. If normally distributed, continuous variables are presented as mean with standard deviation. Sensitivity and specificity with 95% confidence intervals were calculated, for all patients and for subgroups according to location of the lesion. Inter-observer agreement on diagnosis and on level of confidence was calculated using a kappa-coefficient and a weighted kappa-coefficient, respectively.

RESULTS

A total of 86 patients with 93 BCCs and 374 quadrants were scanned. On average, both scanning and image interpretation took between 15 and 20 minutes, depending on the size and location of the tumour.

Thirty-two quadrants had to be excluded because of inaccessible location (n=26) or time shortage prior to surgery (n=6). From the remaining dataset consisting of 83 patients with 90 BCCs and 342 quadrants, 103 cases with positive resection margins and 113 controls with negative resection margins were randomly selected. The reason why the number of cases and controls were not equal was due to a misinterpretation: in five patients a second Mohs' stage was performed, not

because of tumour positive margins, but because of scar tissue. Therefore, these quadrants, that first were assumed to be tumour-positive, were later classified as negative quadrants. Twenty-two quadrants had to be excluded after assessing the OCT images mostly due to an incomplete image (n=17). As a consequence, 194 quadrants (92 OCT-positive quadrants and 102 OCT-negative quadrants) were included for analysis (Figure 4).

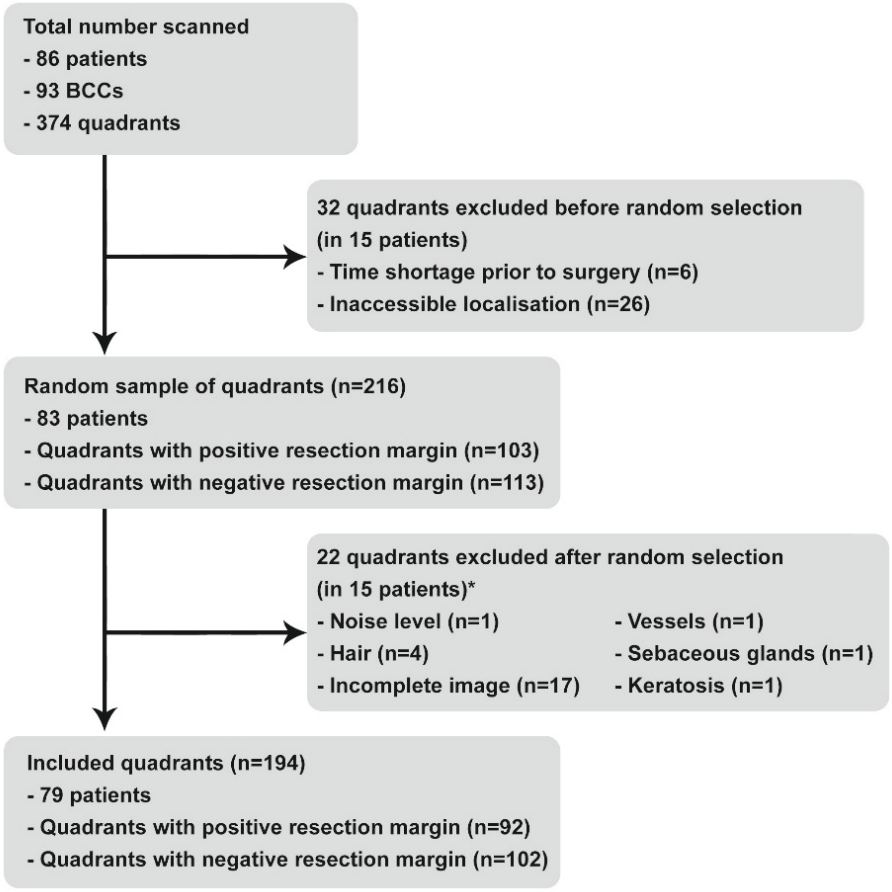

Figure 4. Study flowchart.

Study sample characteristics

The mean age of 52 patients with positive resection margins was 74.0 years (± 12.6) and 57% were male. The mean age of 55 patients with negative resection margins was 74.0 years (± 12.0) and 44% were male. Baseline characteristics of the histology-positive and histology-negative quadrants are shown in Table 1. Most common locations were the nose (cases 40.2% and controls 39.2%) and periocular area (cases 19.6% and controls 16.7%).

Table 1. Overview of baseline characteristics of 92 cases (histology-positive OCT quadrants) and 102 controls (histology-negative OCT quadrants).

Category	Cases (92)	Controls (102)
Clinical length BCC* in mm (sd)	13.3 (8.3)	11.8 (7.4)
Clinical width BCC in mm (sd)	10.8 (6.2)	9.2 (5.5)
Mohs' stages (sd)	2.25 (0.6)	1.05 (0.2)
Location in % (n)		
Nose	40.2 (37)	39.2 (40)
Periocular area	19.6 (18)	16.7 (17)
Forehead	15.2 (14)	14.7 (15)
Helix	13.0 (12)	4.9 (5)
Cheek	8.7 (8)	16.7 (17)
Skin of upper lip/nasolabial fold	3.3 (3)	4.9 (5)
Vertex	0.0 (0)	2.9 (3)
Subtype in % (n)		
Superficial	2.1 (4)	2.1 (4)
Nodular	15.5 (30)_	16.5 (32)
Aggressive (morpheaform/micronodular)	29.4 (57)	31.4 (61)
Basosquamous	0.5 (1)	0.5 (1)
Unknown	-	2.1 (4)

For numeric variables means are given. For categorical variables percentages are given. *BCC: Basal cell carcinoma.

Diagnostic accuracy of OCT

Table 2 shows that there is a trade-off between sensitivity and specificity. Sensitivity decreases and specificity increases, if a higher cut-off value of the confidence score is used to define a positive OCT result. When confidence scores 2-4 were defined as a positive OCT result, sensitivity and specificity of OCT based on consensus opinion was 63.0% (95% CI: 55.1-70.6) and 52.9% (95% CI: 45.8-59.7), respectively (Table 2). Diagnostic accuracy was comparable for both investigators: researcher one reached a sensitivity and specificity of 56.5% (95% CI: 48.6-64.2) and 52.9% (95% CI: 45.8-59.9) and researcher two a sensitivity and specificity of 55.4% (95% CI: 47.5-63.2) and 53.9% (95% CI: 46.8-60.9), respectively.

Diagnostic accuracy of OCT according to location

Subgroup analyses were performed to evaluate the influence of location on diagnostic accuracy of OCT. A distinction was made between locations that are easy and difficult (convex or concave skin surface) to reach with OCT. Skin of upper lip/nasolabial fold, cheek, forehead and vertex were considered as easy to reach locations and nose, periocular area and helix of the ear as difficult to reach

locations. Sensitivity and specificity of OCT for easy to reach locations was 64.3% (95% CI: 48.3-78.4%) and 55.0% (95% CI: 43.8-64.9%), respectively. For difficult to reach locations, sensitivity was 62.5% (95% CI: 53.2-71.3%) and specificity was 51.6% (95% CI: 42.0-60.7%), respectively.

Inter-observer agreement on diagnosis and on level of confidence

With respect to diagnosis (presence versus absence of tumour tissue), the researchers reached agreement in 138 of the 194 quadrants (71.1%). Cohen's kappa coefficient was 0.42 (95% CI 0.30-0.55). The observers had the same confidence level score in 57 of the 194 quadrants (29.4%) and the weighted kappa coefficient (with linear weighting) was 0.30 (95% CI 0.21-0.38).

Table 2.

	Observer 1 (FA)	Observer 2 (EK)	Consensus
Cut-off 1234 versus 0			
Sensitivity	81.5 (75/92)	88.0 (81/92)	-
Specificity	22.5 (23/102)	11.8 (12/102)	-
PPV	48.7 (75/154)	47.4 (81/171)	-
NPV	57.5 (23/40)	52.2 (12/23)	-
Cut-off 234 versus 01			
Sensitivity	56.5 (52/92)	55.4 (51/92)	63.0 (58/92)
Specificity	52.9 (54/102)	53.9 (55/102)	52.9 (54/102)
PPV	52.0 (52/100)	52.0 (51/98)	54.7 (58/106)
NPV	57.4 (54/94)	57.3 (55/96)	61.4 (54/88)
Cut-off 34 versus 012			
Sensitivity	37.0 (34/92)	35.9 (33/92)	-
Specificity	72.5 (74/102)	70.6 (72/102)	-
PPV	54.8 (34/62)	52.4 (33/63)	-
NPV	56.1 (74/132)	55.0 (72/131)	-
Cut-off 4 versus 0123			
Sensitivity	13.0 (12/92)	7.6 (7/92)	-
Specificity	91.2 (93/102)	90.2 (92/102)	-
PPV	57.1 (12/21)	41.2 (7/17)	-
NPV	53.8 (93/173)	52.0 (92/177)	-

Diagnostic parameters for various cut-off values of the confidence score (level of confidence in BCC diagnosis was recorded on a five-point Likert scale (0=certainly not a BCC; 1= suspicion of BCC presence is low; 2= suspicion of BCC is high; 3= surely BCC but uncertain about subtype and 4= surely BCC and certain about BCC subtype)). For example, using a cut-off value of 34 versus 012, only lesions that were surely BCC according to the OCT observer were defined as a positive OCT result, all other scans were defined as a negative OCT result. Consequently, specificity is high in this category at the cost of sensitivity. When using lower cut-off values for the definition of a positive OCT result, sensitivity increases and specificity decreases.

DISCUSSION

Sensitivity and specificity of OCT for the assessment of in vivo-primary BCC resection margins prior to MMS is 63.0% and 52.9% respectively. Hence, diagnostic accuracy is too low for implementation in clinical practice.

A few small-sized studies showed more favorable results with respect to the ability of OCT to correctly predict resection margins. De Carvalho et al. showed that 8 out of 10 BCCs were totally excised in a single MMS stage when margins were previously assessed with use of OCT.[6] In this study, the presence of BCC characteristics was directly evaluated on the OCT images. If any characteristics were visible, margins were enlarged and a new scan was obtained until it showed no suspicious BCC areas anymore, and the lesion was excised. Thus, resection margins were enlarged without histopathological verification. It was only reported whether tumour was excised completely. Wang et al. concluded that in all BCCs which required a second MMS stage (11 out of 52), OCT had correctly predicted that tumour extended outside the clinically estimated margin[16]. In the 41 cases requiring a single MMS stage, the OCT defined margin was always found to be on or within the Mohs defect boundary. In the study of Alawi et al., in 16 out of 19 of the cases the OCT defined margins correctly indicated complete removal of the tumour. The clinical margins never fell below the OCT defined margins.[17] None of these studies reported sensitivity and specificity of OCT for the in-vivo assessment of MMS resection margins for BCC.

The low sensitivity and specificity in the current study contrast with the results of studies on diagnostic performance of OCT in situations where it is important to distinguish BCC from non-BCCs. In these studies, where the center of well visible lesions was scanned, sensitivity varied between 86.0%-96.6% and specificity between 75.3%-98.0%.[7,18–20] When OCT is used to correctly predict resection margins, the periphery of a tumour is scanned and only minimal presence of tumour tissue has to be discovered. This may be an explanation for the low sensitivity. The low specificity and consequently the high number of false positive OCT results may be due to misinterpretation of sebaceous glands for nodular tumour nests and vessels for infiltrative tumour nests (Figure 5). Sebaceous glands are abundant on the nose, and in our study, 39.7% of the included BCCs with an indication for MMS were located on the nose. The dynamic OCT mode is a feature that enables the visualization of the microvasculature of the skin and may make it easier to distinguish infiltrative BCC tumour nests from vessels.[9] In the future it could be helpful to use this feature although using the dynamic OCT mode is more time-consuming since acquiring one scan takes 30 seconds opposed to 10 seconds in the enface mode.

The penetration depth of current OCT devices might also limit an accurate assessment, since the penetration depth of the OCT device is up to 1.5 mm (Figure 5).[8] Because the mean depth of aggressive and nodular BCCs in the head and neck area is 1.5 mm and 1.7 mm respectively, the penetration depth might not be sufficient to detect deeper located BCC tumour nests.[17,21] This might not be a problem when OCT is used for diagnosis of BCC, as a diagnosis can usually be established on the basis of more superficially located nests in the center of the tumour. However, in OCT assessment of resection margins, only the peripheral borders of the tumour are scanned and if only deeper located nests are present there, these nests are invisible on OCT images. A second explanation for the high number of false negative OCT results can be the fact that OCT images are obtained in a perpendicular fashion with a 90°angle whereas a bowl-shaped excision with 45° angles is used in MMS.[12] The clinically drawn margin and the deeper margins of the cutting edge may differ, as the surgeon cuts towards the tumour.

In this study, images were analyzed retrospectively. Due to this study design, there was no possibility to obtain a new image when the previous image was not of sufficient quality. In some cases, a new scan could result in a better scan quality and therefore better assessment and less exclusions.

The usefulness of implementation of OCT in MMS depends on diagnostic accuracy, time needed for evaluation of OCT scans and costs. Based on our findings the accuracy was poor. We also found that the procedure was time consuming and difficult to implement within a well-balanced workflow in MMS. Furthermore, it must be realized that costs include the purchase of the OCT device, training of personnel and extra scanning time.

In conclusion, based on the results of the current study, the use of OCT for the assessment of BCC resection margins prior to MMS cannot be recommended in clinical practice yet.

Acknowledgments
The patients in this manuscript have given written informed consent for the publication of their case details.

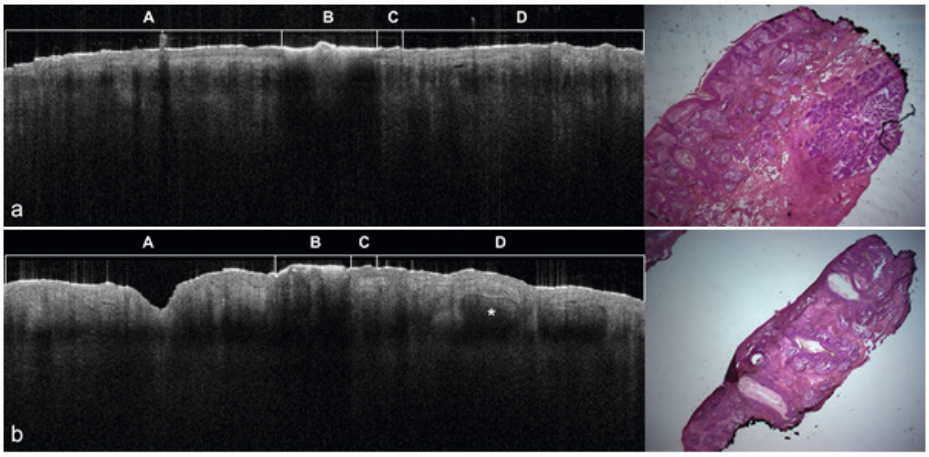

Figure 5. OCT images of a false-negative and a false-positive margin in comparison with the corresponding histology.

A, Tumour tissue and 2-mm safety margin. **B**, White line drawn with POSCA PC-1MV pen visible as a white hyperreflective line, casting a shadow underneath. **C**, Primary resection margin directly next to the white line. **D**, Tissue that was assessed.
(a) False-negative margin in comparison with the corresponding histology. Histology shows a nodular and micronodular BCC in the deep margin. **D**: In this part, no BCC characteristics are visible.
(b) False-positive margin in comparison with the corresponding histology. Histology shows adnexal richness without the presence of BCC nests. **D**: In this part, an adnexal structure (asterisk) was misinterpreted as BCC nest.

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CHAPTER 3.2

The additional diagnostic value of optical coherence tomography in clinically diagnosed basal cell carcinomas undergoing direct surgical excision

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RESEARCH LETTER

Clinical examination appears to be very sensitive for diagnosing BCC (90%), but the specificity is reported to be low (28.6-48.9%).[1, 2] Additional use of dermoscopy can increase specificity to 54.3-55.6% compared to clinical examination alone.[1, 2] With use of optical coherence tomography (OCT), a non-invasive diagnostic method, in addition to clinical and dermoscopic examination, it is possible to further increase the specificity to 76% at a sensitivity of 95%.[1, 3, 4] These results apply to a population of patients with a clinical suspicion of BCC who had an indication for biopsy (e.g. high risk location or uncertainty about diagnosis). However, there are subgroups of patients, such as patients with a very high clinical suspicion for a low risk BCC or patients with multiple BCCs, who undergo direct surgical excision without prior histopathological verification of BCC diagnosis.[5, 6]

The aim of this study was to investigate whether in these subgroups of patients OCT has additional diagnostic value and can help to reduce the risk of misclassification of non-BCC lesions as BCC. Patients were included from August 2019 to January 2021 in one academic and two general hospitals in the Netherlands. The study was approved by the local ethics committee and was conducted according to the Declaration of Helsinki. Written informed consent was obtained from all patients. All included lesions were highly suspicious for BCC based on clinical and dermoscopic examination and were scheduled for surgical excision without prior histopathological verification. Before surgery, an OCT scan was obtained for study purposes and the OCT diagnosis did not influence the treatment decision. A commercially available OCT (< 7.5 µm lateral and < 5 µm axial optical resolution) was used for imaging (VivoSight; Michelson Diagnostics Ltd., Maidstone, UK). Analysis of OCT images was performed by one experienced observer using the morphological characteristics of BCC as previously described.[7] Histopathologic diagnosis was used as gold standard.

In total, 114 patients with a high clinical and dermoscopic suspicion of BCC were included, 59 (51.8%) in an academic hospital and 55 (48.2%) in general hospitals. Median age was 71 (21-91) years and 63 patients were male (55.3%). Lesions were located on the trunk (47.4%), head or neck area (35.1%) and extremities (17.5%). The results with respect to diagnostic accuracy of OCT are summarized in Table 1. According to histopathologic diagnosis, 109 of 114 lesions were BCCs, which corresponds to a positive predictive value (PPV) of clinical and dermoscopic diagnosis of 95.6%. All of the 109 histopathological verified BCCs were identified as such by OCT (sensitivity =100%) and the negative predictive value in case of negative OCT result was 100% (4/4). In only 5 out of 114 lesions (4.4%) histopathology revealed an alternative diagnosis: seborrheic keratosis, solar elastosis, benign lichenoid kerato sis, warty dyskeratoma and squamous cell carcinoma (SCC).

OCT identified 4 of these 5 lesions as non-BCC lesions. A benign lichenoid keratosis was misclassified as BCC by both clinical and dermoscopic examination and OCT. Furthermore, the SCC was excised with a 3mm margin, upon which it was radically removed. The majority (97.4%) of the lesions in this study, all scheduled for excision, were diagnosed as nodular BCCs according to clinical and dermoscopic findings. There were only 3 superficial BCCs, because in superficial BCC non-invasive treatment is usually preferred. Of all 109 BCCs, 11 (10.1%) were superficial, 81 (74.3%) nodular and 17 (15.6%) infiltrative upon histopathology. Clinical and dermoscopic examination misclassified 8/11 (72.7%) superficial BCCs as nodular, whereas with OCT 7/11 (63.6%) were misclassified as mixed superficial/nodular BCC. In total 17 (100%) infiltrative BCCs were misclassified as nodular by clinical and dermoscopical examination and 14 (82.4%) by OCT. With additional use of OCT the PPV increased from 95.6% (without OCT) to 99.2% (109/110) with OCT. The decrease in the percentage of misclassifications was not significant, but a study with enough power to detect differences in this order of magnitude would require a much larger sample size.

In another prospective study, the PPV of an OCT diagnosis that was made with high confidence was only 80%, but the BCC prevalence in that study was also lower (58.2%) than in this study (95.6%). The PPV depends on prevalence and becomes lower if prevalence decreases.[8] Use of OCT in addition to clinical and dermoscopic examination may reduce the risk of misclassification of non-BCC lesions as BCC, but this study also shows that in case of high clinical and dermoscopic suspicion of BCC, this risk is already very low. The gain from additional use of OCT in patients with high clinical suspicion of BCC must be balanced against investments that have to be made for the purchase of an OCT device and the training of OCT users.

Table 1. Diagnostic parameters for OCT in patients with high suspicion of low-risk BCC according to clinical and dermoscopic diagnosis.

	Histology		
	BCC	**No BCC**	**Total**
OCT positive for BCC	109	1	110
OCT negative for BCC	0	4	4
Total	109	5	114

Abbreviations: BCC, basal cell carcinoma; OCT, optical coherence tomography.

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We would like to thank drs. Robert Riedl, pathologist, for his contribution to the manuscipt.

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CHAPTER 3.3

Diagnosis of periocular basal cell carcinoma with optical coherence tomography

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CASE-REPORT

We present a case of periocular basal cell carcinoma (BCC) diagnosed with optical coherence tomography (OCT) prior to Mohs micrographic surgery (MMS).

Basal cell carcinoma is the most common cancer in the periocular region, representing 84-96% of all skin cancers in this region.[1] It can cause significant morbidity due to the close proximity to functional (peri)ocular structures such as the eyelid, lacrimal duct or intraorbital structures. In addition, periocular BCCs may grow more rapidly compared to BCCs occurring at other locations.[2] MMS is the preferred treatment for periocular BCCs as it preserves healthy skin and has higher long-term clearance compared to standard excision.[1] OCT and reflectance confocal microscopy (RCM) are non-invasive imaging techniques that have been used to diagnose BCC (Figure 1). With OCT vertical slides are obtained with a penetration depth of 1.0-1.5mm, whereas RCM provides horizontal slides with a higher resolution, at the cost of the imaging depth of 250µm.[3]

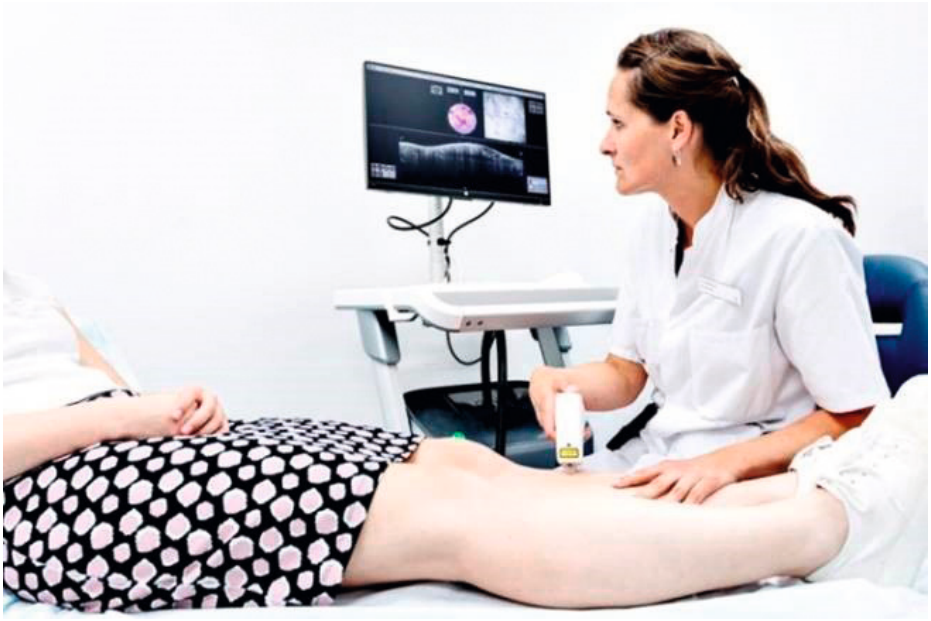


Figure 1. Application of OCT in clinical practice.

OCT can be used to diagnose BCC with a sensitivity of approximately 95% and significantly increases specificity in diagnosis and subtyping of BCC compared to clinical observation alone.[4] In a study including ten patients, the ability to define BCC margins with OCT prior to MMS seems promising.[5] It might also be a valuable technique for diagnosing periocular BCC without the use of a diagnostic

biopsy. However, there is no information regarding the use of OCT in this region in the studies performed to date.[4, 5] Here, we demonstrate the use of OCT in the periocular region and show that it can be helpful to select a representative area for biopsy.

A 52-year-old patient with a history of multiple (>50) basal cell carcinomas, visited the dermatology outpatient clinic of the Maastricht University Medical Center. Three BCCs on the upper left eyelid had been treated in 2005, 2008 and 2012 with cryotherapy and MMS. A skin-colored nodule of 2 mm was visible on her upper left eyelid, without any clinical or dermoscopic signs of BCC (Figure 2). OCT showed a localized protrusion into the upper dermis with a dark border, corresponding to a superficial BCC nest. In the dermis an ovoid grey structure with a dark border was seen, corresponding to a nodular BCC nest (Figure 3). Based on previously defined criteria by Hussain et al., the diagnosis of a superficial/nodular BCC was established.[3] The patient was planned for MMS. On the day of the procedure, a punch biopsy with frozen sections analysis confirmed our diagnosis of a superficial/nodular BCC, and we subsequently performed Mohs micrographic surgery.

Diagnosis and treatment of periocular BCCs can be challenging. In small tumours a diagnostic punch biopsy partially removes the clinically visible BCC. This can make it difficult to find the exact location of the BCC prior to surgery or even lead to a larger surgical defect because of scar tissue formation around the biopsy site. By using OCT in patients with a periocular skin lesion suspicious for BCC, these problems can be avoided. To minimize the risk of overtreatment of benign lesions even further, a frozen section biopsy just before the first Mohs stage can be considered.

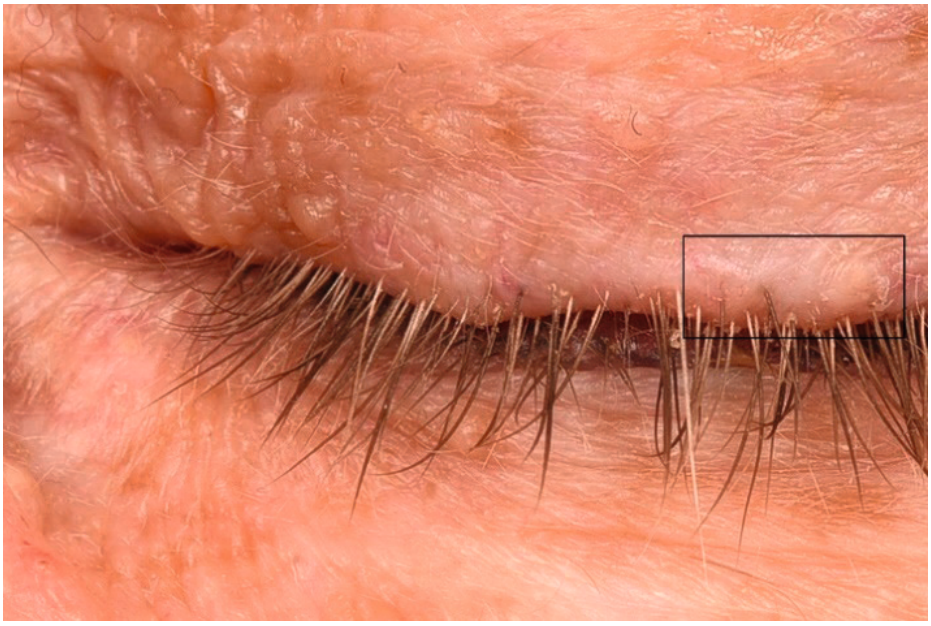
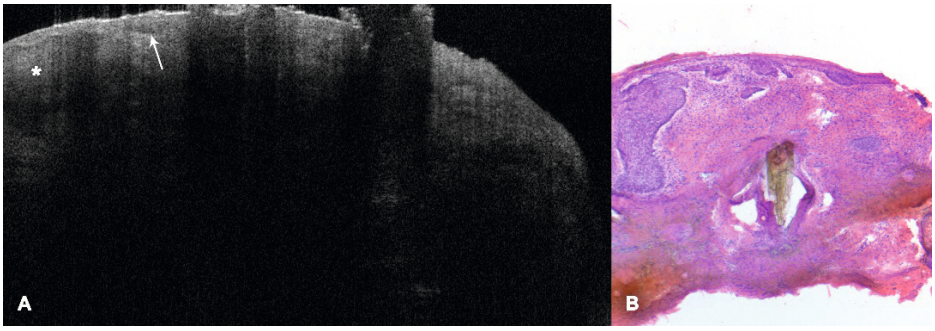


Figure 2. Close up image shows ill-defined area of induration on the upper eyelid. There were no epidermal changes or other evident clues for BCC. The black rectangle represents the area imaged with OCT.



A: OCT image showing a protrusion into the upper dermis with a dark border (white arrow), corresponding to an epidermal bound BCC tumour nest. In the dermis, an ovoid gray structure can be visualized with a dark border (asterisk), corresponding to a nodular BCC tumour nest. B: Corresponding histopathology showing a superficial and nodular BCC.

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CHAPTER 4

Optimization of the diagnostic accuracy of optical coherence tomography for diagnosis of basal cell carcinoma

CHAPTER 4.1

Diagnostic value of optical coherence tomography image features for diagnosis of basal cell carcinoma

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ABSTRACT

Optical coherence tomography (OCT) is a noninvasive diagnostic method. Numerous morphological OCT features have been described for diagnosis of basal cell carcinoma (BCC).

In this study, we evaluate the diagnostic value of established features, and we explore whether the use of a small set of features enables accurate discrimination between BCC and non-BCC lesions and between BCC subtypes. For each lesion, presence or absence of specific features was recorded. Histopathology was used as a gold standard.

Diagnostic parameters were calculated for each feature and multivariate logistic regression analyses were performed to evaluate the loss in discriminative ability when using a small subset of features instead of all features that are characteristic for BCC according to literature.

Results show that the use of a limited number of features allows for good discrimination of superficial BCC from non-superficial BCC and non-BCC lesions. The prevalence of BCC was 75.3% (225/299) and the proposed diagnostic algorithm enabled detection of 97.8% of BCC lesions (220/225). Subtyping without the need for biopsy was possible in 132 of 299 patients (44%) with a predictive value for presence of superficial BCC of 84.3% versus 98.8% for presence of non-superficial BCC.

INTRODUCTION

The incidence of non-melanoma skin cancer (NMSC) has increased over the past decades, with basal cell carcinoma (BCC) being the most prevalent cancer diagnosed in the Caucasian population worldwide.[1-4] Although there are many histopathologic subtypes, a simplified classification by Rippey roughly groups all BCCs into three subtypes: superficial, nodular, and agressive.[5] Currently, the histopathological examination of a punch biopsy is the gold standard to discriminate BCC from alternative diagnoses and to determine the histopathologic subtype.[6]

In recent years, optical coherence tomography (OCT) has emerged as a promising non-invasive diagnostic method for BCC diagnosis, generating real-time in vivo cross-sectional images of tissue microarchitecture with a depth of approximately 1.5 mm.[7] It is based on light interferometry; the interference of two optical beams reflected by tissue produces different shades in the black and white spectrum. Despite the high lateral resolution of <7.5 mm and axial resolution <5 mm, imaging of individual cells is not possible. OCT is therefore suitable for pattern recognition in tissue similar to e.g. ultrasound, allowing for the identification of morphological features of BCC, which have been established in recent years.[8-12] In 2015, Hussain et al. systematically reviewed diagnostic criteria for BCC. The authors evaluated 17 studies and found that in 100% of these studies, rounded dark (hyporeflective) structures in the upper dermis, surrounded by a bright (hyperreflective) halo, sometimes surrounded by a hyporeflective border and disruption of epidermal layering, were described as characteristic for BCC.[8] The rounded dark structures resemble the basaloid cell nests seen in histology, the hyperreflective halo surrounding the rounded structures corresponds to the surrounding tumour stroma and a hyporeflective border at the periphery resembles the peripheral palisading at the margins of basaloid cell nests.[13] Other features that have been described for BCC are presented in Table 1, some of which are illustrated in Figure 1a-c.[9-12]

It remains unknown which features are most discriminative for BCC diagnosis. Therefore, we evaluate the diagnostic value of established morphological OCT features that can be used for diagnosis and subtyping of BCC. The second objective is to explore whether the use of a small set of features with the highest predictive value enables accurate discrimination between BCC and non-BCC lesions and between BCC subtypes.

Table 1. OCT image features with corresponding histopathology features.[8-12, 19]

Optical Coherence Tomography	Histopathology
Epidermis	
Superficial scaling/crust/ulceration	Superficial scaling/crust/ulceration
Atrophy of the epidermis	Atrophy of the epidermis
Thickening of the epidermis	Thickening of the epidermis
Protrusions into the upper dermis with dark rim	Superficial basaloid nests with a firm connection to the epidermis
Dermo-epidermal junction	
Interrupted/poorly defined	Interrupted/poorly defined
Dermis	
Signal-poor ovoid structures/rounded dark structures	Basaloid nests in the dermis
Ovoid structures with bright centre	Basaloid nests with necrotic cell debris in centre
Dark rim/hyporeflective border	Peripheral palisading
Bright (hyperreflective) peritumoural stroma/ hyperreflective halo	Collagen compression between adjacent nests
Prominent vessels	Dilated capillaries
Small ovoid signal-poor structures ‘Shoal of fish’ or ‘Bunch of grapes’	Nests in morpheaform and micronodular basal cell carcinoma, respectively
Dark/black (areflective) areas/cysts	Area of liquefactive necrosis

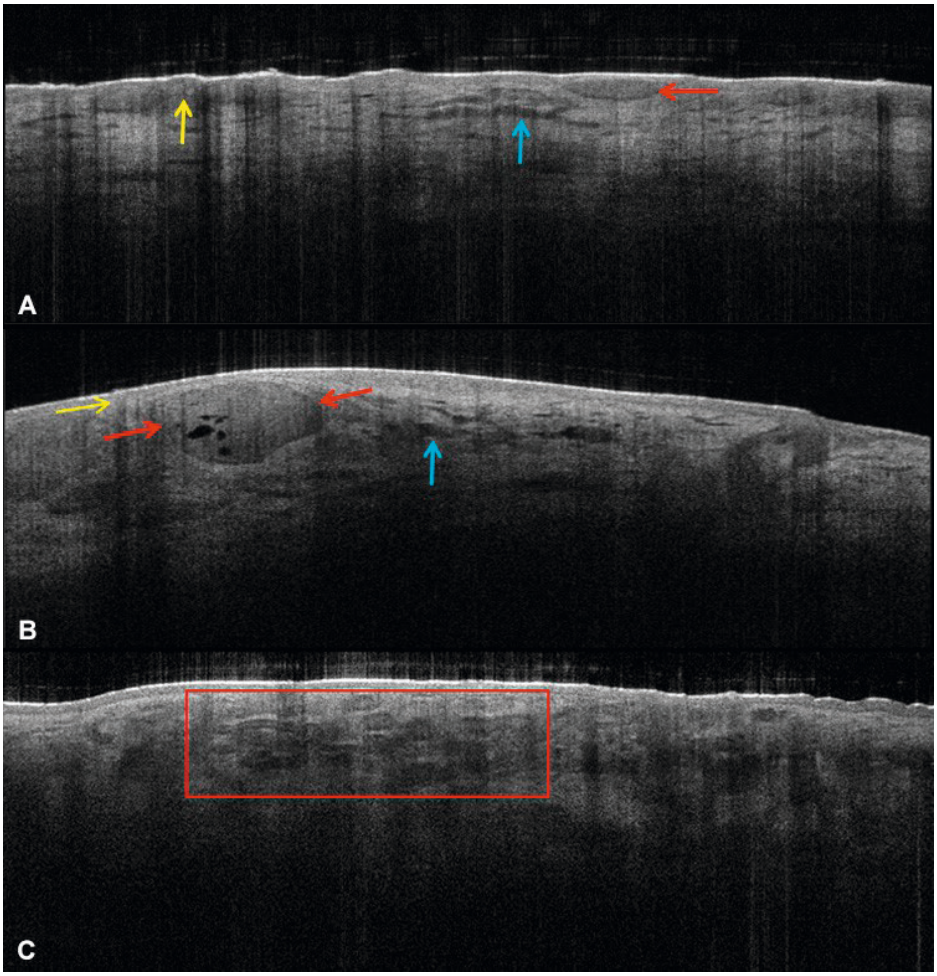

Figure 1 A-C. OCT images of different BCC subtypes. A: OCT image of a superficial BCC. Red arrow points towards a protrusion into the upper dermis with a dark rim, corresponding to a basaloid cell nest with a firm connection to the epidermis.[10] Vessels in the upper dermis are dilated (blue arrow) and the DEJ is disrupted (yellow arrow). B: OCT image of a nodular BCC. Red arrows point towards a fully encompassing signal-poor ovoid structure located in the dermis with a dark rim and bright peritumoural stroma. Inside the nest, well-circumscribed black areas are observed, representing signs of liquefactive necrosis.[19] Vessels in the upper dermis are dilated and directed towards the tumour nests (blue arrow). The epidermis above the nest is atrophic, the DEJ is disrupted (yellow arrow). C: OCT image of an aggressive BCC. Inside the red rectangle, smaller signal-poor ovoid structures with surrounding bright peritumoural stroma are visible, also described as 'shoal of fish' or 'bunch of grapes', indicating an aggressive BCC subtype.[9]

MATERIALS AND METHODS

Data for this study was derived from one arm of a randomised multi-centre non-inferiority trial in one academic and two general Dutch hospitals: the Dermatology outpatient clinic of the Maastricht University Medical Centre+ (Maastricht), Catharina Hospital (Eindhoven) and Zuyderland Medical Centre (Heerlen). Included were consecutive patients (18 years or older) with an indication for skin biopsy of a lesion clinically suspected for BCC. Excluded were patients with a lesion located in the 'H-zone' of the face, patients with a large BCC referred to our head and neck tumour working group and patients who were incompetent to sign informed consent. The marked biopsy area of the clinically most aggressive part was scanned with OCT without any preparations of the skin in advance (Vivosight Multi-beam Swept-Source Frequency Domain OCT, Michelson Diagnostics, Maidstone, Kent, UK).

OCT scanning and interpretations were conducted by one experienced investigator (FA). The investigator used morphological features that are characteristic for identifying BCC lesions using OCT.[8-10, 14] For each lesion, presence or absence of specific morphological features was recorded (Table 1). Features relating to the epidermis included: superficial scaling/crust/ulceration, protrusions into the dermis with a dark rim, atrophy or thickening of epidermis, DEJ interrupted/poorly defined. Features relating to the dermis included: signal-poor ovoid structures, ovoid structures with bright centre, dark rim, bright peritumoural stroma, prominent vessels, black areas or cysts and small signal-poor ovoid structures ('shoal of fish' or 'bunch of grapes' appearance).

The histopathologic result from a punch biopsy or excision biopsy was used as a gold standard. Histopathological examination was performed by a specialized dermato-pathologist, blinded to the OCT diagnosis. BCC subtypes were classified as superficial, nodular, or aggressive BCC.

The study was approved by the local independent Ethics Committee. All patients provided written informed consent.

Statistical analysis

The distribution of baseline characteristics in patients who underwent OCT examination was summarized by absolute numbers and percentages for categorical variables and by mean values with standard deviations or median with range for continuous variables. Diagnostic parameters were calculated for each morphological OCT feature. Diagnostic odds ratios (DOR) with 95% confidence intervals (CI) were calculated using univariate logistic regression analyses.

Subsequently, multivariate logistic regression analyses were performed to evaluate the loss in discriminative ability when using a small subset of morphological features instead of all morphological features that are characteristic for BCC according to literature.[8-10] Histologically verified presence or absence of BCC was the dependent variable. Discriminative ability was expressed as the area under the receiver operating characteristic curve (AUC) and differences in AUCs between models was tested for significance using an algorithm for paired comparison of AUCs from DeLong et al.[15]

Similar analyses were performed to identify subsets of features that can be used for accurate subtyping. These analyses were restricted to the subgroup of patients with histologically confirmed BCC. Discrimination between superficial BCC and non-superficial BCC subtypes was considered most relevant for clinical practice, since superficial BCC can be treated non-invasively. Therefore, histologically verified superficial BCC versus non-superficial BCC subtypes was used as dependent variable. A DOR> 1 indicates that the presence of a feature is indicative for presence of superficial BCC (sBCC). P-values ≤ 0.05 were considered to indicate statistical significance. Analyses were performed using IBM SPSS Statistics version 24 and Stata version 14.

RESULTS

A total of 598 patients with 598 skin lesions clinically suspicious for BCC were included in the randomised trial between March 2019 and September 2020. The data of 299 patients, who were randomised to the OCT group, were used for this study. According to histological diagnosis, 225 (75.3%) patients had a BCC and 74 had an alternative diagnosis. Of these 225 BCCs, 66 were superficial, 79 nodular and 12 aggressive. The remaining 68 BCCs were of a mixed subtype (Table 2).

Discrimination between BCC and non-BCC

For each morphological feature that has been described to be relevant for discrimination between BCC and non-BCC lesions, diagnostic parameters are presented in Table 3. A dark rim is the strongest positive predictor of BCC (odds ratio (OR): 64.11, 95% CI 27.02-152.11), followed by bright peritumoural stroma (OR: 49.38, 95% CI 20.09-121.40). Protrusions into the upper dermis with a dark rim (OR: 15.18, 95% CI 6.66-34.59), poorly defined/interrupted DEJ (OR: 9.17, 95% CI 4.98-16.88) and signal-poor ovoid structures (OR: 9.08, 95% CI 4.82-17.12) were also strongly associated with presence of BCC. The odds ratios were statistically significant ($p< 0.0001$) for all features but superficial scaling/crust/ulceration ($p=0.79$) and prominent vessels ($p=0.89$).

The AUC of the full multivariate logistic model including all twelve morphological features was 95.2% (95% CI 91.8-98.7%), The AUC of a model including the five features with the highest diagnostic odds ratios was 94.7% (95% CI 91.4-98.1%). A final model included the four features which were most discriminative based on clinical experience of the investigator: signal-poor ovoid structures, dark rim, bright peritumoural stroma and protrusions into the upper dermis with a dark rim. For this model, the AUC decreased to 94.1% (95% CI 90.8-97.3%). However, the decreases in AUC when using four or five features instead of all features were minor and non-significant (p=0.49 and p=0.24, respectively). Based on these results we conclude that four features can be used to discriminate between BCC and non-BCC without significant loss in diagnostic performance.

Table 2. Baseline characteristics of 299 patients who underwent OCT examination.

Characteristic	
Median age (SD)	72 (21-95)
Sex, n (%)	
Male	164 (54.8)
Female	135 (45.2)
Localization, n (%)	
Head/neck	94 (31.4)
Upper anterior chest	37 (12.4)
Trunk	89 (29.8)
Extremities	79 (26.4)
Histologic diagnoses, n (%)	
BCC	225 (75.3)
No BCC	74 (24.7)
BCC subtypes, n (%)	
Superficial	66 (29.3)
Nodular	79 (35.1)
Aggressive (morpheaform/micronodular)	12 (5.3)
Mixed	68 (30.2)
Other diagnoses (non-BCC), n (%)	
Benign[1]	34 (11.4)
Actinic keratosis	24 (8.0)
Bowen's disease	9 (3.0)
SCC	5 (1.7)
Superficial spreading malignant melanoma	1 (0.3)
Atypical fibroxanthoma	1 (0.3)

Abbreviations: BCC, basal cell carcinoma; SCC, squamous cell carcinoma.
[1]Including: sebaceous gland hyperplasia and/or adenoma, dermatofibroma, dermal nevus, seborrhoic keratosis, scar, benign lichenoid keratosis, acute folliculitis, neurofibroma, trichofolliculoma, venous stasis dermatitis, sclerosing dermatitis, excoriation, dilated hair follicle, angioma, chronic inflammation, eczema, apocrine hidrocystoma, epidermoid cyst.

Discrimination between superficial BCC and non-superficial BCC subtypes

Table 4 shows the frequency of each morphological feature, as well as diagnostic parameters for discrimination between superficial BCC and non-superficial BCC

subtypes. The diagnostic odds ratio was significant for eight features. The high DOR for 'protrusions into the upper dermis with a dark rim' and an 'interrupted DEJ' indicates that presence of these features is strongly indicative for presence of superficial BCC (sBCC).

The other six features 1) atrophy of the epidermis, 2) signal-poor ovoid structures, 3) ovoid structures with bright centre, 4) bright peritumoural stroma, 5) shoal of fish/bunch of grapes and 6) black areas, cysts are associated with DORs which are significantly lower than 1, indicating that these features are highly predictive for non-superficial BCC.

The AUC of the full multivariate logistic model including all twelve features was 95.5% (95% CI: 93.0-98.0). The use of a model including the eight features that were associated with a significantly increased or decreased DOR resulted in a comparable AUC (p=0.88) of 95.6% (95% CI 93.2%-98.0%). Use of subset of six of these eight features (excluding 'bright peritumoural stroma' and 'interrupted DEJ') led to a significant (p=0.01) decrease of the AUC (94.0% with 95% CI: 91.1%-97.0%)

Diagnostic algorithm

Figure 2 proposes a diagnostic algorithm for diagnosis and subtyping of BCC. First, to discriminate between BCC and non-BCC lesions, one can use the four features: 1) dark rim, 2) bright peritumoural stroma, 3) protrusions, 4) signal-poor ovoid structures. If one or more of these features are present, the probability of BCC is 89.4% (PPV), whereas if all four features are absent the probability of a non-BCC lesion is 90.6% (NPV).

With respect to subtyping, absence of the feature 'protrusions into the upper dermis with a dark rim' indicates a high probability of non-superficial BCC. However, when this feature is present, both superficial and non-superficial BCC can be present. For further discrimination, additional features that increase the probability of non-superficial BCC can be used: 1) atrophy of the epidermis, 2) signal-poor ovoid structures, 3) ovoid structures with bright centre, 4) shoal of fish/bunch of grapes or 5) black areas, cysts. If all five features are absent and protrusions are present (n=51), the probability of superficial BCC is 84.3%. If one or more of the five features are present and protrusions are absent (n=81), there is a high probability of non-superficial BCC subtype (98.8%). In case of other combinations, there remains too much doubt and a punch biopsy is still necessary to determine the histological subtype. For instance, when both protrusions and one or more of the other five features are present, the probability of superficial BCC and non-superficial BCC is 23% and 77%, respectively.

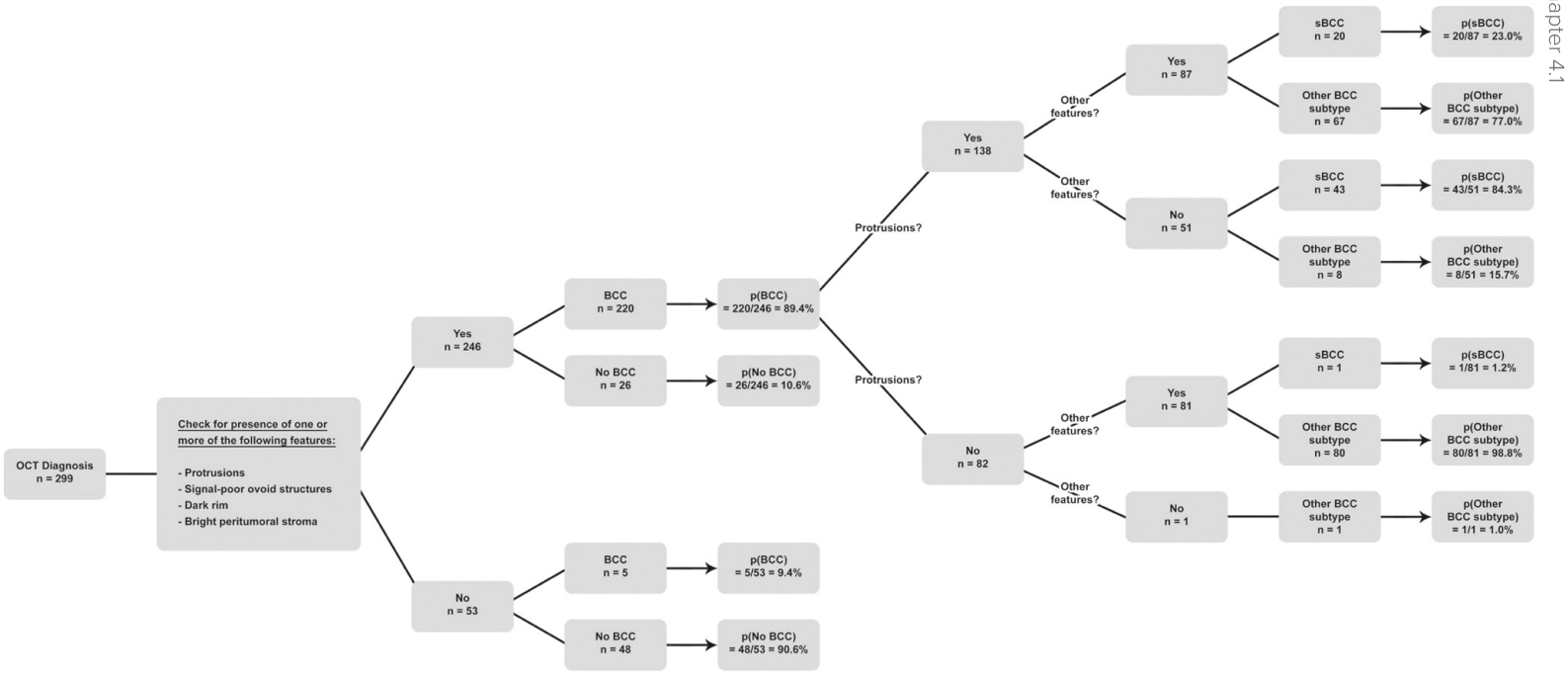

Figure 2. Diagnostic algorithm for diagnosing and subtyping of BCC. Check for presence of one or more 'other features': atrophy of the epidermis, signal-poor ovoid structures, ovoid structures with bight centre, shoal of fish/bunch of grapes and black areas/cysts. p(sBCC) stands for probability of superficial BCC and p(Oher BCC subtype) for probability of non-superficial BCC.

Table 3. Diagnostic parameters for discrimination between BCC and non-BCC.

Characteristic	Presence in BCC (%)* (Sensitivity)	Absence in non-BCC (%)** (Specificity)	Probability of BCC if present (PPV)	Probability of non-BCC if absent (NPV)	Odds ratio (95% CI)	p-value
Epidermis						
Superficial scaling/crust/ulceration	57.3 (129/225)	40.5 (30/74)	74.6 (129/173)	23.8 (30/126)	0.92 (0.54-1.56)	0.79
Atrophy of the epidermis	40.9 (92/225)	94.6 (70/74)	95.8 (92/96)	34.5 (70/203)	12.11 (4.27-34.32)	< 0.0001
Thickening of the epidermis	16.0 (36/226)	31.1 (23/74)	41.4 (36/87)	10.8 (23/212)	0.09 (0.05-0.16)	< 0.0001
Protrusions into upper dermis with dark rim	61.3 (138/225)	90.5 (67/74)	95.2 (138/145)	43.5 (67/154)	15.18 (6.66-34.59)	< 0.0001
Dermo-epidermal junction						
Dermo-epidermal junction poorly defined/ interrupted	74.7 (168/225)	75.7 (56/74)	90.3 (168/186)	49.6 (56/113)	9.17 (4.98-16.88)	< 0.0001
Dermis						
Signal-poor ovoid structures	69.8 (157/225)	79.7 (59/74)	91.3 (157/172)	46.5 (59/127)	9.08 (4.82-17.12)	< 0.0001
Ovoid structures with bright centre	28.9 (65/225)	93.2 (69/74)	92.9 (65/70)	30.1 (69/229)	5.61 (2.16-14.53)	< 0.0001
Dark rim	96.4 (217/225)	70.3 (52/74)	90.8 (217/239)	86.7 (52/60)	64.11 (27.02-152.11)	< 0.0001
Bright peritumoural stroma	81.3 (183/225)	91.9 (68/74)	96.8 (183/189)	61.8 (68/110)	49.38 (20.09-121.40)	< 0.0001
Prominent vessels	56.9 (128/225)	44.6 (33/74)	75.7 (128/169)	25.4 (33/130)	1.06 (0.63-1.80)	0.89
Shoal of fish/bunch of grapes	22.2 (50/225)	97.3 (72/74)	96.2 (50/52)	29.1 (72/247)	10.29 (2.44-43.40)	< 0.0001
Black areas, cysts	27.6 (62/225)	95.9 (71/74)	95.4 (62/65)	30.3 (71/234)	9.00 (2.73-29.64)	< 0.0001

Odds ratios (OR) of univariate logistic regression analyses and p-values.
*Data subset includes 225 BCCs and 74 non-BCCs.

Table 4. Prevalence of morphological features in superficial BCC and non-superficial BCC subtypes in patients with histologically verified BCC.

Characteristic	Presence in sBCC (%) n=66 (Sensitivity)	Absence in non-superficial BCC subtype (%) n=159 (Specificity)	Probability of sBCC if present (PPV)	Probability of non-superficial BCC subtype if absent (NPV)	Odds ratio* (95% CI)	p-value
Epidermis						
Superficial scaling/crust/ulceration	56.1 (37/66)	42.1 (67/159)	28.7 (37/129)	69.8 (67/96)	0.93 (0.52-1.66)	0.88
Atrophy of the epidermis	9.1 (6/66)	45.9 (73/159)	6.5 (6/92)	54.9 (73/133)	0.09 (0.04-0.21)	<0.001
Thickening of the epidermis	22.7 (15/66)	86.8 (138/159)	41.7 (15/36)	73.0 (138/189)	1.93 (0.93-4.04)	0.11
Protrusions into upper dermis with dark rim	95.5 (63/66)	52.8 (84/159)	45.7 (63/138)	96.6 (84/87)	23.52 (7.09-78.04)	<0.001
Dermo-epidermal junction						
Dermo-epidermal junction poorly defined	98.5 (65/66)	35.2 (56/159)	38.7 (65/168)	98.2 (56/57)	35.34 (4.78-261.55)	<0.001
Dermis						
Signal-poor ovoid structures	19.7 (13/66)	9.4 (15/159)	8.3 (13/157)	22.1 (15/68)	0.03 (0.01-0.06)	<0.001
Ovoid structures with bright centre	9.1 (6/66)	62.9 (100/159)	9.2 (6/65)	62.5 (100/160)	0.17 (0.07-0.42)	<0.001
Dark rim	95.5 (63/66)	3.1 (5/159)	29.0 (63/217)	62.5 (5/8)	0.68 (0.16-2.94)	0.70
Bright peritumoural stroma	65.2 (43/66)	11.9 (19/159)	23.5 (43/183)	45.2 (19/42)	0.25 (0.13-0.51)	<0.001
Prominent vessels	43.9 (29/66)	37.7 (60/159)	22.7 (29/128)	61.9 (60/97)	0.48 (0.27-0.85)	0.01
Shoal of fish/bunch of grapes	0.0 (0/66)	68.6 (109/159)	0.0 (0/50)	62.3 (109/175)	0 (cannot be calculated)	<0.001
Black areas, cysts	9.1 (6/66)	64.8 (103/159)	9.7 (6/62)	63.2 (103/163)	0.18 (0.08-0.45)	<0.001

Odds ratios (OR) of univariate logistic regression analyses and p-values. *An odds ratio >1 indicates higher probability of superficial BCC and an odds ratio<1 indicates higher probability of non-superficial subtype.

Summarizing, the use of the diagnostic algorithm enabled detection of 97.8% of BCC lesions (220/225). In 132 of 299 patients (44%) a diagnosis of subtype could be made with high predictive value for presence of superficial BCC (84.3%) versus presence of non-superficial BCC (98.8%) without the need for biopsy.

DISCUSSION

This study evaluated the diagnostic values of morphological OCT features for BCC diagnosis. With the features 1) dark rim, 2) bright peritumoural stroma, 3) protrusions into the upper dermis with a dark rim and 4) signal-poor ovoid structures (all with PPV > 90%) a good discrimination between BCC and non-BCC lesions is possible. With regard to subtyping, 'protrusions into the upper dermis with a dark rim' are visible in the vast majority of superficial BCCs and absence of this feature is highly predictive of non-superficial BCC when other BCC features are present. However, if 'protrusions' are present, a conclusive diagnosis cannot be made. In this situation, five other features that are highly predictive of non-superficial BCC have to be used.

Accurate diagnosis of BCC with OCT, or other non-invasive methods, would enable the use of a one-stop shop approach in patients with a lesion clinically suspected for BCC. This approach requires that superficial BCCs can be distinguished from non-superficial BCCs in a substantial part of patients. Patients with superficial BCC can be treated non-invasively (i.e. imiquimod cream, photodynamic therapy). Therefore, these patients would benefit most from accurate non-invasive diagnosis.

In case of high suspicion of sBCC, non-invasive treatment can be discussed with the patient and initiated in the same visit and an invasive procedure with risk of pain, bleeding and scar formation can be prevented. In case of high suspicion of non-superficial BCC, a surgical excision can be planned immediately. This one-stop shop approach could result in reduction of the number of biopsies and is expected to be more efficient, patient friendly and cost-effective than regular care, where diagnosis and treatment is based on the histopathological result of a punch biopsy.[16, 17]

The proposed diagnostic algorithm offers a systematic approach towards discrimination of superficial BCC from non-superficial BCC and non-BCC lesions. The first step, discrimination of BCC from non-BCC lesions, is possible with four features (PPV of 89.4) There is a risk (of approximately 10%) that a lesion that is diagnosed as BCC by OCT is not a BCC. The prevention of misclassification of another cutaneous malignancy as BCC is the biggest challenge. Cheng et

al. evaluated the diagnostic accuracy of OCT in lesions for which sBCC was considered in the differential diagnosis. In their series, one amelanotic melanoma was misclassified as sBCC. In our study cohort, one patient had a superficial spreading malignant melanoma, clinically highly suspected for BCC. However, upon OCT examination, no BCC features were present, so this case was diagnosed as a non-BCC lesion with an indication for biopsy. The risk of misclassification of an amelanotic melanoma as BCC is small, but future research on morphological OCT features that can help to distinguish melanoma from BCC is needed.

The proposed diagnostic algorithm also enables discrimination of sBCC from non-superficial BCCs and non-BCC lesions, but there remains a chance of 15.7% that non-superficial BCC is misclassified as sBCC, if protrusions are present and one or more of the other five features are absent. Such misclassifications may result in a higher risk of residual or recurrent BCC if non-invasive treatment is chosen. To verify that misclassifications by OCT do not compromise patient safety, the results of a randomised controlled trial, which compares effectiveness of an OCT guided diagnosis with regular care (biopsy) have to be awaited.

The diagnostic algorithm uses six features for subtyping, although the discriminative ability of a multivariate diagnostic model using six features was significantly worse than that of a model with eight features, which also included 'bright peritumoural stroma' and 'interrupted DEJ'. Still, the latter two features were not used in the algorithm. The reason is that the multivariate model evaluates the probability of sBCC given all combinations of the presence and/or absence of features, whereas the diagnostic algorithm considers only a limited number of possible combinations. Absence of 'protrusions' in combination with one or more of features indicative of non-superficial BCC results in high probability of non-superficial BCC being present. However, in this respect, addition of the feature 'bright peritumoural stroma', which is indicative of non-superficial BCC, was not helpful, because its prevalence is also high in superficial BCC. The feature 'interrupted DEJ' was also left out. It had little added value, because it occurred nearly always in combination with the feature 'protrusions into the upper dermis with a dark rim' (except for two lesions).

In this study, the selection of features that are useful for diagnosis and subtyping of BCC was based on reports from the literature. A problem is that there is high variability in the use of terminology in the literature. Describing the four features that discriminate well between BCC and non-BCC lesions, Hussain et al. referred to 1) rounded dark structures in the upper dermis (corresponding to signal-poor ovoid structures) 2) surrounded by a hyperreflective halo (bright peritumoural stroma) 3) possibly surrounded by a hyporeflective border (dark rim) and 4) disruption of epidermal layering (poorly defined/interrupted DEJ).[8] Cheng et al.[10] concluded

that ‘protrusions into the upper dermis with a dark rim’ are highly predictive for superficial BCC, and described this feature as ‘hyporeflective ovoid structures with firm attachment to the DEJ and a clefting region focused or solely visible at the inferior margin’. The high prevalence of an atrophic epidermis and ovoid structures with bright centre (referred to as intranodular small bright dots) in nodular BCC has also been reported by other studies.[11, 12] Since the evidence for the use of OCT for BCC diagnosis is increasing, more uniformity in terminology is desirable for future implementation of OCT for BCC diagnosis. In this study, we used conventional OCT, whereas the use of dynamic OCT provides additional information by visualizing the vascular patterns and thus allows for better differentiation between BCC subtypes. Therefore, with use of dynamic OCT the proposed diagnostic algorithm may lead to even better diagnostic performance, which can be evaluated in future studies.[18]

A limitation of this study is that predictive values, such as PPV and NPV, are highly dependent on the prevalence of BCC and sBCC subtypes in a study population. In this study, patients were included with lesions suspected for BCC, based on clinical and dermoscopic examination by a dermatologist, leading to a study population with a high BCC prevalence (75.3%). In other study populations, where for BCC suspected lesions would have been selected by physicians with less experience in clinical and dermoscopic examination, the BCC prevalence might be lower, which could result in different predictive values of the morphological features. Furthermore, the proposed diagnostic algorithm is meant as support for OCT users that do not yet have much experience with interpretation of OCT images. The trial had the aim to evaluate whether OCT guided diagnosis and treatment does not compromise patient safety when compared with regular care (always biopsy). For ethical reasons, it was decided not yet to include high-risk patients with large lesions or lesions in the ‘H-zone’. Furthermore, in the H-zone surface areas are often convex or concave, making it more difficult to obtain an OCT image of sufficient quality. More studies are therefore required to determine whether OCT is suitable in this subpopulation. Also, the diagnostic algorithm needs to be validated in future studies.

In conclusion, the results of this study show that the use of a limited number of morphological features allows for good discrimination of superficial BCC from non-superficial BCC and non-BCC lesions with the potential to obviate the need for punch biopsy in 44% of patients. Hence, novice OCT assessors who start with OCT training could first focus on recognizing these selected features.

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CHAPTER 4.2

Topical application of glycerol increases penetration depth in optical coherence tomography in diagnosis of basal cell carcinoma

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ABSTRACT

Optical coherence tomography (OCT) is a non-invasive imaging technique allowing high-resolution in-vivo imaging of skin. Although OCT is promising for diagnosing basal cell carcinoma (BCC), its limited penetration depth may impede BCC subtyping. This study evaluated whether topical application of glycerol can increase penetration depth and improve the image quality and visibility of characteristic BCC features.

Sixty-one patients with a total of 72 BCCs were included. OCT scans were obtained before and after application of an 85% glycerol solution. The average penetration depth of each OCT scan was acquired by automatically tracing both skin surface and the point of signal loss using a custom-made MATLAB program. Average penetration depth increased from 883±108 to 904±88 μm before and after glycerol application, respectively (p=0.005).

Topical application of glycerol leads to a significant 2.4% increase in penetration depth. However, no significant differences in image quality and visibility of BCC features were found.

INTRODUCTION

The incidence of non-melanoma skin cancer (NMSC) is increasing globally with basal cell carcinoma (BCC) being the most prevalent skin cancer diagnosed among the Caucasian population.[1] Histopathological examination of a punch biopsy remains the gold standard for confirming BCC diagnosis and subtype.[2, 3] However, a punch biopsy is a minor invasive procedure.

Optical coherence tomography (OCT) emerged as a promising non-invasive diagnostic imaging technique for the diagnosis of BCC, showing improved specificity and sensitivity when used in addition to clinical examination and dermoscopy.[3-6]

OCT uses the reflection of an optical beam to acquire real-time cross-sectional images of the skin with a < 7.5 μm lateral and < 5 μm axial optical resolution, and a penetration depth of approximately 1 – 1.5 mm. Based on the optical reflections, the epidermis, dermis, and skin appendages can be distinguished.[6, 7] However, as the average tumour depth of aggressive BCC subtypes, including infiltrative and micronodular BCC, is estimated at approximately 1.5 mm the penetration depth may be insufficient to detect deeper located and smaller BCC tumour nests.[8]

By reducing light scattering in OCT scans, the penetration depth may be enhanced. Light scattering mainly occurs at the tissue interfaces whose refractive indices mismatch, such as the surface of skin and the dermal-epidermal border. In pursuance of enhancing OCT image quality and penetration depth, hyperosmotic chemical agents, called optical clearing agents (OCAs), have been applied to the skin to match refractive indices. These OCAs reduce light scattering and thereby enhance optical penetration depth.[9, 10] Glycerol, a hydrophilic trihydroxy alcoholic substance, has been used as OCA in multiple studies, demonstrating increased penetration depth and enhanced contrast in OCT diagnostics.[9-14] However, the reported increase in penetration depth has not yet been quantified.

In this study, we evaluated whether topical application of glycerol solution on BCCs improves optical penetration depth. Additionally, the effect of glycerol application on image quality and visibility of characteristic BCC features was evaluated.[15]

MATERIALS & METHODS

Patients, aged 18 years or older, visiting the department of dermatology of the Maastricht University Medical Centre+ (MUMC+) with one or more histopathologically confirmed BCCs were included between January 2019 and May 2019. The study was approved by the local ethics committee (METC 16-4-197) and was conducted according to the Declaration of Helsinki. Written informed consent was obtained from all patients prior to inclusion.

OCT imaging

OCT imaging of the BCC(s) was performed both before and immediately after topical application of glycerol 85% (0.01 ml) solution on the skin lesion. All OCT scans were acquired by a single physician using a commercially available OCT device (VivoSight; Michelson Diagnostics Ltd., Maidstone, United Kingdom) equipped with a 6 mm probe (axial resolution 15 μm). Prior to OCT imaging, a medical photograph was taken of each lesion.

Image analysis

For all OCT images the average penetration depth was assessed using a custom-made MATLAB (version 2018b; The Mathworks, Natick, MA, USA) script. This program automatically traced the skin surface and the point of signal loss for each location in the image based on the (differences in) signal intensity, represented by a blue and red line in the OCT image, respectively (Figure 1). Penetration depth was defined as the mean distance between these two lines. Subsequently, all OCT images were presented in random order to three observers who were blinded to any patient data and did not know whether the OCT image was taken before or after glycerol application: one dermatologist with extensive OCT imaging experience (EvL) and two dermatology residents with moderate OCT imaging experience (EO and GD).

Observers scored: 1) overall image quality (determined by the noise level and shadows casted by keratosis and/or crusts/ulcerations), and 2) visibility of the most common BCC features (as previously identified by Hussain et al.).[15]

Both parameters were scored separately using a 4-point Likert-scale (1: Low, 2: Medium, 3: High, and 4: Very high). Since lower image quality has been reported for BCCs presenting with keratosis and with crusts and/or ulcerations, lesions with these features were classified into two subgroups based on clinical presentation.[16]

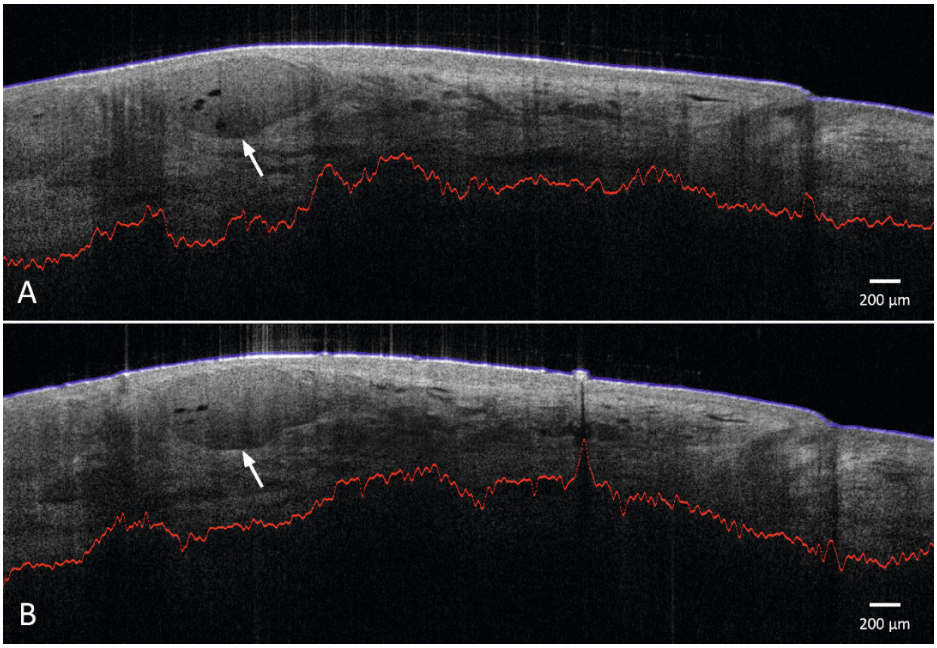

Figure 1. OCT images of the same basal cell carcinoma (BCC) acquired before (image A) and after (image B) glycerol application. A custom-made MATLAB program was used to automatically analyse the images: the blue line traces the skin surface, while the red line traces the point of signal loss. The distance between these two lines was calculated at all positions to obtain mean penetration depth. A signal-poor ovoid nest (corresponding to a basaloid cell nest) is indicated by the white arrow. At approximately 1mm depth the signal intensity drastically decreases which might make it difficult to identify BCC features in deeper skin layers. *Note that images A and B slightly differ in image position as the OCT imaging probe was removed for glycerol application.*

Statistical analysis

The sample size calculation was based on the mean penetration depth, the primary outcome measure. An increase in mean penetration depth after glycerol application of half the standard deviation of the difference (effect size=0.5) was considered as minimally clinically relevant. To enable detection of such a difference between the two conditions (before and after topical application of glycerol) with a power of 80% and two-sided alpha of 5%, 64 BCCs were required. To account for a 10% drop out rate 72 BCCs were included.

Results are expressed as mean ± standard deviation or as percentage unless otherwise specified. Differences in mean penetration depth before and after topical glycerol application were evaluated using either a paired-samples Student's *t*-test (in case of normally distributed data) or a non-parametric Wilcoxon signed-rank test (in case of non-normally distributed data). Normality of the data was

evaluated using the Shapiro-Wilk test. Differences in image quality and visibility of characteristic BCC features before and after topical glycerol application were evaluated using the McNemar's test for paired proportions. The proportions of OCT scans with higher scores after glycerol application (improved outcome) were compared with percentages with lower scores after glycerol application (worsened outcome). Separate analyses were performed for the two subgroups of BCCs presenting with keratosis, or crusts and/or ulcerations and for superficial BCCs. All statistical analyses were performed using SPSS Statistics 25 (International Business Machines (IBM), Armonk, NY, USA). Two-sided p-values < 0.05 were considered statistically significant.

RESULTS

Sixty-one patients (35 male, median age 70 years-old, range 44 - 95) with a total of 72 BCCs were included. Baseline characteristics of the study sample are summarized in Table 1. OCT imaging was successfully performed before and after topical application of glycerol in all patients.

Table 1. Baseline characteristics of the study sample (61 patients with a total of 72 basal cell carcinomas (BCCs).

Sex (male), *n* (%)	35 (57.4%)		
Age, median (range)	70 (44-95) years		
Number of lesions per patient *n* (%)	1 lesion	53	(86.9%)
	2 lesions	6	(9.8%)
	3 lesions	1	(0.02%)
	4 lesions	1	(0.02%)
Lesion location *n* (%)	Head and neck region	23	(31.9%)
	Upper chest	6	(8.3%)
	Back/abdomen	27	(37.5%)
	Extremities	16	(22.2%)
BCC subtype	Superficial	26	(36.1%)
	Nodular	28	(38.9%)
	Mixed nodular/superficial	13	(18.1%)
	Infiltrating/morpheaform	2	(2.8%)
	Mixed nodular/morpheaform	1	(1.4%)
	Mixed superficial/micronodular Mixed	1	(1.4%)
	nodular/micronodular	1	(1.4%)

The mean penetration depth significantly increased after topical glycerol application (883 ± 108 vs 904 ± 88 µm, p=0.005). The 21 µm difference represented an increase by 0.34 standard deviations of the difference corresponding with an effect size of 0.34.

The numbers and proportions of BCCs with improved and reduced scores on the 4-point Likert scale, with respect to overall image quality and visibility of characteristic BCC features after glycerol application, are shown in Table 2. Regarding overall image quality after glycerol application, no significant improvement was found for observer 1 and 3. For observer 2, the proportions with improved scores were substantially higher than the proportions with reduced scores, but only statistically significant for BCC with crusts and/or ulcerations (p=0.04). Regarding the visibility of BCC features, there was a trend toward improved scores for observers 1 and 2, but the results were not statistically significant. For observer 1, the visibility of BCC features for superficial BCCs significantly decreased (p=0.01).

Table 2. Proportions of improved score, equal score, and reduced score for overall image quality and visibility of basal cell carcinoma (BCC) features after topical glycerol application for all three observers (1: EvL, 2: EO, and 3: GD). Results are presented as % (n) with corresponding p-values (McNemar's test).

Outcome	Observer	Improved score[a] *% (n)*	Equal Score *% (n)*	Reduced score *% (n)*	*p*-value
All (n=72)					
Overall image quality after glycerol application	1	26.4% (19)	41.7% (30)	31.9% (23)	0.79
	2	38.9% (28)	45.8% (33)	15.3% (11)	0.08
	3	22.2% (16)	47.2% (34)	30.6% (22)	0.20
Visibility of BCC features after glycerol application	1	25.0% (18)	56.9% (41)	18.1% (13)	0.70
	2	38.9% (28)	31.9% (23)	29.2% (21)	0.49
	3	18.1% (13)	56.9% (41)	25.0% (18)	0.06
Keratosis (n=42)					
Overall image quality after glycerol application	1	28.6% (12)	45.2% (19)	26.2% (11)	0.84
	2	42.9% (18)	45.2% (19)	11.9% (5)	0.07
	3	19.0% (8)	57.1% (24)	23.8% (10)	0.64
Visibility of BCC features after glycerol application	1	28.6% (12)	52.4% (22)	19.0% (8)	0.22
	2	42.9% (18)	31.0% (13)	26.2% (11)	0.51
	3	19.0% (8)	54.8% (23)	26.2% (11)	0.37
Crust / ulceration (n=19)					
Overall image quality after glycerol application	1	26.3% (5)	47.4% (9)	26.3% (5)	0.48
	2	52.6% (10)	31.6% (6)	15.8% (3)	0.04
	3	26.3% (5)	52.6% (10)	21.1% (4)	0.56
Visibility of BCC features after glycerol application	1	26.3% (5)	57.9% (11)	15.8% (3)	0.77
	2	47.4% (9)	36.8% (7)	15.8% (3)	0.16
	3	21.1% (4)	57.9% (11)	21.1% (4)	0.76
Superficial BCC (n=26)					
Overall image quality after glycerol application	1	38.5% (10)	19.2% (5)	42.3% (11)	0.15
	2	38.5% (10)	46.2% (12)	15.4% (4)	0.46
	3	15.4% (4)	65.4% (17)	19.2% (5)	0.74
Visibility of BCC features after glycerol application	1	27.0% (7)	15.4% (4)	57.7% (15)	0.01
	2	46.2% (12)	15.4% (4)	38.5% (10)	0.88
	3	19.2% (5)	61.5% (16)	19.2% (5)	0.78

[a] An improved score is defined as an increase on the 4-point Likert scale.

DISCUSSION

The main objective of this study was to evaluate whether topical application of glycerol increases the optical penetration depth, which may aid the detection of deeper located BCC tumour nests. This study demonstrates that glycerol application increases penetration depth from 883µm to 904µm, corresponding to an effect size of 0.34. This limited increase, however, may not be sufficient to detect aggressive BCC tumour nests, which can reach an estimated average depth of 1500µm.

The observed penetration depth was remarkably lower than expected, as a systematic review reports a mean penetration depth of 1.2-2 mm with the same OCT device used in the current study.[17] We found that beyond 1 mm depth the signal intensity drastically decreases (Figure 1), even after glycerol application. Reported penetration depths of other devices vary from 1-1.6 mm (Thorlabs, Newton, New Jersey, USA), 1.3 mm (Risø National Laboratory, Roskilde, Denmark) and 2.0-2.5 mm (an OCT device developed at the Technical University of Denmark).[17]

Despite the increase in penetration depth, no improvement in image quality and visibility of BCC features was found. This may be explained by the fact that resolution, more than penetration depth, determines image quality and how well BCC features can be distinguished from surrounding tissue.

Although OCAs may be useful for OCT imaging, Welzel et al. concluded that topical treatment of the skin prior to OCT imaging is not imperative but gives a non-specific increase in optical penetration depth due to the lower surface reflectivity.[14] They found that a decrease of the light attenuation coefficient implies an increase in optical penetration depth, though this increase was not exactly quantified. Different solutions, including glycerol, ultrasonic gel, urea, petrolatum and paraffin oil were tested on healthy skin of the fingertips in 15 patients. OCT images were obtained directly after application and compared with the untreated fingertips of the other hand.[14] All investigated solutions resulted in a comparable decrease in surface reflectivity and increase in optical penetration depth. Wang et al. used a combined liquid paraffin and glycerol mixture to reduce light scattering in tissue and achieve more optical penetration depth.[10] Eight OCT images of human fingers were obtained at 0 to 40 minutes after application with a 5 minute interval between each image. The time to reach the optimal optical clearing effect, defined as an OCT image with enhanced contrast, was around 10 - 30 min after application of a mixture with 70% glycerol concentration. The authors concluded that applying the liquid paraffin and glycerol mixture led to an OCT scan with enhanced contrast and assumed that this indicated an increase in optical penetration depth, although this increase was not exactly quantified.

Even though abovementioned studies report an increase in optical penetration depth and enhanced contrast after glycerol application, it was not reported whether these findings led to improved image quality and visibility of BCC features in OCT images. Wang et al. observed enhanced contrast after glycerol application, but in our study an improvement in of image quality and visibility of BCC features was not observed.[10]

Conclusion

Topical application of glycerol increases the optical penetration depth in OCT imaging of skin lesions suspected for BCC. However, this limited increase may not be clinically relevant. No significant differences in image quality and visibility of BCC features after topical glycerol application were found.

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CHAPTER 4.3

Detection and subtyping of basal cell carcinoma with optical coherence tomography: the additional value of distant diagnosis by an expert

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Submitted for publication.

ABSTRACT

Background: Diagnostic accuracy of optical coherence tomography (OCT) for the detection of basal cell carcinoma (BCC) varies among assessors due to differences in experience.

Objective: This study compared the diagnostic accuracy of a novice assessor who obtained OCT scans within the clinical setting with an OCT expert without visual and clinical information on the lesion.

Methods: A cohort study was conducted among patients undergoing a biopsy for lesions suspect for non-melanoma skin cancer. Both OCT assessors recorded the level of suspicion for BCC presence on a 5-point Likert-scale and suspected subtype. The histological results based on punch biopsy were used as gold standard.

Results: The specificity, defined as the ability to detect non-BCC lesions was 96% for both assessors. Sensitivity, defined as ability to detect BCC lesions, was significantly higher (82.2%) for the OCT expert than for the novice assessor (71.8%) (P=0.005). Sensitivity of the expert to detect nodular and aggressive BCCs was also significantly higher (89.2% vs 97.6%, P=0.016).

Conclusion: Supervision by a distant expert without direct visual inspection of the lesion may improve diagnostic performance and could be valuable for clinical implementation of OCT.

INTRODUCTION

Current guidelines recommend to obtain biopsies of lesions clinically suspected for basal cell carcinoma (BCC) in order to verify BCC presence and subtype.[1] Histological subtyping is required to decide on treatment options. Non-invasive treatment is suitable for superficial BCC (sBCC), whereas nodular and aggressive BCCs (nBCC/aBCC) require excision.[2] Optical coherence tomography (OCT), a non-invasive tool based on light interferometry, generates real-time in-vivo cross-sectional images of the skin with a depth of approximately 1.00-1.5mm.[3] The interference of optical beams that are reflected by skin tissue, result in an image with shades within the black and white spectrum. This allows OCT assessors to identify morphological BCC characteristics.[4]

It has been suggested that OCT diagnosis, if established with high confidence, could replace biopsies, thereby enabling a so-called one-stop-shop approach.[5, 6] Following this approach, diagnosis and discussion of treatment options can be combined in one and the same consultation, but only if the OCT diagnosis can be made with high confidence. In the remaining patients, a biopsy is still needed.

In a study by Sinx, van Loo, Tonk *et al*, a high confidence OCT diagnosis could be made in 30% of patients and was associated with a sensitivity of 58.6% and specificity of 94.8% for discrimination between BCCs and non-BCCs.[2] With additional use of OCT, a higher proportion of patients with sBCC could be detected than with clinical and dermoscopic evaluation alone. Yet, the proportion of nBCC/aBCCs detected by OCT was similar to that of clinical and dermoscopic examination.[2] This study assessed OCT scans retrospectively in conjunction with clinical photographs, but without direct visual inspection of the lesion. It was hypothesized that combining OCT with visual inspection could improve sensitivity since recognition of nodular BCCs is aided by characteristic features such as elevation, a pearly translucent margin, and telangiectasia. These are clearer during clinical examination of a patient.

The level of experience level of OCT assessors is also an important determinant of diagnostic performance. Olsen, Themstrup, De Carvalho *et al*. reported that the diagnostic accuracy varies greatly among assessors, partially due to differences in experience.[7] Re-assessment of OCT scans by a distant OCT expert may improve the diagnostic process, yet experts are not readily available in all dermatology departments. As these experts will have to interpret OCT scans without visual information on the suspected lesion, the question rises to what extent they can optimize the diagnostic process.

Firstly, this study aimed to assess the diagnostic accuracy of high confidence OCT diagnosis by a novice assessor, who obtained and interpreted OCT scans in combination with direct visual inspection of the lesion. Secondly, this study compared the diagnostic performance of the novice assessor and an OCT expert who had no visual or clinical information on the suspected lesion.

MATERIALS AND METHODS

A cohort study was conducted at the Dermatology outpatient clinic of the Maastricht University Medical Centre+, Maastricht, The Netherlands (MUMC+). Patients (18 years or older) with an indication for punch biopsy of a lesion clinically suspected for non-melanoma skin cancer or a premalignant skin lesion were included. Ineligible were patients who were unable to sign informed consent.

The treating physician recorded the confidence level in BCC diagnosis using a 5-point Likert-scale, based on visual and dermoscopic evaluation (Heine Delta 20T) of the suspected lesion (Table 1). The suspected BCC subtype was documented if there was any suspicion of BCC. Diagnosis of BCC and subtype were based on characteristic features (such as shiny borders, telangiectasia, ulceration) and dermoscopic findings (such as ovoid nests or telangiectasia). The clinically most aggressive part was marked for biopsy. A photograph was taken by a medical photographer (Nikon D750) for documentation.

Subsequently, the novice assessor scanned the marked biopsy area with OCT (Vivosight Multi-beam Swept-Source Frequency Domain OCT, Michelson Diagnostics, Maidstone, Kent, UK; specifications: class 1 eye safe, resolution <7.5 mm lateral, <5 mm axial, depth of focus 1.0 mm, scan area 6 x 6 mm^2). The novice assessor was aware of the clinical information provided by the treating physician and subsequently evaluated the OCT scan based on BCC characteristics as described by Hussain, Themstrup and Jemec.[8] The novice assessor (TW) was a medical student trained as OCT assessor at the dermatology department of MUMC+. Training included studying literature, an online atlas on OCT and assessing over 200 scans supervised by the expert assessor. Cumulative sum (CUSUM) analysis was used to evaluate the diagnostic performance in diagnosing BCC.[9] The expert assessor (FA) evaluated all OCT scans without visual or clinical information on the lesion. The expert had received a comparable training, but also attended an OCT convention.[10] Additionally, she had already assessed over 1500 scans.

Both assessors documented their confidence level in OCT diagnosis on a 5-point Likert-scale (Table 1). A confidence score of 4 reflects certainty about BCC

diagnosis and subtype (positive test result), whereas confidence scores 0-3 were considered uncertain (negative test result). OCT images were coded and saved anonymously.

Table 1. Likert-scores and their respective level of certainty.

Likert-score 0	Certain it is no BCC
Likert-score 1	Low suspicion for BCC
Likert-score 2	High suspicion for BCC
Likert-score 3	Certain of BCC but uncertain of subtype
Likert-score 4	Certain of BCC and certain of subtype

Following the OCT scan, a 3mm punch biopsy was taken and the histological result from biopsies served as gold standard. Both investigators were blinded to the results of histological examination, which was performed by an independent and experienced dermato-pathologist, who was blinded to the OCT diagnosis. BCC subtypes were classified as superficial or nodular/aggressive (micronodular or infiltrating). In case of mixed subtypes, the most aggressive subtype was used for analysis. This study was approved by the local independent Ethics Committee.

Statistical analysis

One lesion per patient was included to ensure independence of observations. Diagnostic performance was expressed by sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic odds ratio (DOR). For BCC subtyping, sensitivity was defined as the proportion of patients with a nBCC/aBCC (excision required) correctly identified by OCT as nBCC/aBCC. Specificity was defined as the proportion of patients with a sBCC (non-invasive therapy optional) correctly identified as sBCC by OCT. Differences in diagnostic parameters were tested for statistical significance using the McNemar test for paired proportions. SPSS (version 24) was used for statistical analyses. Two-sided *P* values of 5% were considered to indicate statistical significance.

RESULTS

The analysis was based on 287 lesions of which 163 were histologically confirmed BCC (56.8%). A total of 38 (23.3%) were sBCCs, whereas 125 (76.7%) were nBCC/aBCCs. Patient and lesion characteristics are displayed in Table 2. The diagnostic process and the consequences of correct and incorrect OCT diagnoses are illustrated in Fig. 1.

Table 2. Baseline characteristics of 287 patients

Characteristic	
Mean age (SD)	70.6 (11.6)
Sex, n (%)	
Male	149 (51.9)
Female	138 (48.1)
Localization, n (%)	
Head/neck	151 (52.6)
Trunk	73 (25.4)
Extremities	63 (22.0)
Histologic diagnoses, n (%)	
BCC	163 (56.8)
No BCC	124 (43.2)
BCC subtypes, n (%)	
Superficial	38 (23.3)
Non-superficial	125 (76.7)
Nodular	91 (55.8)
Aggressive (morpheaform/micronodular)	34 (20.9)
Other diagnoses (non-BCC), n (%)	
Actinic keratosis	29 (23.4)
Bowen's disease	16 (12.9)
SCC	21 (16.9)
Other	58 (46.8)
Benign[1]	52 (91.4)
Melanoma in situ	2 (3.4)
In transit/satellite metastasis of melanoma	1 (1.7)
Fibroxanthoma	1 (1.7)
Sebaceous tumour	1 (1.7)

Abbreviations: BCC, basal cell carcinoma; SCC, squamous cell carcinoma.
[1]Including: Apocrine hydrocystoma, Benign lichenoid keratosis, clavus, dermal naevi, eczema, erosions, folliculitis, inflammation, interphase dermatitis, hypertrophic scar, lentigo simplex, lentigo solaris, lichen amyloidosis, melanocytic atypia, osteoma cutis, prurigo nodularis, rosacea, scar tissue, seborrheic keratosis, solar elastosis, stasis dermatitis, reactive ulceration, verruca.

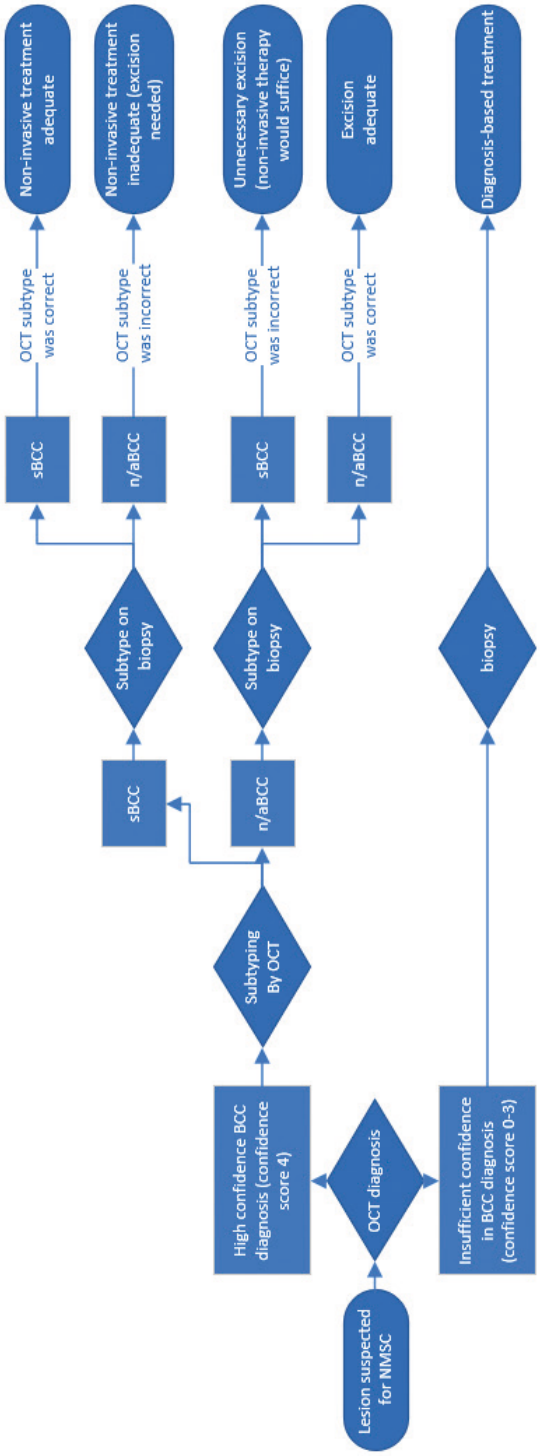

Figure 1. Diagnostic process

Abbreviations: BCC, basal cell carcinoma; n/aBCC, nodular or aggressive basal cell carcinoma; NMSC, non-melanoma skin cancer; OCT, optical coherence tomography; sBCC, superficial basal cell carcinoma.

Ability to distinguish between BCC and non-BCC lesions

The diagnostic parameters of a high confidence OCT diagnosis using a cut-off value of score 4 versus scores 0-3 are shown in Table 3 for both the novice and expert assessor. The results showed that both assessors were able to detect non-BCCs with a high specificity of 96%. The PPV was high for both the novice (95.9%) and expert assessor (96.4%) (*P*=0.838). This implies that if the assessor is certain of BCC presence and subtype (Likert-score 4), the chance that the lesion is indeed a histological BCC is high. Both OCT assessors misclassified five non-BCC lesions as BCC. According to biopsy, the novice assessor misclassified two cases of squamous cell carcinoma, two cases of actinic keratosis and one case of interphase dermatitis. The expert assessor misclassified the same two cases of squamous cell carcinoma as well as one case of actinic keratosis. Furthermore, the expert misclassified one apocrine hydrocystoma and one case of rosacea.

The DOR indicated better performance by the expert (DOR=110) than by the novice assessor (DOR=60.5). The main reason being the significantly higher sensitivity for BCC detection of the expert (82.2%) compared to the novice assessor (71.8%) (*P*=0.005). The expert detected 25 BCCs with high confidence that were not detected by the novice assessor (Table 5). Closer evaluation of the discrepant cases revealed that 40% of the discrepancies arose from uncertainty on BCC subtype rather than BCC presence. The NPV was 72.1% for the novice and 80.4% for the expert assessor (*P*=0.087). In other words, the risk that lesions classified as non-BCC by OCT, turn out to be BCC after histological verification is 27.9% for the novice and 19.6% for the expert assessor. However, when using this diagnostic strategy, cases classified as non-BCC are always referred for biopsy and will still be detected on histology. The difference in sensitivity at similar specificity mainly affects the proportion of patients in whom biopsies could be omitted. This proportion was 42.5% for the novice assessor and 48.4% for the expert assessor (*P*=0.16).

Table 3. Diagnostic performance of BCC detection with OCT by a novice OCT assessor and an expert OCT assessor. The absolute number of BCCs is given.

	Novice assessor % (x/n)	CI	Expert assessor % (x/n)	CI	*P* value
Sensitivity	71.8 (117/163)	(68.1 - 73.7)	82.2 (134/163)	(78.7 - 84.1)	0.005
Specificity	96.0 (119/124)	(91.2 - 98.5)	96.0 (119/124)	(91.3 - 98.5)	1.000
PPV	95.9 (117/122)	(91.0 - 98.4)	96.4 (134/139)	(92.3 - 98.5)	0.838
NPV	72.1 (119/165)	(68.5 - 74.0)	80.4 (119/148)	(76.5 - 82.5)	0.087
DOR	60.5	(22.0 – 180.2)	110	(38.8 – 336.9)	

Abbreviations: BCC, basal cell carcinoma; CI, confidence interval; DOR, diagnostic odds ratio; NPV, negative predictive value; PPV, positive predictive value.

Ability to distinguish between subtypes of basal cell carcinoma

The diagnostic parameters for BCC subtyping are listed in Table 4. There were 163 histological BCCs and the novice and expert assessor identified 117 and 134 of these BCCs respectively. A total of 109 histologically verified BCCs were diagnosed with high confidence by both assessors and were used to compare the diagnostic parameters for subtyping between both assessors.

Sensitivity to detect nBCC/aBCC was 89.2% for the novice and 97.6% for the expert assessor (*P*=0.016). The NPV represents the probability that histological nBCC/aBCC is indeed absent in case a sBCC has been diagnosed by OCT. The NPV of 90.9% was higher for the expert than for the novice assessor, who had a NPV of 70% (*P*=0.078). Hence, the risk that a lesion classified as sBCC on OCT turns out to be a more aggressive subtype after histological verification, was lower for the expert (9.1%) than for the novice assessor (30%): the expert had a higher ability to rule out the presence of nBCC/aBCCs.

Specificity to detect sBCC was 80.8% for the novice and 76.9% for the expert. The difference in specificity was not significant (*P*=1.000). The PPV represents the probability of presence of histological nBCC/aBCC in case nBCC/aBCC has been diagnosed by OCT. The PPV was 93.7% for the novice and 93.1% for the expert (*P*=0.883). Hence, the risk that lesions classified as nBCC/aBCC on OCT turn out to be a superficial subtype after histological verification was similar for the novice (6.3%) and the expert (6.9%).

The DOR indicated a better performance in BCC subtyping by the expert assessor (DOR=135) compared to the novice assessor (DOR=34.5). The main reason being the significantly higher sensitivity to detect nBCC/aBCCs of the expert (97.6%) compared to the novice assessor (89.2%) (*P*=0.016). The expert assessor detected 7 nBCC/aBCC that were not detected by the novice assessor (Table 4). Hence, supervision by a distant OCT expert may reduce the risk of misclassification of nBCC/aBCC as sBCC which subsequently leads to a reduced risk of inadequately prescribing non-invasive treatment for nBCC/aBCC.

Table 4. Diagnostic performance of BCC subtyping by a novice OCT assessor and an expert OCT assessor. The absolute number of BCCs is given.

	Novice assessor % (x/n)	CI	Expert assessor % (x/n)	CI	*P* value
Sensitivity (nBCC/aBCC)*	89.2 (74/83)	(83.9 – 92.4)	97.6 (81/83)	(93.0 – 99.6)	0.016
Specificity (sBCC)	80.8 (21/26)	(63.9 – 91.8)	76.9 (20/26)	(62.2 – 83.2)	1.000
PPV	93.7 (74/79)	(88.1 – 97.3)	93.1 (81/87)	(88.7 – 95.0)	0.883
NPV	70.0 (21/30)	(55.4 – 79.6)	90.9 (20/22)	(73.5 – 98.3)	0.078
DOR	34.5	(9.2 – 140.1)	135	(21.8 – 1110.2)	

Abbreviations: CI, confidence interval; DOR, diagnostic odds ratio; nBCC/aBCC, nodular/aggressive basal cell carcinoma; NPV, negative predictive value; PPV, positive predictive value; sBCC, superficial basal cell carcinoma. *for analysis, nodular and aggressive BCC subtypes (requiring surgery) were considered one group.

Table 5. Agreement and discrepancies for diagnosing and subtyping BCC.

	Novice + Expert +	Novice - Expert -	Novice - Expert +	Novice + Expert -	*P* value (Mc Nemar)
Discrimination between BCC vs non-BCC lesions (n=287)					
Histologically BCC (n=163)	109	21	25	8	0.005
Histologically no BCC (n=124)	117	3	2	2	1.000
Discrimination between non-sBCC vs sBCC (n=109 histologically verified BCCs)					
Histologically nBCC/aBCC* (n=83)	74	2	7	0	0.016
Histologically sBCC (n=26)	18	3	2	3	1.000

+ indicating correct detection, - indicating misclassification, *p*-value McNemar test. *for analysis, nodular and aggressive BCC subtypes (requiring surgery) were considered one group.

DISCUSSION

This study indicates that a novice assessor who obtains scans in a clinical setting and has both visual and clinical information on the lesion has a high diagnostic accuracy. The high PPV and specificity indicated a low risk of misclassifying non-BCC as BCC. The expert assessor had a similar PPV and specificity, but sensitivity to detect BCC and nBCC/aBCC subtypes was significantly higher compared to the novice assessor. However, if a high confidence diagnosis cannot be established, a biopsy will be obtained. Thus, the lower sensitivity to detect BCC of the novice assessor has no harmful clinical consequences. Supervision by an expert assessor could result in higher sensitivity to detect BCC leading to a higher proportion of BCCs being eligible for a one-stop-shop approach. Moreover, due to higher

sensitivity to detect nBCC/aBCC misclassifications of more aggressive BCC as sBCC can be prevented. Based on the results of this study, supervision by an OCT expert seems especially indicated in cases where the novice assessor has a high suspicion of BCC being present but is not certain of the BCC subtype.

The results of this study have relevance for successful implementation of OCT in clinical practice. Correct interpretation of OCT scans requires substantial training and there should be no diagnostic misclassifications resulting from a lack of experience. A problem is that experts are not readily available. We therefore propose a supervision system in which a novice assessor consults an expert who is not on-site. Presence in the clinic is no necessity since our study showed a high diagnostic accuracy of an expert without any clinical information. The novice assessor can send the OCT scan, preliminary diagnosis and clinical information to the expert assessor, after which the expert re-evaluates the scan. This method is fast and efficient, and one expert could provide supervision for multiple centers. For future implementation of such a supervision system, it may be more cost-effective and convenient to outsource the task of obtaining and interpreting scans to non-dermatologists. A study by Fuchs, Ortner, Mogensen *et al* reported consensus among experts that dermatologists should acquire and interpret OCT scans.[11] However, this consensus is challenged by the results of this study, in which neither of the assessors were dermatologist.

High confidence OCT diagnosis was associated with a small risk of misclassification. Both assessors misclassified 5 non-BCCs as BCC. The same two squamous cell carcinomas were misclassified as nBCC/aBCC by both assessors. These misclassifications had no clinical consequences since both diagnoses require excision. Both assessors classified the same case of actinic keratosis as sBCC, but non-invasive treatment for sBCC can be suitable for actinic keratosis. In addition, the novice assessor classified another case of actinic keratosis as sBCC. The novice assessor misclassified one case of interphase dermatitis as sBCC, which would have led to inadequate treatment. The expert assessor misclassified one apocrine hydrocystoma and one case of rosacea as nBCC/aBCC. Although excision may be considered as an adequate treatment option for apocrine hydrocystomas, it is inadequate for rosacea.

A limitation of this study is that the results are based on the comparison of diagnoses made by a single novice and expert assessor. The added value of supervision will largely depend on the diagnostic skill of both novice and expert assessors. In clinical practice, substantial inter-observer variation in diagnostic performance among novice assessors can be expected.

In conclusion, this study showed that a novice assessor has a high diagnostic accuracy for detecting BCC. The chance of misclassifying non-BCC as BCC was low and clinical consequences were limited. The expert assessor had a higher sensitivity to detect BCC and nBCC/aBCCs than a novice assessor. Improved detection of nBCC/aBCCs may lead to a reduced risk of inadequate treatment. Hence, supervision of the novice assessor by an expert assessor may be valuable for future clinical implementation of OCT.

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PLAIN LANGUAGE SUMMARY

Basal cell carcinoma (BCC) is the most common form of cancer among fair-skinned people: of those, one in six will develop this form of skin cancer. Although BCC rarely leads to death, it grows over time and therefore treatment is indicated. Several treatments are available, among which non-invasive therapies. Treatment is based on BCC subtype, usually determined by biopsies. Obtaining a biopsy can be painful and examination of the tissue takes time. Optical coherence tomography (OCT) is a non-invasive method that enables BCC detection. A part of the biopsies may be replaced by OCT, but high certainty of both BCC presence and subtype is essential. Since superficial BCCs can be treated with creams, and do not require surgery as other subtypes, correct subtyping with high certainty can lead to a non-invasive care strategy in which diagnosis and treatment prescription are done during one consultation. Detection and subtyping BCC on OCT is challenging and requires training. Especially when starting, supervision is essential. Yet, OCT experts are not available in every clinic. Therefore, we conducted a study in The Netherlands to find out if an OCT expert at a distance is better at detecting and subtyping BCC compared to a newly trained OCT assessor who uses the OCT scanner in the clinic. We found that even without direct clinical evaluation, the OCT detected more BCCs, and more specifically the subtypes requiring surgery. We therefore concluded that distant supervision by experts may be valuable when OCT is used in the future.

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CHAPTER 5

General discussion and summary

In this chapter, the most important conclusions of this thesis will be summarized and discussed. Moreover, an interpretation of the results will be provided as well as implications for clinical practice and future research.

Currently, guidelines recommend referring patients with a skin lesion that is suspicious of BCC for biopsy to establish the diagnosis.[1, 2] In the past years, imaging technologies have become available for non-invasive diagnosis of BCC. These non-invasive diagnostic tools make it possible to confirm and subtype BCC with high confidence, thereby obviating the need for a punch biopsy in a substantial part of patients.[3-5] In this way, BCC diagnosis and treatment can be accomplished in one day, enabling a so-called one-stop-shop approach. Implementation of non-invasive diagnostic tools into clinical practice is thus expected to lead to more efficient and patient friendly care. In addition, it is expected to save costs and reduce the workload for dermatologists caused by the high number of punch biopsies and post-biopsy (telephone) consultations.

In recent years, a Cochrane systematic review was published on the use of OCT for diagnosing skin cancer.[6] Initial data suggested that conventional OCT may have a role for the diagnosis of BCC in clinically challenging lesions. The meta-analysis showed a higher sensitivity and specificity of OCT when compared to clinical examination and dermoscopy. However, the small number of studies and varying methodological quality meant that implications to guide clinical practice could not be drawn.
This Cochrane systematic review did not include a recent observational cohort study, in which there was no need for punch biopsy anymore in 30% of patients where OCT diagnosis could be made with high confidence.[5] Owing to the design of this study, the OCT assessors only had photographs in which clinical characteristics of BCC, such as elevation and shiny appearance are obviously less clear. Recognition of (nodular) BCC might improve when OCT is used directly during clinical examination.

Hence, as well-conducted prospective randomised controlled trials were lacking, the necessary high-level evidence required for implementing OCT in clinical practice was not yet available.

(Cost-)effectiveness of optical coherence tomography for non-invasive diagnosis of basal cell carcinoma
Aim and study design - We conducted the **ROCTI-trial**, a multicentre prospective randomised controlled non-inferiority trial to evaluate whether use of OCT-guided diagnosis and treatment is non-inferior to regular care, where diagnosis and treatment is always based on a biopsy. An absolute decrease in the one-year probability of remaining free from a recurrent or residual (pre-)malignant skin lesion

≤ 10% was considered acceptable (non-inferiority margin). Another aim of the trial was to evaluate whether OCT guided diagnosis and treatment is a cost-effective alternative to regular care.

In the ROCTI-trial, 598 patients were randomised to either OCT-guided diagnosis and treatment or regular care. The OCT-guided diagnosis and treatment is a diagnostic strategy, where diagnosis and treatment is based on OCT diagnosis only when an OCT diagnosis of BCC can be made with high confidence. All patients, for whom a high confidence OCT diagnosis of BCC cannot be made, have to be referred for a punch biopsy to establish a diagnosis. As such, patients with an OCT diagnosis of non-BCC will also be referred for biopsy.

Eligible for inclusion were patients with lesions that were possibly BCC based on clinical and dermoscopic examination, i.e. BCC was included in the differential diagnosis and there was thus an indication for punch biopsy. This ranged from lesions in which BCC diagnosis was considered, but where another benign or (pre-)malignant diagnosis was also suspected to lesions which were very suspect for BCC, but where doubt remained concerning the BCC subtype. Patients in whom the diagnosis of BCC was so evident, that the lesion could be treated directly without prior biopsy were excluded. In the Netherlands, 63-90% of BCCs are diagnosed by biopsy.[7, 8] There can, however, be significant variation between different hospitals for setting the indication for punch biopsy. We aimed to make our results generalizable by including three different hospitals in this study: two general and one academic hospital.

Results - The results indicate that OCT-guided diagnosis and treatment is non-inferior to regular care and does not compromise patient safety (**Chapter 2.1**). In the OCT group 94.4% (253/268) patients were free from a recurrent or residual (pre-)malignant lesion, compared to 93.3% (266/285) in the regular care group. According to the modified intention-to-treat analysis, the absolute difference (OCT versus regular care) was +1.07 (95% CI: -2.93 to 5.06) where the lower limit of the 95% CI does not exceed the predefined non-inferiority margin of 10%. In the OCT group, a punch biopsy could be omitted in 196/299 (65.6%) of patients. The sensitivity and specificity of high confidence OCT diagnosis was 85.3% and 94.6%, respectively.

Not only was an OCT-guided diagnosis and treatment non-inferior in terms of clinical effectiveness, a cost-effectiveness analysis after 12 months follow-up indicated that OCT-guided diagnosis and treatment is also a dominant cost-effective strategy compared to regular care punch biopsy (**Chapter 2.1**). The total mean costs for OCT guided diagnosis and treatment were €689 compared to €758 for regular care punch biopsy based diagnosis, with lower one-year probability of treatment

failure in the OCT group. These differences in costs are mainly attributable to cost savings in patients where a high confidence OCT diagnosis can be made, because costs associated with performing a punch biopsy, histopathological examination and a post-biopsy (telephone) consultation to discuss the results can be saved. These cost savings were larger than the extra costs of OCT scans in this study arm that were abundant in patients where no high confidence OCT diagnosis could be made. Within the cost analysis, we used cost prices specific for the Dutch healthcare system and these might differ depending on specific prices in different countries. Therefore, data on resource use was added, allowing others to determine applicability to their own situation.

Discussion - In the ROCTI-trial we found that the percentage of punch biopsies that could have been avoided (65.6%) was much higher than the reduction of 30% achieved in a recent observational study that was conducted before the start of the randomised controlled trial (RCT).[5] In the RCT, all OCT scans were evaluated directly in a clinical setting, which allows the investigator to directly make a new scan of the area if the first scan is of insufficient quality, or slightly shift the scanning area if a BCC tumour nest could not be visualized in a lesion with a high clinical suspicion.

Although the necessity for a punch biopsy is greatly reduced, both studies showed that correct subtyping of BCCs is still difficult with OCT. Among the 192 BCCs that were identified by OCT in the RCT, OCT correctly identified 47/72 histologically superficial BCCs (specificity=65.3%) and 113 of 120 other more aggressive subtypes (sensitivity=94.2%). The difficulty in subtyping could be due to the limited resolution of OCT, which makes it harder to differentiate in cases where subtypes show overlap. It is often difficult to determine whether a BCC is only superficial or already starting to grow nodular. This is, however, difficult for pathologists as well and remains a matter of clinical interpretation.

In the ROCTI-trial, a punch biopsy was chosen as the reference standard according to (inter-)national guidelines.[1, 2] It is known that biopsies, either punch or shave, do not always represent the entire lesion.[9, 10] In the RCT, 25 superficial BCCs diagnosed by punch biopsy, were over classified as non-superficial BCC by OCT diagnosis and treated with surgical excision. However, in 13 of these patients the non-superficial subtype as seen on OCT appeared to be missed by punch biopsy because it was present in the excision specimen. This illustrates that OCT can also have an advantage over a 3 mm punch biopsy, as the entire lesion is visualized instead of only 3mm.

The results generated by the ROCTI-trial provide the required high-level evidence for future implementation of OCT in clinical practice. However, it remains important

to realize that misclassification by OCT, which is not verified by punch biopsy, harbours the risk of over- or undertreatment. There is a small risk that non-BCC lesions are misdiagnosed as BCC, which leads to unnecessary treatment in case of a benign lesion or possibly inadequate treatment in case of another type of (pre-) malignant skin lesion. Another risk is that nodular or aggressive BCC subtypes are underdiagnosed as superficial BCC by OCT, which may result in the decision to treat such a lesion non-invasively, whereas surgery is indicated. This might not be a problem when a low-risk nodular BCC is treated with imiquimod cream, as the 5-year sustained clearance is still 81% with recurrences being detected early during follow-up.[11] However, for aggressive BCCs, treatment with imiquimod cream seems more harmful.[12] There is also a small risk that superficial BCCs are misclassified as a nodular or aggressive BCC, which results in a decision to treat the lesion surgically. Although surgery is a very effective treatment for superficial BCC, the choice for a non-invasive treatment would then be wrongfully withheld.[1] Misclassification of histopathogical BCC as a non-BCC lesion by OCT has no clinical consequences, because it is part of the OCT strategy to always refer patients with an OCT diagnosis of non-BCC for a punch biopsy. In the process of implementation, we consider it important to adhere to this strategy to ensure patient safety. In the current study, none of the misclassifications that occurred had severe clinical implications and none of the 15 recurrences in the OCT group were due to misclassification by OCT. However, the risk of over- or undertreatment must always be carefully weighed against the advantage of treatment without delay and less invasive procedures.

Patient preferences

Aim and study design - From a patient's perspective, an OCT-guided strategy might be an attractive option, because an invasive procedure can be omitted, and BCC treatment can be initiated immediately in case of a high confidence OCT diagnosis of BCC. However, prior to implementation of new technologies in clinical practice, it is important to obtain insight into patient preferences.[13, 14] A discrete choice experiment (DCE) is a survey method used to measure preferences, in which an intervention or treatment is described by different characteristics, or attributes, with different levels. Based on the attributes and their levels, hypothetical choice sets are generated. We performed a labelled DCE alongside the RCT, to examine patient preferences for OCT or punch biopsy as diagnostic strategy for BCC **(Chapter 2.2)**. Patients were asked to choose between OCT or punch biopsy based on six attributes with varying levels per choice set. Three attributes were associated with diagnostic accuracy (sensitivity, false positive rate, and physicians' confidence in diagnosis), two with side-effects (bleeding and infection, painfulness of procedure), and one with waiting time to diagnosis.

Results - In total, 344 patients completed the DCE between May 2019 and September 2020. Median age was 72 (21–92) years. Patients preferred a biopsy strategy in 55% of the choice sets, and an OCT strategy in 45% of the choice sets. When the highest levels for attributes relating to diagnostic accuracy were applied for OCT in a simulation analysis (sensitivity 94%, confidence in diagnosis 50% and false positive rate 6%), the proportions changed only slightly, and OCT as an initial diagnostic strategy was preferred by patients in 58% of choice sets.

Discussion - In the aforementioned RCT we found a sensitivity of 85.3%, a physicians' confidence in the diagnosis in 65.5% and a false positive rate of 5.4% and therefore these "highest" levels presented in the DCE appeared to be realistic. The results of the DCE therefore indicate the potential uptake of OCT in clinical practice, but do not convincingly demonstrate that an OCT strategy is currently preferred by patients in a vast majority of the choice sets. For this, we consider three possible explanations. First, the levels of the attributes associated with diagnostic accuracy were always very high for biopsy, whereas for OCT there was greater variation in the levels of these attributes. Side effects of a biopsy (bleeding and infection) only occur in a small percentage of the patients and pain is short-lasting. It is possible that part of the patients are willing to undergo a biopsy, as long as an accurate and confident diagnosis can be made.

Second, it is known from literature that respondents usually ascribe more value to the things they have experienced, knows as status quo bias.[5] Since the DCE was performed after treatment completion and patients therefore had experience with OCT and biopsy (when randomised to the OCT group) or only biopsy (when randomised to regular care), experience with the diagnostic strategy impacted their preference. At last, completion difficulties of the DCE often occurred in this elderly population and have been reported by 24% of the patients in this study, as in the study by Tinelli et al.[15] (22.4%). Although explained in the DCE, concepts such as "sensitivity" and "false-positive rate", might still be difficult to understand, since it requires patients to oversee the consequences of a wrongly diagnosed skin lesion.

In the DCE, the highest level for the attribute "physicians' confidence in diagnosis" was 50%, meaning that 50% of biopsies could have been omitted. When this percentage increases to the achieved percentage in the RCT of 65.6%, or maybe even higher in the future, it is likely that more patients prefer OCT as initial diagnostic strategy. However, part of the patients will continue to prefer a biopsy.

Exploring other applications of OCT

We hypothesised that OCT might also be useful for other purposes than diagnosis of skin lesions clinically suspected for BCC. This led us to explore the potential

of use of OCT for determining BCC resection margins prior to Mohs micrographic surgery (MMS).

MMS is a surgical procedure which is typically applied for lesions which are located in the H-zone of the face, and lesions located in this area were excluded in the ROCTI-trial. Since BCCs at this location have a higher risk of aggressive behaviour, it was decided not yet to include these high-risk patients in a trial that had the aim to evaluate whether OCT-guided diagnosis and treatment does not compromise patient safety when compared with regular care biopsy.[1] However, we were interested in the potential of use of OCT to diagnose a BCC in the H-zone (specifically in the periocular region) prior to MMS and for selection of a representative area for biopsy in this area, and therefore we applied OCT in a single patient. Furthermore, we evaluated whether OCT has additional value in patients with clinically evident BCCs that are immediately surgically removed without prior biopsy, who were excluded from the ROCTI-trial. In this subgroup of patients, OCT might be of value in order to reduce the risk of misclassification of non-BCC lesions as BCC.

Possibilities for application of optical coherence tomography in Mohs surgery and specific populations

Aim and study design - As MMS is a labour-intensive and time-consuming procedure, correct estimation of resection margins with OCT prior to MMS could reduce the average number of stages required for complete excision. A systematic review on the use of OCT in the diagnosis and management of BCC provides recommendations on the use of OCT to estimate resection margins prior to MMS based on two case reports and five case series.[10] In-vivo use of OCT to reduce the number of stages of MMS has shown promising results, but ex-vivo use of OCT in MMS is not recommended based on current studies. These few small-sized in-vivo studies provide no estimates of sensitivity and specificity, and therefore we conducted a case-control study (**Chapter 3.1**).

Results - We showed that in 58/92 quadrants with positive resection margins, tumour was visible on the OCT image, corresponding with a sensitivity of 63.0% (95% CI: 55.1-70.6). In 54/102 quadrants with negative resection margins, no tumour tissue was visible on the OCT image corresponding with a specificity of 52.9% (95% CI: 45.8-59.7).

Discussion - Our study showed poor diagnostic performance of OCT compared to the few available other studies, which showed more favorable results with respect to the ability of OCT to correctly predict resection margins prior to MMS. For instance, De Carvalho et al. showed that 8 out of 10 BCCs were totally excised in a single MMS stage when margins were previously assessed with use of OCT.[6]

In this study, the presence of BCC characteristics was directly evaluated on the OCT scans. If any BCC characteristics were visible, margins were enlarged and a new scan was obtained until it showed no suspicious BCC areas anymore, and the lesion was excised. Thus, resection margins were enlarged without histopathological verification, therefore not allowing determination of sensitivity and specificity estimates of OCT.

The low sensitivity and specificity for the distinction between presence and absence of BCC in resection margins stands in stark contrast with the much better results that were observed in the ROCTI-trial and other studies on diagnostic performance of OCT used to distinguish BCC from non-BCC lesions. In these studies, the center of well visible lesions was scanned, whereas when OCT is used to correctly predict resection margins, the periphery of a tumour is scanned and only minimal presence of tumour tissue must be discovered, which may be an explanation for the low sensitivity. Furthermore, OCT might miss small, microscopic tumour islands of an infiltrative BCC subtype because of the low resolution. The low specificity and consequently high number of false positive OCT results may be due to misinterpretation of sebaceous glands for nodular tumour nests and vessels for infiltrative tumour nests. Distinguishing infiltrative BCC tumour nests from vessels may be improved by using the dynamic OCT mode, a feature that enables the visualization of the microvasculature of the skin.[9]

The penetration depth of the OCT device (up to 1.5mm) might also limit an accurate assessment. This limited penetration depth might not be a problem when OCT is used for diagnosis of BCC, as BCCs contain more superficially located nests in the centre of the tumour.[5] However, for margin estimation, only peripheral borders of tumours are scanned. If only deeper located tumour nests are present there, these are invisible on OCT images.

Images in this study were analysed retrospectively and due to this study design, there was no possibility to obtain a new image when the quality of the previous image was not sufficient. In some cases, a new scan could have resulted in a better image quality and therefore allow for an improved assessment. Also, slight adjustment to the angle in which the OCT scan is obtained might result in a better view of the scanned tissue. Conducting a prospective study, where the OCT assessor directly interprets the scans, might be of benefit because the tissue can then be scanned in the free-run mode which allows for exact localization of the scanned tissue, compared to the en-face mode, where a ‘multislice’ area of 6x6 mm^2 is scanned. Apart from the poor diagnostic performance of OCT in determining BCC resection margins prior to MMS, we also found that obtaining and assessing the OCT scans was time consuming and thus difficult to implement within a well-balanced workflow in MMS. Based on the results of our study, the

use of OCT for determining BCC resection margins prior to MMS cannot be recommended in clinical practice yet.

Chapter 3.2 presents a case report on a patient who presented to the clinic with a scar-like lesion of 2 mm on her upper left eyelid (H-zone region) without any clinical or dermoscopic signs of BCC. In the same region, three BCCs had been treated with cryotherapy and MMS. With OCT, we were able to diagnose this scar-like lesion as BCC. A punch biopsy with frozen sections analysis confirmed the initial OCT diagnosis, after which MMS was performed. Diagnosis and treatment of periocular BCCs can be difficult. Especially in small tumours, a diagnostic punch biopsy removes the clinically visible BCC, making it challenging to find the exact location of the BCC prior to surgery. Furthermore, scar tissue formation around the biopsy site may even lead to a larger defect of the surgical procedure. By using OCT in patients with a periocular skin lesion suspicious for BCC, these problems can be avoided. In our case-report, we demonstrated that use of OCT in the periocular region can be helpful to select a representative area for a biopsy that was performed on the day of MMS, so that scar formation was prevented. Larger studies are needed to establish the diagnostic value of OCT in this sensitive skin region.

With the study described in **Chapter 3.3** we add information on the additional value of using OCT in a specific population of patients who were excluded from the RCT, namely patients in whom the diagnosis of BCC was clinically so evident, that the lesion could be directly excised without prior biopsy.

Aim - Especially in general hospitals, clinically suspected BCCs are often directly treated without prior biopsy. The aim of this study was to investigate whether in these subgroups of patients OCT has additional diagnostic value and can help to reduce the risk of misclassification of non-BCC lesions as BCC.

Results – Therefore, a study including 114 patients was performed in one academic and two general hospitals in the Netherlands. According to histopathologic diagnosis, 109/114 lesions were BCCs, which corresponds to a positive predictive value (PPV) of clinical and dermoscopic diagnosis of 95.6%. All 109 histopathological verified BCCs were identified as such by OCT (sensitivity =100%) and the negative predictive value (NPV) in case of negative OCT result was 100% (4/4). In only 5 out of 114 lesions (4.4%), histopathology revealed an alternative diagnosis. With OCT, it was possible to identify 4 out of 5 lesions as non-BCC lesions. The results show that, with additional use of OCT, the PPV increased from 95.6% (without OCT) to 99.2% (109/110) with OCT. Hence, use of OCT in addition to clinical and dermoscopic examination reduces the risk of misclassification of non-BCC lesions as BCC. However, this risk is already low in case of high clinical and dermoscopic suspicion of BCC.

Discussion - In this study, clinical and dermoscopic BCC diagnoses were made by dermatologists with extensive experience in skin cancer diagnosis. The risk of misclassification of non-BCC lesions as BCC might increase when BCC diagnosis is made by dermatologists and physicians with less experience in clinical and dermoscopic examination of BCC. Also, misclassification of non-BCC lesions as BCC may occur in clinically evident BCC for which non-invasive treatment is initiated without prior biopsy. It is, however, not possible to determine the risk of misclassification as non-BCC lesion in this subgroup, because histopathological verification is not available. Based on our results, we recommend that the limited gain from additional use of OCT in patients with high clinical suspicion of BCC, should be considered against the required investments for the purchase of an OCT device and the training of OCT users.

Exploring ways to optimize diagnostic accuracy of OCT for diagnosis of BCC
Another question that was addressed in this thesis was whether there are ways to optimize the diagnostic accuracy of OCT for diagnosis of BCC. With this aim, we conducted three different studies.

During the interpretation of OCT scans, the assessor tries to identify morphological features that are typical for BCC. We evaluated which features are most discriminative for BCC and proposed a diagnostic algorithm.

In addition, we searched for ways to improve OCT image quality and the visibility of these BCC features. Since penetration depth of the OCT device (up to 1.5mm) might limit an accurate assessment, we evaluated whether topical application of glycerol, a so-called optical clearing agent, improved OCT image quality.

At last, we investigated to what extent supervision of an OCT expert who is not on-site, can improve the diagnostic process in clinical practice. This distant expert, who is not on-site to directly inspect the patient and thus has no visual and clinical information, can be consulted by novice OCT assessors for re-assessment of OCT scans. Supervision from experts for novice OCT assessors might be valuable, since the ability to establish accurate diagnoses with OCT requires training and experience with this technique and may vary between future OCT assessors, especially novice OCT assessors. In the face of more widespread implementation of OCT-guided diagnosis and treatment, the diagnostic process might benefit from incorporation of support by an expert OCT assessor.

Optimization of the diagnostic accuracy of optical coherence tomography for diagnosis of basal cell carcinoma
OCT provides a resolution which is not high enough to distinguish cells, but it is suitable for pattern recognition in tissue similar to ultrasound. Consequently,

morphological features of BCC can be identified on an OCT image. These features have been established in recent years.[16-20] It is, however, not known which features are most discriminative for BCC.

Aim - In **Chapter 4.1** we aimed to evaluate the diagnostic value of these established OCT features and explored whether use of a small set of OCT features enables accurate discrimination between BCC and non-BCC lesions and between BCC subtypes. Accurate diagnosis of BCC with OCT would enable a one-stop-shop approach, as described previously.

Results - With the features: (*1*) dark rim, (*2*) bright peritumoural stroma, (*3*) protrusions into the upper dermis with a dark rim, and (*4*) signal-poor ovoid structures (all with PPV > 90%), a good discrimination between BCC and non-BCC lesions is possible. With regard to subtyping, 'protrusions into the upper dermis with a dark rim' are visible in the vast majority of superficial BCCs and absence of this feature is highly predictive of non-superficial BCC when other BCC features are present. However, if 'protrusions' are present, a conclusive diagnosis cannot be made. In this situation, 5 other features that are highly predictive of non-superficial BCC have to be used. In this study, which included data derived from the OCT group of the RCT, the prevalence of BCC was 75.3% (225/299). We proposed a diagnostic algorithm that enabled detection of 97.8% of BCC lesions (220/225). Subtyping without the need for biopsy was possible in 132 of 299 patients (44%), with a predictive value for presence of superficial BCC of 84.3% vs 98.8% for presence of non-superficial BCC.

Discussion - The proposed diagnostic algorithm is intended as support for OCT users who do not yet have much experience with interpretation of OCT images. Hence, novice OCT assessors commencing OCT training could focus initially on recognizing a small set of OCT features.

The study had two limitations. First, conventional OCT was used, whereas the use of dynamic OCT provides additional information by visualizing the vascular patterns and thus might allow for better differentiation between BCC subtypes.[21] Second, predictive values were high, but are highly dependent on the prevalence of BCC and BCC subtypes in a study population. The prevalence of BCC in the study population was 75.3% and may be lower in in other study populations, where lesions suspicious of BCC are selected by physicians with less experience with clinical and dermoscopic examination. When the prevalence of BCC is lower, this might result in different predictive values of the morphological features.

Since the resolution of OCT is rather low, and it can thus be difficult to identify BCC features on an OCT scan, we searched for ways to improve OCT image quality

and the visibility of these BCC features (**Chapter 4.2**). Also, penetration depth of the OCT device (up to 1.5mm) might limit an accurate assessment.

Aim - Consequently, we evaluated whether topical application of glycerol, a so-called optical clearing agent, improved OCT image quality. The main objective was to evaluate whether topical application of glycerol increases the optical penetration depth of OCT, which may aid the detection of deeper located BCC tumour nests.

Results - Sixty-one patients with a total of 72 BCCs were included. OCT scans were obtained before and after application of an 85% glycerol solution. The average penetration depth of each OCT scan was acquired by automatically tracing both skin surface and the point of signal loss using a custom-made MATLAB program. We demonstrated that glycerol application increases penetration depth from 883µm to 904µm (p=0.005), corresponding to an effect size of 0.34. This limited increase, however, may not be sufficient to detect aggressive BCC tumour nests, which can reach an estimated average depth of 1500µm.

Discussion - We found that beyond 1 mm depth the signal intensity drastically decreases, even after glycerol application. Despite the slight increase in penetration depth, no improvement in image quality and visibility of BCC features was found. This may be explained by the fact that resolution, more than penetration depth, determines image quality and how well BCC features can be distinguished from surrounding tissue. Therefore, application of glycerol prior to OCT imaging, is not recommended in clinical practice. Future improvements of the OCT device, leading to a higher resolution, is desired.

Since the level of experience of OCT assessors may influence the diagnostic accuracy of OCT, as shown in a study by Olsen et al., back up from OCT experts for novice assessors might be valuable.[22] Consequently, a clinical scenario is imaginable in which OCT experts at a distance are consulted for re-assessment of OCT scans.

Aim and study design- In **Chapter 4.3**, we describe a cohort study in which we evaluated whether supervision from experts for novice OCT assessors can optimize the diagnostic process in clinical practice. We compared the diagnostic accuracy of a novice assessor who obtained OCT scans within the clinic to the diagnostic accuracy of an OCT expert without visual information of the suspected lesion.

Results - We included 287 patients with a lesion clinically suspected for non-melanoma skin cancer or a (pre-)malignant skin lesion and an indication for punch biopsy. The results showed that a novice OCT assessor, who is provided with

visual and clinical information on the suspected skin lesion, is able to reach a high diagnostic accuracy. The risk of misclassification of non-BCCs as BCC was small resulting in a high specificity (96.0%) and a high probability that a lesion diagnosed as BCC by OCT was actually a histological BCC (PPV=95.9%). The high PPV implies that if the assessor is certain of BCC presence and subtype, the chance that the lesion is indeed a histopathological BCC is high. The expert assessor was not on-site and assessed the scan without visual information on the lesion, but reached a similar PPV and specificity. Moreover, sensitivity to detect BCCs was significantly higher (82.2% vs 71.8%, p=0.001) and sensitivity to identify nodular and aggressive BCCs was also higher (97.6% vs 89.2%, p=0.016).

Discussion - Hence, supervision by a distant expert assessor may lead to more BCCs being eligible for a proposed one-stop-shop approach. In addition, supervision by a distant expert may decrease the risk of nodular and aggressive BCC being falsely diagnosed as superficial BCC and subsequently being inadequately treated non-invasively. Although we established the potential value of expert supervision, experts are not readily available. We therefore propose a supervision system in which a novice assessor consults an expert who is not on-site. Presence in the clinic is no necessity since the expert achieved a high diagnostic accuracy without any clinical information. The novice assessor can send the OCT scan as well as the preliminary diagnosis and clinical information to the expert assessor, after which the expert re-evaluates the scan. This method is fast and efficient, which is a requisite for a one-stop-shop approach. This strategy enables a distant expert assessor to re-evaluate scans for multiple centers, potentially resolving the lack of available experts.

A limitation of this study is that the results are based on the comparison of diagnoses made by a single novice and expert assessor. The added value of supervision will largely depend on the diagnostic skill of both novice and expert assessors. In clinical practice, substantial inter-observer variation in diagnostic performance among novice readers can be expected. To validate the value of our proposed supervision system, we suggest that a study is conducted in which more expert and novice assessors participate, possibly in different countries.

Recommendations for implementation of OCT

We showed that OCT-guided diagnosis and treatment of BCC is non-inferior compared to regular care, does not compromise patient safety and substantially reduces the need for biopsy. OCT is a cost-effective strategy, and it allows for more efficient and patient friendly care because an invasive procedure can be omitted. Implementation of OCT in clinical practice enables a one-stop-shop approach, where BCC diagnosis and treatment can be accomplished all in one day. In the

paragraph below we describe the steps which we believe are necessary in the process of implementation of OCT in clinical practice.

1. Incorporation into guidelines and involvement of patients

First of all, for successful implementation of OCT in clinical practice, (inter) national dissemination and implementation activities will need to be conducted. Dissemination activities are of high importance to create awareness within the dermatological community and among patients. The evidence generated by the ROCTI-trial justifies the incorporation of OCT in (inter)national guidelines. We recommend that OCT is incorporated as initial diagnostic strategy, omitting the need for a punch biopsy only if a high confidence BCC diagnosis can be established. In cases where a high confidence BCC diagnosis cannot be made, a biopsy is still needed to ensure patient safety. Patients also play an important role in the implementation of this new strategy in clinical practice. Since the DCE demonstrated that an OCT strategy was not preferred by patients in a large majority of choice sets, a re-design of the DCE based on the newly available evidence from the ROCTI-trial is suggested. This new DCE can be conducted among patients in clinical practice, instead of in a research setting, in order to provide new insights. Based on our experience, it might be of value to provide (elderly) patients, who experience difficulties in filling out the DCE, help from a research nurse in order to increase the reliability of the DCE results. Apart from conducting a DCE, patients' preferences may be elicited by providing patients information about OCT at the clinic and simply asking patients about their thoughts on OCT and their experience. Subsequently, patients should be offered the choice between an OCT-guided diagnostic strategy or punch biopsy. Relevant patient associations (for example Huidpatienten Nederland) can be involved in the development of dedicated patient information folders and information videos about OCT. Involving patient organizations that represent patient interests is important prior to the process of implementation, as well as when OCT is implemented in clinical practice. This may provide important insights about the implementation process, allowing for adjustments where needed.

2. Development of expertise with OCT

Another important condition for successful implementation of OCT in clinical practice is the development of expertise with OCT among professionals who are involved in the diagnosis and treatment of patients BCC.[23] The results achieved in the RCT hinge on the OCT diagnoses made by a single physician, who was well trained and had evaluated 500 scans before the start of the study. Experience with and high confidence in OCT diagnoses of BCC is needed to realize a one-stop-shop approach with few misclassifications in a substantial part of patients. Therefore, proper training of novice OCT assessors is essential. This training can be supported by an E-learning environment which should be widely assessable

to trainees. Such an E-learning environment should include a sufficiently diverse number of OCT-images of both BCC and non-BCC lesions, demonstrating typical morphological features of BCC. Herein, the focus can lie on recognizing a small set of morphological BCC features, as demonstrated in **Chapter 4.1.** Since the terminology for morphological BCC features used in the literature was not uniform until recently, a Delphi consensus reported on a condensed set of OCT terms to describe these morphological BCC features.[24] The suggested E-learning environment should also contain a module in which the diagnostic performance of the trainee is assessed, with histopathological results made available after trainee evaluation. Here it is critical to set criteria for adequate diagnostic performance and to quantify the time and training required to achieve a sufficient level of diagnostic performance. In a former study performed at our department, it was illustrated how cumulative sum analysis can be used to train novice assessors and to monitor the level of diagnostic performance over time.[23] Herein, the minimal number of OCT scans that need to be assessed depends on pre-set acceptable and unacceptable error rates, but also on cut-off values for the definition of a positive test result. OCT trainees report their diagnosis on a 5-point confidence scale, which enables us to monitor performance for different thresholds for a positive test result for OCT. It is important to monitor the ability to make both accurate and confident (confidence score 4) OCT diagnoses. Because dermatologists are already trained in the analysis of histopathological slides, which resemble the OCT images, we expect a relatively short learning curve. Moreover, dermatologists are already used to perform imaging techniques during their outpatient clinic, such as ultrasound diagnostics for varicose disease.

Even though the learning curve provides insight into the learning process and the level of diagnostic performance of an OCT trainee, the diagnostic performance of OCT also depends on the skills of the observer.[22] To facilitate and optimize the diagnostic performance of OCT and accelerate implementation of OCT in clinical practice, automated analysis of OCT images using artificial intelligence (AI) seems to be a promising perspective. AI is already applied in various medical specialties such as radiology, pathology, orthopaedics, obstetrics and gynaecology and now also in dermatology.[25-27] It is expected that increasingly sophisticated computer-aided diagnostics will find its way into dermatological practice. Extra guarantees for adequate diagnostic performance can be built in by provisions to get support from an OCT expert who can oversee and double-check the process of (automatic) integration of all available information to establish a well-considered diagnosis and treatment plan.

3. Re-organization of dermatology outpatient clinics

Use of OCT will require re-organization of current clinical practice. In part of the patients, in whom OCT diagnosis can be made with high confidence, the clinical

process will change from a punch biopsy with one to two weeks waiting time for the results, to a one-stop-shop approach without the need for a punch biopsy. In these patients, the pathologist will be bypassed. The first consultation will partly change, because with OCT the diagnosis and treatment are directly discussed with the patient. The proposed redesign of care is mostly an organizational change involving the de-implementation of routines (biopsy) and thereby adding new services (OCT). The dermatology outpatient clinic should be organized in a way that OCT scanning of patients is made possible. This requires that skin cancer patients are clustered in consultations and that a care-pathway is developed which includes OCT. To develop such a care pathway training of personnel is the first step. In a recent 2021 international consensus statement on OCT for BCC, there was consensus among experts that dermatologists should acquire and interpret OCT scans.[24] This consensus is challenged by the results of our studies, in which neither of the assessors were dermatologists, but were either a research physician or a medical student. It has several advantages when a dermatologist performs OCT examinations, for instance it might be easier for dermatologists to interpret an OCT scan since they already have knowledge on the histopathological appearance of BCC. In addition, the dermatologist is the person that performs clinical and dermoscopic examination and decides on the treatment plan. On the other hand, dermatologists are costly and scarce, and therefore it might be beneficial to train other personnel, such as dermatology residents, technicians, medical students or researchers.[24] Another example is a specialised dermato-oncology nurse, who exclusively performs consultations with skin cancer patients, so that he or she can acquire and assess OCT scans under supervision of a dermatologist. Although the organization of clinical practice varies between hospitals and clinics, we believe that one care pathway can be developed to serve as a basis for implementation of OCT.

4. Reimbursement

In the Netherlands, a DBC care product (Diagnose Behandel Combinatie, or in English, Diagnosis Treatment Combination, i.e. your care path) is linked to BCC diagnosis and treatment. A DBC care product represents a sequence of medical activities (care-activities) that are performed during the treatment of a patient. Hence, a DBC care product describes a complete care episode rather than a single activity.[28] There are fixed tariffs for DBC care products that hospitals may charge to health insurers and patients.

Currently, the DBC care product tariff linked to BCC diagnosis and treatment includes the costs of performing a biopsy (€51,58) and histopathological assessment by the pathologist (€75,12), which adds up to a total price of €126,70 for a punch biopsy. A punch biopsy is a so-called care-activity (Zorgactiviteit) which is registered in the hospital information system. A combination of care-activities

leads to a DBC care product. All the activities that have been performed for the DBC care product are registered separately by the administrative departments. Each care-activity is labelled with a code that classifies what has been done.

As mentioned above, the clinical process will change when OCT is implemented. It is important that a care-activity can also be registered (and labelled with a code) for OCT, leading to the same DBC care product as to which a punch biopsy is linked. This requires that a cost-price is determined for OCT. In our cost-effectiveness analysis, we calculated a cost-price of OCT based on the methodology described in the Dutch manual for costing.[29] These calculations include equipment costs (€75.000), annual maintenance costs and depreciation period (total €12.875), the annual number of procedures and personnel costs. General hospital overhead of 38% were additionally allocated to the direct costs since it is an in-hospital procedure.[29] Based on the cost-price of OCT (calculated at €31.35) a margin should be determined by the hospital financial department and a sales price will have to be negotiated with health insurance companies. This sales prices determines the reimbursement the hospital receives for the delivered care. We expect that hospitals will only be interested in OCT when reimbursement is provided for the care-activity OCT. However, based on the results of our cost-effectiveness analysis (**Chapter 2.1**), implementation of OCT will eventually lead to cost-savings for health insurance companies, which justifies the availability of reimbursement for OCT.

Advantages of OCT over other methods, future improvements of OCT devices and recent developments

In the paragraph below we compare OCT to another non-invasive diagnostic tool, and we provide suggestions for improvements to the OCT device, which would ease the use and interpretation of OCT scans. We also describe recent developments in the field.

OCT is a convenient diagnostic tool for use in daily clinical practice. Compared to reflectance confocal microscopy (RCM), a more rapid implementation of OCT may be expected. The vertical OCT scans resemble histopathological slides and are therefore easier to interpret than the horizontal RCM images, making OCT more accessible to a broader group of dermatologists. In a study at our department, monitoring the learning process of diagnosing BCC with OCT using cumulative sum analysis, we showed that an adequate level of diagnostic performance was reached after assessing 183 to 311 scans.[23] In comparison, to reach a good level of confidence and expertise with RCM (VivaScope), a minimum of 6 months full-time training, including the evaluation of more than 4000 cases, is required to obtain an adequate level of diagnostic performance and confidence with RCM, according to Pellacani et al.[30] However, a direct comparison between OCT training and RCM training time is difficult, as in our study OCT training focuses on the diagnosis of

BCC, whereas the study on RCM focuses on the detection of melanoma, a different and far more aggressive form of skin cancer.[23, 30] No studies on RCM training time needed to diagnose BCC are available, and since OCT is mainly used to diagnose non-melanoma skin cancer, OCT training time needed to diagnose melanoma is also not at hand.

We also tried to compare the time needed to acquire and interpret OCT scans and RCM images. Acquiring and interpreting OCT scans only takes an average time of 4 minutes and 20 seconds, based on time measurement which were conducted during the clinical trial. Based on expert opinion, the average time needed to acquire and interpret RCM images of skin lesions suspicious of cancer is 15 and 10 minutes for RCM, using Vivascope 1500 and 3000, respectively.[31] The VivaScope 3000 is a handheld RCM device that allows imaging of lesions that are inaccessible by the VivaScope 1500, a stationary device.

Future improvements of the OCT device might further enhance the utility of OCT in clinical practice. Although the Vivosight OCT device is a wired device, it easily moves around the outpatient clinic and has a short start-up time. However, some hospitals have more than one outpatient clinic at different locations. In this situation, translocating the OCT device from one location to another is costly, time consuming and inefficient. As the technology of OCT improves, the construction of a portable, handheld, OCT device becomes realistic. A portable device could increase the utility and expand OCT into clinical settings where use is currently inhibited due to costs and the difficulty of translocating the device. Portable OCT devices, specifically designed for use in the field of ophthalmology, are already available.[32] Just recently, a portable RCM connected to a smartphone has been developed.[33] Therefore, this bodes well for portable OCT devices in the field of dermatology.

Another technical aspect of the OCT device, which requires mention, is that current devices are equipped with a flat, removable headpiece. In the H-zone of the face, surface areas are often convex or concave, making it more difficult to obtain an OCT image of sufficient quality. In the RCT we excluded patients with lesions located in the ‘H-zone’ of the face. More studies are therefore required to determine whether OCT is suitable in this subpopulation. Because of the difficulty to scan convex or concave skin areas, we received a grant and started a collaboration with the Department of Innovation Space of the Technical University of Eindhoven. Four students from this department investigated different innovative solutions to improve OCT image quality in convex or concave surface areas. The most promising solution was the incorporation of an optic solid lens in the OCT headpiece. The lens redirects the light, which is backscattered from the subsurface skin. This has a flattening effect on the curvature of the OCT image in convex/

concave skin areas. The difference in contrast which is the result of incorporation of a lens in the OCT headpiece, can be corrected by an Image Processing App which is able to change the contrast of the OCT images. Although not yet ready for use in clinical practice, the proposed solution might be of interest for future improvements of the OCT device.

A recent development in the field of non-invasive imaging technologies is line-field OCT (LC-OCT). This emerging technology combines the principles of both OCT and RCM. It combines the high resolution of RCM in a vertical plane similar to OCT. The penetration depth of the device is up to 500 µm (compared to 1.0-1.5 mm for OCT and 250 µm for RCM). However, the image quality degrades beyond 400 µm due to decreased resolution and increased noise. Overall, due to the combination of the advantages of OCT (high penetration depth and visualization of the vertical plane) and RCM (high resolution), LC-OCT appears promising for BCC diagnosis and subtyping.[34, 35] A recent study by Suppa et al. proposed morphologic LC-OCT criteria, which could be potentially useful for diagnosis and subtyping of BCC.[34] Whether LC-OCT results in better diagnostic accuracy for BCC diagnosis and subtyping compared to OCT and RCM is not clear and should be investigated in future studies.

Conclusions

The research presented in this thesis shows that OCT-guided diagnosis and treatment of BCC is a non-inferior and cost-effective strategy compared to regular care, because it obviates the need for biopsy in a substantial part of patients. Based on the results of our DCE, careful monitoring of patient preferences and experiences with OCT is suggested. The study population of the RCT consisted of patients presenting with a lesion clinically suspected for BCC and an indication for biopsy to verify the diagnosis. The potential of use of OCT in other patient populations was also evaluated. In patients undergoing MMS, the diagnostic accuracy of OCT for the assessment of BCC resection margins prior to MMS was poor. In patients with clinically evident BCC undergoing direct surgical excision, the added diagnostic value of use of OCT was limited, because diagnosis based on clinical and dermoscopic examination was already associated with very low risk of misclassification of non-BCC lesions as BCC. Thus, the main indication area for use of OCT is to establish an initial diagnosis in patients who visit the dermatology outpatient clinic with a lesion clinically suspected for BCC but still have an indication for biopsy to enable histological verification of the clinical diagnosis.

In clinical practice, the performance of novice assessors can be further improved by back-up of an OCT expert. OCT experts are not always at the site to visually inspect the suspected skin lesion, but our results indicate that supervision by

an OCT expert at a distance helps to improve diagnostic performance of an OCT assessor with less experience. The research presented in this thesis lays the foundation for implementation of OCT in clinical practice. However, several challenges need to be overcome for successful implementation of OCT in clinical practice.

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CHAPTER 6

Dutch summary

SAMENVATTING

Basaalcelcarcinoom (BCC) is de meest voorkomende vorm van huidkanker. Ongeveer 1 op de 5 à 6 Nederlanders krijgt een BCC in zijn of haar leven. Er worden grofweg drie subtypen onderscheiden: oppervlakkig (superficieel), nodulair en agressief. De behandeling is afhankelijk van het subtype van het BCC. Een superficieel BCC kan niet-invasief behandeld worden (met bijvoorbeeld imiquimod crème), terwijl nodulaire en agressieve subtypes vrijwel altijd behandeld worden met chirurgische excisie. De diagnose BCC wordt bij klinische verdenking voorafgaand aan de behandeling in principe gesteld door middel van een biopt. Een biopt is echter een kleine chirurgische ingreep, waarbij klachten zoals pijn, bloeden en infectie kunnen optreden. Het verkrijgen van de uitslag van histopathologisch onderzoek duurt minimaal een week. Met optische coherentie tomografie (OCT) kan het BCC vaak direct worden herkend, waardoor een biopt bij een groot deel van de patiënten achterwege gelaten kan worden. De uitslag van het onderzoek kan direct na de OCT scan worden besproken en behandeling kan worden ingezet. Dit zogenoemde one-stop-shop principe leidt naar verwachting tot efficiëntere en patiëntvriendelijkere zorg. Door implementatie van OCT in de klinische praktijk kunnen mogelijk kosten worden bespaard en kan de werklast voor dermatologen verminderd worden. Aangezien het BCC langzaam groei en bijna nooit uitzaait, is het een type huidkanker dat bij uitstek geschikt is voor een niet-invasieve aanpak. Dit proefschrift richt zich specifiek op niet-invasieve diagnostiek van het BCC.

In **hoofdstuk 1** wordt een algemene inleiding gegeven over de epidemiologie, ontstaanswijze, kliniek, diagnostiek en behandeling van het BCC. Daarnaast worden in dit hoofdstuk de doelstellingen van dit proefschrift beschreven.

De afgelopen jaren is er veel onderzoek gedaan naar verschillende tools voor niet-invasieve diagnostiek van het BCC. Eén van deze diagnostische tools is OCT, een beeldvormende techniek die real-time in-vivo dwarsdoorsnedebeelden van huidlaesies genereert tot een diepte van 1,0-1,5 mm. OCT is gebaseerd op het principe van lichtinterferometrie. Onlangs werd in een eerder onderzoek van de afdeling dermatologie van het MUMC+ aangetoond dat de diagnostische accuratesse van OCT (in combinatie met de klinische blik en dermatoscopie) voor het diagnosticeren van een BCC hoger is dan de diagnostische accuratesse van de klinische blik gecombineerd met dermatoscopie. Ongeveer 40% van de biopten kan achterwege gelaten worden wanneer OCT wordt toegepast. In dit onderzoek werden OCT-scans, in combinatie met klinische foto's, retrospectief beoordeeld. Aangezien een grote, goed uitgevoerde prospectieve gerandomiseerde geconroleerde trial ontbrak, was het onduidelijk of een OCT-geleide diagnose en behandeling niet zou resulteren in een onacceptabel verhoogd risico op falen van

de behandeling (residu of recidief van een (pre-)maligne huidlaesie) vergeleken met het reguliere zorg biopt.

In **hoofdstuk 2.1** worden de resultaten van de ROCTI-trial gepresenteerd. Dit is de eerste multicenter gerandomiseerde prospectieve non-inferioriteitsstudie waarin OCT werd vergeleken met het reguliere biopt. Tussen februari 2019 en september 2020 werden 598 patiënten geïncludeerd, verdeeld over 3 ziekenhuizen in Zuid-Nederland. Patiënten met een indicatie voor een biopt van een huidlaesie klinisch verdacht voor BCC, werden at-random toegewezen aan één van de twee diagnostische strategieën. In de OCT groep werd de diagnose en behandeling gebaseerd op OCT, maar alleen als met zekerheid de diagnose BCC en het subtype kon worden vastgesteld. Bij twijfel over de diagnose werd er alsnog een biopt afgenomen en werd de behandeling gebaseerd op de uitslag van het biopt. In de reguliere zorg groep werd de diagnose en behandeling altijd gebaseerd op de het biopt. Om veiligheidsredenen werd ook bij de patiënten met een zekere OCT diagnose een biopt afgenomen. De histopathologische uitslag van het biopt diende als gouden standaard. De primaire uitkomstmaat was de proportie patiënten vrij van een recidief of residu (pre-)maligne laesie 12 maanden na behandeling. We vonden dat 12 maanden na behandeling 253 van de 268 (94.4%) patiënten in de OCT groep vrij waren van een recidief of residu (pre-) maligne laesie ten opzichte van 266 van de 285 (93.3%) patiënten in de reguliere zorg groep. Op basis van de gemodificeerde intention-to-treat analyse was het absolute verschil (OCT versus reguliere zorg) +1.07 (95% betrouwbaarheidsinterval (BI): -2.93 tot 5.06) waarbij de onderste grens van het 95% BI niet de vooraf gedefinieerde non-inferioriteitsmarge van 10% overschrijdt. Daarnaast vonden we dat een substantieel deel (65.6%) van de biopten achterwege gelaten had kunnen worden. De sensitiviteit en specificiteit van een zekere OCT diagnose was respectievelijk 85.3% en 94.6%.

Door de toenemende incidentie resulteert de diagnostiek en behandeling van het BCC in een aanzienlijke socio-economische belasting voor de (dermatologische) gezondheidszorg. Voor het gevolg op de kosten van de gezondheidszorg is het belangrijk om te weten of een OCT-geleide diagnose en behandeling kosteneffectief is ten opzichte van de reguliere zorg. In **hoofdstuk 2.1** worden tevens de resultaten van de kosten-effectiviteitsanalyse beschreven. Om te kunnen bepalen of een OCT-geleide diagnose en behandeling van het BCC kosteneffectief is ten opzichte van het reguliere biopt, werd een economische evaluatie uitgevoerd vanuit een gezondheidszorgperspectief. Alle kosten behorende tot de diagnostische fase, de behandelfase en de nabehandeling fase werden verzameld. We vonden dat de gemiddelde kosten voor een OCT-geleide diagnose en behandeling lager waren (€689), vergeleken met €758 voor

de reguliere zorg (biopt). De resultaten toonden aan dat OCT een kosteneffectieve diagnostische strategie is in vergelijking met het reguliere biopt.

Hoofdstuk 2.3 beschrijft de resultaten van een discrete choice experiment dat parallel aan de RCT werd uitgevoerd. In deze vragenlijst werden op basis van een aantal attributen, in dit geval sensitiviteit, fout positieven, zekerheid van de arts over diagnose, wachttijd, neveneffecten en pijnlijkheid van de procedure, met verschillende levels (bijvoorbeeld percentage fout positieven), keuzesets voorgelegd aan de patiënten. Elke keuzeset verschilde in de levels van de attributen en per keuzeset werden de patiënten gevraagd voor welke diagnostische strategie zij de voorkeur hadden: OCT of biopt. Uit de resultaten bleek dat, voor OCT, een hogere sensitiviteit en een lager percentage fout positieven als de belangrijkste attributen werden gewaardeerd. Voor het biopt gaven patiënten de voorkeur aan een hogere zekerheid van de arts over diagnose, een langere wachttijd, terwijl de attributen veel, kortdurende pijn en een hoger percentage fout positieven negatief werden gewaardeerd. Patiënten kozen in 55% van de keuzesets voor biopt en in 45% voor OCT. Wanneer de hoogste levels werden toegepast voor OCT in een simulatie analyse (sensitiviteit 94%, zekerheid van de arts over diagnose 50%, percentage fout positieven 6%), dan veranderden de proporties en kozen patiënten in 58% van de keuzesets voor OCT.

In **hoofdstuk 3** hebben wij de toepasbaarheid van OCT onderzocht bij Mohs micrografische chirurgie en bij specifieke subpopulaties. Met de case-control studie beschreven in **hoofdstuk 3.1** bepaalden wij de sensitiviteit en specificiteit van OCT, ingezet voor het bepalen van de resectiemarges van in-vivo BCC voorafgaand aan Mohs micrografische chirurgie. We toonden aan dat er bij 58 van de 92 kwadranten met positieve resectiemarges, tumour zichtbaar was op de OCT scan. Dit correspondeert met een sensitiviteit van 63.0% (95% BI: 55.1-70.6). Bij 54 van de 102 kwadranten met negatieve resectiemarges was er tumour zichtbaar op de OCT scan, wat overeenkomt met een specificiteit van 52.9% (95% BI: 45.8-59.7). Op basis van deze lage diagnostische accuratesse van OCT, en het feit dat het maken en het beoordelen van OCT scans tijdrovend bleek te zijn, concluderen wij dat OCT in de klinische praktijk (nog) niet aangeraden wordt voor het bepalen van de resectiemarges van in-vivo BCC voorafgaand aan Mohs micrografische chirurgie.

Daarnaast beschreven we in **hoofdstuk 3.2** een casus waarbij we OCT hebben ingezet voor het diagnosticeren van perioculair BCC voorafgaand aan Mohs micrografische chirurgie. Patiënte presenteerde zich op de polikliniek met een litteken-achtige laesie ter plaatse van haar linker bovenooglid, waarbij er op basis van kliniek en dermatoscopie geen aanwijzingen waren voor een BCC. Met OCT werd deze laesie gediagnosticeerd als een superficieel en nodulair BCC.

Middels een vriescoupe biopt werd de OCT diagnose bevestigd, waarna Mohs micrografische chirurgie plaatsvond.

In **hoofdstuk 3.3** hebben we OCT ingezet bij patiënten met een klinisch evident BCC, waarvoor direct chirurgische excisie werd ingepland zonder voorafgaand biopt. We onderzochten of OCT een toegevoegde diagnostische waarde heeft in deze specifieke patiëntenpopulatie en of met OCT het risico op misclassificatie van niet-BCC laesies als BCC kan worden gereduceerd. In totaal werden er 114 patiënten geïncludeerd in drie ziekenhuizen in Zuid-Nederland. Volgens de histopathologische diagnose, waren 109 van de 114 laesies BCCs, wat overeenkomt met een positief voorspellende waarde (PPV) van 95.6% op basis van kliniek en dermatoscopie. Alle 109 histopathologisch bevestigde BCCs werden als zodanig herkend met OCT (sensitiviteit = 100%) en de negatief voorspellende waarde (NPV) in het geval dat er op OCT geen BCC zichtbaar was, was 100% (4/4).
In slechts 5 van de 114 laesies (4.4%) was er op basis van histopathologie sprake van een alternatieve diagnose. Middels OCT was het mogelijk 4 van die 5 laesies als niet-BCC laesies te herkennen.

Concluderend laten de resultaten zien dat met gebruik van OCT, de PPV toeneemt van 95.6% (kliniek en dermatoscopie zonder OCT) naar 99.2% (kliniek en dermatoscopie met OCT). Wanneer OCT gebruikt wordt in combinatie met de klinische blik en dermatoscopie neemt het risico op misclassificatie van niet-BCC laesies als BCC af. Het risico op misclassificatie is echter al laag in het geval van een zeer hoge klinische en dermatoscopische verdenking op BCC. De toegevoegde diagnostische waarde van OCT bij patiënten met een klinisch evident BCC zal in de praktijk moeten worden afgewogen ten opzichte van de kosten voor aanschaf van OCT en het trainen van personeel.

Tot slot hebben wij **hoofdstuk 4.1, hoofdstuk 4.2** en **hoofdstuk 4.3** gewijd aan de optimalisatie van de diagnostische accuratesse van OCT voor het diagnosticeren van BCC.

De resolutie van OCT is niet hoog genoeg om individuele cellen te kunnen onderscheiden op een OCT scan, maar deze is wel geschikt voor patroonherkenning. Daarom is het mogelijk om morfologische kenmerken van BCC te identificeren op een OCT scan. De afgelopen jaren zijn er in de literatuur verschillende kenmerken vastgesteld en beschreven. Het was echter nog niet bekend welke van deze kenmerken het meest onderscheidend zijn voor BCC.

In **hoofdstuk 4.1** hebben we daarom de diagnostische waarde bepaald van vastgestelde morfologische OCT kenmerken voor het diagnosticeren en het subtyperen van BCC. We onderzochten of het met een klein aantal OCT

kenmerken mogelijk is om een onderscheid te maken tussen BCC en niet-BCC laesies en tussen verschillende subtypes BCC. Wij gebruikten hiervoor de data van 299 patiënten in de OCT groep van de prospectieve multicenter gerandomiseerde non-inferioriteitstrial. Voor iedere laesie werd de aan- of afwezigheid van specifieke OCT kenmerken vastgelegd. De histopathologische diagnose werd als gouden standaard gebruikt.

De resultaten laten zien dat het met de kenmerken: (*1*) donkere rand, (*2*) helder peri-tumouraal stroma, (*3*) protrusies in de bovenste dermis met een donkere rand en (*4*) signaal-arme ovoïde structuren (allen met een PPV > 90%), een goed onderscheid mogelijk is tussen BCC en niet-BCC laesies. Met betrekking tot subtyperen, is het kenmerk 'protrusies in de bovenste dermis met een donkere rand' zichtbaar in de grote meerderheid van de superficiële BCCs. Afwezigheid van dit kenmerk is zeer voorspellend voor een nodulair of agressief BCC, wanneer er andere kenmerken aanwezig zijn. Er kan echter geen conclusieve diagnose worden gesteld wanneer 'protrusies in de bovenste dermis met een donkere rand' aanwezig zijn. In dit geval moeten er nog vijf andere kenmerken worden gebruikt die zeer voorspellend zijn voor een nodulair of agressief BCC. In onze studie was de BCC prevalentie 75.3% (225/299) en we stelden een diagnostisch algoritme voor waarmee het mogelijk is om 97.8% van de BCCs (220/225) te herkennen. Subtyperen zonder de noodzaak van een biopt is hiermee mogelijk in 132 van de 299 patiënten (44%), met een voorspellende waarde van 84.3% voor de aanwezigheid van superficieel BCC versus 98.8% voor de aanwezigheid van een nodulair of agressief BCC. Het voorgestelde diagnostische algoritme is bedoeld ter ondersteuning voor nieuwe OCT gebruikers, waarbij er in de OCT training gefocust kan worden op het herkennen van een klein aantal OCT kenmerken.

In **hoofdstuk 4.2** onderzochten we of het topicaal op de huid aanbrengen van glycerol, een zogenoemde optisch verhelderende agent, leidt tot een toegenomen beeldkwaliteit van OCT scans. Het primaire doel was om te onderzoeken of door het topicaal op de huid aanbrengen van glycerol de optische penetratiediepte van OCT toeneemt, waardoor het mogelijk wordt om dieper gelokaliseerde BCC tumournesten te herkennen. Er werden 61 patiënten met in totaal 72 BCCs geïncludeerd. OCT scans werden voorafgaand en na het aanbrengen van een 85% glycerol oplossing gemaakt. De gemiddelde penetratiediepte werd op iedere OCT scan bepaald door het automatisch traceren van het huidoppervlak en het punt waarbij er op de OCT scan signaalverlies optreedt. Hiervoor werd een speciaal ontworpen MATLAB programma gebruik. Daarnaast werden alle OCT scans in random volgorde gepresenteerd aan drie OCT beoordelaars, welke geblindeerd waren voor patiënten data en niet wisten of een OCT scan voorafgaand of na het aanbrengen van glycerol was gemaakt. De OCT beoordelaars werd gevraagd om de beeldkwaliteit van de OCT scans en de zichtbaarheid van typische OCT

kenmerken van BCC te scoren op een 4-punt Likert-schaal. De resultaten laten zien dat het topicaal op de huid aanbrengen van glycerol leidt tot een toegenomen optische penetratiediepte van 883µm naar 904µm (p=0.005). Waarschijnlijk is deze beperkte toename in penetratiediepte klinisch niet relevant, aangezien wij geen significante verbetering van de beeldkwaliteit en verbeterde zichtbaarheid van OCT kenmerken van BCC vonden na het topicaal aanbrengen van glycerol. Het aanbrengen van glycerol voorafgaand aan het maken van een OCT scan wordt daarom niet aangeraden.

Diagnostische accuratesse van OCT voor het detecteren van BCC verschilt tussen beoordelaars ten gevolge van verschillen in ervaring. In **hoofdstuk 4.3** bepaalden we de diagnostische accuratesse van een zekere OCT diagnose van een nieuwe beoordelaar, die OCT scans maakten en beoordeelden in combinatie met directe visuele inspectie van de laesie. We onderzochten of de diagnostische prestatie verbeterd kon worden door back-up van een OCT expert op afstand, die geen visuele informatie had over de verdachte laesie. We voerden een cohortstudie uit bij patienten die een biopt ondergingen vanwege een laesie verdacht voor niet-melanoom huidkanker. Beide OCT beoordelaars legden hun verdenking op BCC en het verdachte subtype vast op een 5-punt Likert-schaal. De histopathologische uitslag van het biopst diende als gouden standaard. De specificiteit, gedefinieerd als het vermogen om niet-BCC laesies te detecteren, was 96% voor beide beoordelaars. Sensitiviteit, gedefinieerd als het vermogen om BCC-laesies te detecteren, was significant hoger (82.2%) voor de OCT expert dan voor de nieuwe beoordelaar (71.8%) (*P*=0.005). Sensitiviteit van de expert om nodulaire en agressieve BCCs te detecteren was ook significant hoger (89.2% vs 97.6%, *P*=0.016). We concluderen dat supervisie door een expert op afstand, die geen directe visuele informatie heeft over de laesie, de diagnostische prestatie kan verbeteren en waardevol kan zijn voor de klinische implementatie van OCT.

Hoofdstuk 5 besluit het proefschrift met een interpretatie van de resultaten van de verrichte onderzoeken. We bediscussiëren welke plaats deze resultaten innemen in de dagelijkse klinische praktijk en we bespreken toekomstperspectieven.

CHAPTER 7

Impact paragraph

RESEARCH

This thesis focused on the (cost-)effectiveness of optical coherence tomography (OCT)-guided diagnosis and treatment of skin lesions clinically suspected for basal cell carcinoma (BCC) compared to regular care punch biopsy. We provided insight in patient preferences and investigated the potential of using OCT in alternative subgroups of patients. In addition, we searched for ways to improve the interpretation of OCT scans by investigators.

SCIENTIFIC IMPACT AND RESULTS

Basal cell carcinoma (BCC) is the most common form of skin cancer. Nowadays, one in five to six people in the Netherlands will develop a BCC during their lifetime.[1-3] Sometimes BCC diagnosis is evident based on the clinical appearance, but most often a punch biopsy is performed under local anaesthesia to verify the clinical diagnosis and to determine the BCC subtype.[4, 5] A punch biopsy is a small invasive procedure. The injection of the anaesthetic can be painful and there is a small chance of complications (bleeding, infection and scar formation). Awaiting the results takes approximately one to two weeks, after which the results are discussed with the patient in a (telephone) consultation. This causes treatment delay and the uncertainty during this period may be stressful for patients. In this thesis we investigated whether in part of the patients who visit the outpatient dermatology clinic with a skin lesion suspected for BCC, an accurate non-invasive diagnosis can be made with OCT. Hence, in part of the patients, BCC diagnosis and treatment can be accomplished in one day. Implementation of OCT into clinical practice is thus expected to lead to a more efficient, patient friendly and potentially cost-saving healthcare.

As large, well-conducted randomised controlled trials were lacking, it was unclear whether a diagnostic strategy using OCT for diagnosis and treatment does not result in an unacceptable increase in treatment failure (residual or recurrent (pre-) malignant skin lesion) when compared to regular care using punch biopsy in the large majority of patients. Therefore, we conducted the first randomised controlled non-inferiority trial (the ROCTI-trial) in which we compared the 1-year probability of treatment failure of an OCT-guided diagnosis and treatment to regular care punch biopsy. Costs of both strategies were also compared and to get a good impression of the perspective from patients their preferences were explored. In order to make the results generalizable, the trial was conducted in three hospitals: two general hospitals and one academic hospital.

We showed that OCT-guided diagnosis and treatment is non-inferior compared to regular care. It leads to more efficient healthcare since almost two-third of

biopsies (65.6%) could be omitted. OCT is also a cost-effective strategy compared to regular care punch biopsy. Patient preferences show that OCT is accepted by patients in clinical practice. As the ROCTI-trial was only performed in patients with an indication for biopsy, the results were not entirely generalizable for all BCC patients. Therefore, we also evaluated the added value of OCT in patients in whom there was no indication for a biopsy, namely patients with clinically evident BCCs that are immediately surgically removed without prior biopsy. These patients were not included in the ROCTI-trial, but it is possible that a skin lesion which is actually not a BCC, is incorrectly diagnosed as BCC by the clinician. We found that OCT was able to slightly reduce the risk of incorrect diagnosis by clinicians of non-BCC lesions as BCC. However, the risk of incorrect BCC diagnosis by clinicians is already very low and the value of OCT thus seems limited.

Apart from using OCT for diagnosis of BCC, we explored the application of OCT in alternative subgroups of patients. As such, we evaluated if OCT was helpful for determining the resection margins of BCC prior to Mohs micrographic surgery (MMS), a specialised surgical method. This method is performed in stages by removing the tumour together with a thin (1-2mm) layer of surrounding normal tissue. As MMS is a labour-intensive and time-consuming procedure, correct estimation of resection margins with OCT prior to MMS could reduce the average number of stages required for complete tumour removal. Unfortunately, OCT was not accurate when used for this purpose and we also found that obtaining and interpreting the OCT scans was time consuming.

Besides looking in which subgroups of patients OCT can be applied, we searched for ways to improve the interpretation of the OCT scans by the investigator. During the assessment of OCT scans, the investigator tries to identify features that are typical for BCC. We conducted a study in which we evaluated which features are most discriminative for BCC, as using only a small set of features can ease the interpretation of OCT scans by the investigator.

Finally, we investigated whether we could improve the quality of the OCT scan in order to facilitate correct diagnosis. We tried to improve OCT image quality and thereby visibility of BCC features by application of glycerol on the skin, a so-called optical clearing agent. We found that although application of glycerol on the skin increased the optical penetration depth, it did not have a clinically relevant effect and therefore was not recommended.

Lastly, the level of experience of the investigators who interprets OCT scans may influence their ability to make both accurate and confident diagnoses with OCT. We showed that for novice OCT assessors, distant supervision from an OCT expert who is not on-site and has no clinical information, is still valuable.

The ROCTI-trial generated the necessary high-level evidence for implementation of OCT in clinical practice. The other studies in this thesis contributed knowledge about the applicability of OCT in alternative subgroups of patients, as well as ways to improve the interpretation of OCT scans.

SOCIETAL IMPACT

In the Netherlands, approximately 50.000 patients were diagnosed with a new BCC in 2021. About one-third of these patients were or will be diagnosed with more than one BCC. The number of biopsies that are performed in patients with a skin lesion clinically suspected for BCC, is much higher and approximately 90.000 each year. These numbers continue to rise, illustrating that BCC is a public health problem that puts a substantial burden on the resources of healthcare systems, as well as a heavy economic burden on society, being associated with significant direct medical costs.

We found that with OCT, almost two-third (65.6%) of biopsies are no longer needed. The results of this thesis are relevant for patients who visit the dermatology outpatient clinic with a lesion suspected for BCC for which a biopsy needs to be taken. An invasive procedure can be omitted and treatment can often be discussed and started immediately. For clinicians, use of OCT is relevant because it can decrease the workload caused by the high number of biopsies and post-biopsy (telephone) consultations. And from a healthcare perspective the results are also of interest given their aim to make the diagnostic process more efficient, patient friendly and less costly.

RECOMMENDATIONS FOR IMPLEMENTATION

The research presented in this thesis lays the foundation for implementation of OCT in clinical practice.

To facilitate successful implementation of OCT in clinical practice, (inter) national dissemination and implementation activities will need to be conducted. Dissemination activities are of high importance to create awareness within the dermatological community and among patients. A summary of the most important results of the ROCTI-trial can be accessed by patients on the website of ZonMw. Relevant patient associations (Huidpatiënten Nederland) can be involved in the development of dedicated patient information folders and information videos about OCT. To increase awareness of the results among the dermatological community, we presented and discussed the results of the studies on national and international (scientific) meetings.

The results of the studies in this thesis have been published in scientific research journals, which increases the worldwide availability of the results, and may aid researchers designing future studies. To allow for the results to be implemented in daily care, (inter)national BCC guidelines need to be adapted. OCT can be incorporated in the guideline as initial diagnostic strategy, omitting the need for a punch biopsy only if a BCC diagnosis can be made with high confidence. In cases where a BCC diagnosis cannot be made with high confidence, a biopsy is still needed.

Use of OCT will require re-organization of current clinical practice. In part of the patients (65.6%), in whom an OCT diagnosis can be made with high confidence, the clinical process will change from a punch biopsy with one to two weeks waiting time for the results, to a so-called one-stop-shop approach without the need for a punch biopsy. The first consultation will then partly change, because with OCT diagnosis and treatment are directly discussed with the patient. The proposed redesign of care is mostly an organizational change involving the de-implementation of routines (biopsy) and thereby adding new services (OCT). For that reason, it is essential that reimbursement becomes available for hospitals for the task of obtaining and interpreting OCT scans. Currently, no declaration can be performed for obtaining an OCT scan, whereas obtaining a punch biopsy is reimbursed. Consequently, it is important to involve the hospital financial department and health insurance companies. Another requirement for successful implementation of OCT in clinical practice is that users of OCT are well-trained. Therefore, it is essential to set criteria for adequate diagnostic performance and to quantify the time and training required to achieve a sufficient level of diagnostic performance.

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ADDENDUM

CURRICULUM VITAE

Fieke Adan is geboren op 2 augustus 1993 te Eindhoven. Zij groeide op in Eindhoven en verhuisde op de leeftijd van 8 jaar met haar ouders en broer naar Amerika (Chapel Hill, North Carolina) waar zij met veel plezier een jaar heeft gewoond. Na het cum laude behalen van haar Atheneum diploma aan het Pleincollege Bisschop Bekkers te Eindhoven in 2011, begon zij aan de opleiding geneeskunde aan de Universiteit van Amsterdam (UvA).

Vanwege haar interesse voor het vak dermatologie, heeft zij haar laatste jaar van de opleiding geneeskunde zowel haar wetenschappelijke stage als haar semi-arts stage bij de vakgroep Dermatologie van het Antoni van Leeuwenhoek ziekenhuis/Nederlands Kanker Instituut te Amsterdam voltooid. Haar opleiding geneeskunde sloot zij af met een keuze-coschap tropendermatologie aan de Universitas Gadjah Mada in Yogyakarta, waarna zij een mooie reis maakte door Indonesië.

In januari 2018 behaalde zij haar artsendiploma, waarna zij gedurende een half jaar als ANIOS interne geneeskunde/oncologie heeft gewerkt in het Antoni van Leeuwenhoek ziekenhuis/Nederlands Kanker Instituut te Amsterdam. Vanwege haar interesse in onderzoek en voor het vak dermatologie, begon zij in september 2018 aan een promotietraject onder begeleiding van Dr. Mosterd, Dr. Kelleners-Smeets en Dr. Nelemans in het Maastricht Universitair Medisch Centrum (MUMC+), hetgeen leidde tot dit proefschrift. In 2021 won Fieke de eerste prijs op het Pélerin Wetenschapssymposium in het MUMC+ voor haar onderzoek naar optical coherence tomography als niet-invasieve diagnostiek van het basaalcelcarcinoom.

Vanaf september 2021 is zij gedurende vijf maanden als ANIOS werkzaam geweest op de afdeling dermatologie van het Catharina ziekenhuis te Eindhoven. Vanwege haar behoefte om langer de tijd te hebben voor haar patiënten en haar al lang bestaande interesse in de psyche van de mens, besloot zij om een ander carrièrepad te kiezen. Fieke startte daarom op 1 april 2022 met de opleiding tot psychiater in de geestelijke gezondheidszorg te Eindhoven (GGzE).

LIST OF PUBLICATIONS AND PRESENTATIONS

Related to this thesis

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OCT als niet-invasieve diagnostiek van BCC. Oral presentation at Dutch Society for Dermatology and Venereology (NVDV) webinar: De Dermatoloog Draait Door, Amsterdam, the Netherlands, April 2021

OCT als niet-invasieve diagnostiek van BCC. Oral presentation at 356th Scientific Meeting of Dutch Society for Dermatology and Venereology (NVDV), Maastricht, the Netherlands, November 2021

OCT als niet-invasieve diagnostiek van BCC. Oral presentation at Pélerin Wetenschapssymposium, Maastricht, the Netherlands, October 2021

OCT for non-invasive diagnosis of BCC. Oral presentation at European Association of Dermato Oncology (EADO) congress, Sevilla, Spain, April 2022

Awards

First price of €5000, Pélerin Wetenschapssymposium, Maastricht, the Netherlands, October 2021

Grants

Travel grant of €800, *Nijbakker-Morra Stichting*, Amsterdam. Visiting OCT experts for training purposes:

Prof. G. Pellacani (Università degli Studi di Modena e Reggio Emilia, Modena, Italy, December 2018) and Prof. J. Welzel (Universitätsklinikum Augsburg, Germany, January 2019).

Travel grant, *Erasmus+ 'staff exchange' program*, €480 (Germany) and €765 (Italy). Visiting OCT experts for training purposes: Prof. G. Pellacani (Università degli Studi di Modena e Reggio Emilia, Modena, Italy, December 2018) and Prof. J. Welzel (Universitätsklinikum Augsburg, Germany, January 2019).

Research grant of €2000, *University Fund Limburg/SWOL*, Project: Optical Coherence Tomography assisted diagnosis of periocular basal cell carcinoma prior to Mohs' micrographic surgery – Design of a new convex OCT headpiece, March 2020.

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