Through the looking glass

Confocal microscopy imaging of basal cell carcinoma

Kadouch, D.J.

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Download date: 21 Feb 2020
Through the looking glass: confocal microscopy imaging of basal cell carcinoma

Daniel J. Kadouch
Through the looking glass: confocal microscopy imaging of basal cell carcinoma

Daniel J. Kadouch
THROUGH THE LOOKING GLASS: 
CONFOCAL MICROSCOPY IMAGING OF 
BASAL CELL CARCINOMA

ACADEMISCH PROEFSCHRIFT 
ter verkrijging van de graad van doctor 
aan de Universiteit van Amsterdam 
op gezag van de Rector Magnificus 
prof. dr. ir. K.I.J. Maex 
ten overstaan van een door het College voor Promoties ingestelde commissie, 
in het openbaar te verdedigen in de Aula der Universiteit op 
woensdag 18 oktober 2017 te 13:00 uur

door

Daniel Jan Kadouch 
geboren te Senlis, Frankrijk
PROMOTIECOMMISSIE

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Faculteit der Geneeskunde
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GENERAL INTRODUCTION
INTRODUCTION

Skin cancer

Skin cancer is on the rise as the most common cancer currently diagnosed in humans worldwide.\(^1\) It is generally divided into melanoma and non-melanoma, of which keratinocyte cancer (KC) is the most important group. Currently between 2-3 million KC and 132,000 melanoma skin cancer occur globally each year.\(^2\) Although KC accounts for approximately 95% of all skin cancers,\(^3\) melanoma remains by far the most aggressive form with the worst prognosis due to metastasis.\(^4\) On the contrary basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), the most common types of KC, can normally be well treated.\(^5,6\) The studies presented in this thesis further focus on BCC.

Basal cell carcinoma

BCC is the most common form accounting for roughly 80% of all skin cancers.\(^7\) In the past decades, the European BCC rates have annually increased with approximately 5% compared to 2% per year in the U.S.A.\(^1,8\) The increased incidence is most probably a result of an increased awareness in the general population and among physicians, improved registration, an ageing population and more intensive ultraviolet radiation (UVR) exposure.\(^9\) The latter is supported by previous published epidemiological literature indicating that UVR is an important risk factor for BCC with a significant increase among outdoor workers\(^10,11\) However not only outdoor workers are affected. With the increase of recreational sun exposure and indoor tanning more BCCs are diagnosed among young adults.\(^12\) At the same time it has been shown that the actual BCC occurrence is being underestimated due to limited available data from only a few cancer registries, mostly only registering the first BCC in patients.\(^13\)

Burden for patients

BCC is a slow-growing skin neoplasm and is often mistaken by patients for a sore with recurrent crusting and bleeding. Clinical appearances and morphology are diverse, but most BCC are characterized by small, translucent, or pearly papules, with raised edges, and small blood vessels (telangiectasia) in sun-exposed skin of head and neck areas.\(^14\) Although BCC is the least aggressive form of skin cancer, delay in diagnosis and appropriate treatment may result in substantial morbidity and cosmetic disfigurement. Patients suffering from BCC attach greatest importance to low risk of recurrence, high chances of cure and favorable cosmetic outcomes.\(^15\) As shared-decision making is becoming more common, physicians should inform each individual patient on all aspects of different treatment options for BCC, allowing them to make their own decision.\(^16\)

Healthcare costs

In addition to the patients’ burden of disease, the rapidly increasing BCC incidence in recent decades has become an important public health problem.\(^17,18\) In general, there is a significant cost burden of skin cancer for many countries and consequently health care costs are growing accordingly.\(^19\) Relative to the size of their populations, the annual direct health system costs for skin cancer were
highest for Australia, New Zealand, Sweden and Denmark (2013 Euros). Furthermore it has been shown that early detection and adequate treatment can reduce treatment complexity and cost, and offer the best chance for control.

**Diagnosis**

Historically, BCC has been classified into various histopathological subtypes. Each subtype is recognized by its distinct morphological growth pattern and has an associated biological behavior that can affect the likelihood of tumor recurrence. While many subtypes have been reported, recent international guidelines recommend a simplified classification that distinguishes between superficial, nodular and aggressive (micronodular, infiltrative and basosquamous subtypes) BCC. Of those, nodular and superficial subtypes are the most frequently diagnosed. Moreover in daily dermatology practice mixed histologic BCC subtypes, defined as the presence of more than one subtype within the same lesion, are frequently observed.

Ideally, BCC diagnosing and subtyping should be performed non-invasively by a painless procedure leading to an immediate diagnosis and treatment. This seems to be particularly relevant to the growing use of topical treatments as nonsurgical first-line therapy for superficial BCC (sBCC). Current available diagnostic tools for diagnosing and subtyping BCC include dermoscopy, histopathological examination of a routine punch biopsy, and novel non-invasive skin imaging techniques.

**Dermoscopy**

Previous studies have shown that the histopathological BCC subtypes seem to correspond to specific macroscopic features. In addition to naked-eye-examination, dermoscopy is nowadays fully integrated in dermatology practice. Studies have shown that dermoscopy enhances clinical diagnostic accuracy of BCC with a reported sensitivity range between 86% and 97% and specificity range between 71% and 99%. More recently, several dermoscopy signs, such as arborizing vessels and blue-grey ovoid nests have been associated with BCC. While dermoscopy has been shown to accurately discriminate BCC from other of clinically suspected lesions new studies have further reported on using dermoscopy to discriminate sBCC from other subtypes.

Lallas et al. found specific dermoscopic features such as ‘maple leaf-like areas’, presence of ‘short fine telangiectasias’, and ‘shiny white-red structureless areas’ to be more prevalent in sBCC. Ahnlide et al. added the clinical observation of ‘flat surface’ and confirmed the dermoscopic feature ‘multiple small erosions’ to identify sBCC.

**Punch biopsy**

Current standard care implies a punch biopsy of clinically suspected BCC to confirm clinical diagnosis and classify into histological subtypes prior to treatment selection. The most aggressive subtype that is identified by biopsy serves as an important factor of treatment selection. However, despite local anesthesia, a diagnostic punch biopsy generally leads to some degree of pain, scarring and discomfort for patients. In addition, there is a doctor’s delay in the diagnosis due to tissue processing time. Compared to clinical diagnosis using dermoscopy, a punch biopsy has been found
to be more accurate in subtyping BCC. However studies have also reported on discordances between histology reports of punch biopsy and final excision specimen.

Skin cancer imaging
In this rapidly developing medical technological era, non-invasive imaging devices have emerged as useful tools for diagnosing skin cancer. In addition to diagnosing, medical imaging has also dramatically transformed how clinicians evaluate, monitor, and treat skin malignancies. Non-invasive skin imaging techniques such as high-frequency ultrasonography, optical coherence tomography (OCT), Raman spectroscopy, terahertz pulsed imaging, positron emission tomography, high-resolution magnetic resonance imaging and reflectance confocal microscopy (RCM) have been developed to assist the diagnostic process for BCC. Of those, RCM is an optical imaging technique that is capable of directly imaging a skin lesion at high-resolution.

Reflectance confocal microscopy
RCM relies on a low-power laser that emits near-infrared light at 830 nm and focuses the light through a gating pinhole before it enters the detector. With optical sectioning of 2-5 µm and a resolution of 0.5-1.0 µm, imaging of the epidermis and underling papillary dermis is routinely performed in small fields-of-view of 0.5x0.5mm and down to depths of 100-200 µm. Strong signal and bright contrast is primarily obtained from melanin, keratin and collagen. The acquired black and white RCM optical section images are comparable to high magnification histopathology at about X30. In contrast to vertical histopathology sectioning of the skin, RCM images are acquired horizontal and parallel (en face) to the skin. Besides RCM images can be watched in stack mode (enabling an overview of each consecutive skin layer from the epidermis down to the papillary dermis) and in mosaic mode (stitching together of multiple optical sections to create a larger image of a specific skin layer). Currently there are two in vivo RCM models available, namely the traditional wide-probe RCM (VivaScope 1500; CaliberID, Rochester, NY, USA) and the more recent introduced handheld-RCM probe (VivaScope 3000; CaliberID, Rochester, NY, USA). The wide-probe RCM is suitable for flat body areas and has the ability of piecing together optical sections into a 8x8 mm mosaic image. The handheld RCM can be used for concave body areas such as the nose, ear and peri-orbital regions but has a smaller field of view of 1x1 mm.

Treatment
BCC treatment is needed due to local invasive, aggressive and destructive effects on the skin and surrounding tissues. If left untreated, or inadequately treated, BCC can cause extensive tissue destruction, and may even infiltrate underlying nerves, muscles and bones. Therefore treatment should be aimed at tumor clearance. To achieve optimal treatment, important factors such as size, location, histological subtype, patient preferences and availability of an appropriate therapy should be taken into consideration for each patient. BCC management can be divided into surgical and non-surgical treatments.
Surgical
Surgery is considered as the gold standard in treatment of BCC. Surgical treatments include conventional surgical excision with an adequate surgical margin and Mohs’ micrographic surgery (MMS). According to the Dutch evidence-based guidelines for BCC treatment a surgical margin of 3mm is recommended for non-aggressive (superficial or nodular) BCC less than 10mm in size while 5mm margin should be used for aggressive BCC, non-aggressive BCC >10mm in size or recurrences. Five-year recurrence rates after conventional surgical excision are 4% to 10% for primary and 17% for recurrent BCC. MMS is an elegant tissue sparing surgical technique that uses direct frozen-tissue sectioning with complete margin control, avoiding unnecessary excision of uninvolved tissue. Compared to conventional surgical excision, MMS has higher cure rates but the procedure is more time-consuming, labor intensive, and thus more expensive. Indications for Mohs’ micrographic surgery are a primary BCC on high-risk facial areas (depending on tumor size) and recurrent or previously incompletely excised BCC in all facial areas.

Non-surgical
Non-surgical treatments can be used as alternatives for sBCC. This histological growth pattern makes the tumor well accessible for local destructive techniques such as curettage and cautery, cryotherapy, photodynamic therapy, laser surgery and radiotherapy, as well as topical immune modulators such as imiquimod and 5 fluorouracil. For example, photodynamic therapy and topical immune modulators are increasingly being used because of their favorable cosmetic outcome compared to surgery. However, disadvantages of these treatments are the lack of histological control and higher recurrence rates. Therefore non-surgical treatments are not recommended for recurrent BCC, aggressive BCC or for BCC located within high-risk facial areas of the face.

In addition, vismodigib (systemic inhibitor of the hedge-hog pathway) can be indicated in patients suffering from rare metastatic or locally advanced BCC not suitable for surgical or radiation treatment.

AIMS OF THE THESIS
This thesis focuses on improving diagnosis and treatment for patients suffering from BCC. Although some aspects of optical coherence tomography imaging and Raman spectroscopy will be discussed in this thesis, it focuses mainly on incorporating reflectance confocal microscopy (RCM) as non-invasive skin imaging tool in a new treatment pathway for BCC.

The principal aim of this thesis is to assess the efficacy of a one-stop-shop concept with RCM for surgical management of BCC. One-stop-shop implies that diagnosis and treatment both take place on the day of initial outpatient clinic consultation.

Chapter 2 summarizes diagnostic accuracy (sensitivity and specificity) of a punch biopsy in subtyping basal cell carcinoma. Current management of BCC relies on the histopathological subtype of a routine punch biopsy and becomes more and more relevant due to the increasing use of non-surgical treatments. However, the reliability of a punch biopsy in subtyping BCC has been questioned.
Chapter 3 reviews diagnostic accuracy of RCM to judge its usefulness in accurately diagnosing BCC. While RCM has been proposed as a novel accurate tool for non-invasive real-time diagnosis of BCC, we found that a systematic overview and quality assessment of RCM for diagnosing BCC was missing.

In Chapter 4.1 the study protocol of our randomized controlled trial (RCT) is being described. In this open-label, parallel-group, non-inferiority, multicenter RCT we enrolled patients with clinically suspected BCC at two tertiary hospitals in Amsterdam, the Netherlands. The clinical trial was designed to assess the efficacy of a one-stop-shop concept with RCM for surgical management of clinically suspected BCC compared to standard care. The outcomes of the trial could possibly lead to a more efficient disease management strategy for patients suffering from BCC. Chapter 4.2 discusses the main outcomes of our RCT such as the proportion of patients with tumour-free margins after surgical excision of BCC, the proportion accurately diagnosed BCC by RCM and a punch biopsy (including the most aggressive histological subtype), patients’ throughput time, patient’s satisfaction and adverse events.

Chapter 5 further assesses the clinical value and utility of RCM versus a punch biopsy in diagnosing and subtyping BCC according to the Standards of Reporting of Diagnostic Accuracy (STARD) guidelines. In Chapter 6 the intra- and interrater agreement of RCM images is assessed to evaluate the reliability in correctly diagnosing and subtyping BCC. Since RCM relies on individual morphologic pattern recognition that might vary among users, a critical appraisal of the diagnostic procedure is needed. Chapter 7 concludes the thesis with a general discussion on the main findings and future considerations on skin imaging in dermatological practice are being discussed.
REFERENCES


IS A PUNCH BIOPSY RELIABLE IN SUBTYPING BASAL CELL CARCINOMA? A SYSTEMATIC REVIEW
DEAR EDITOR, Basal cell carcinoma (BCC) is the most prevalent type of skin cancer. Numerous studies have reported on the rising incidence of BCC causing a major burden on current healthcare systems. Current management relies on the histopathological subtype of a punch biopsy, and this is becoming more and more relevant due to the increasing use of nonsurgical treatments. However, the reliability of a punch biopsy in subtyping BCC has been questioned. The aim of this systematic review was to judge the reliability of a punch biopsy in accurately subtyping primary BCC.

We searched Medline, Embase, the non-Medline subset of PubMed and Google Scholar for studies on the diagnostic accuracy of punch biopsy for subtyping primary BCC (Figure 1). The databases were searched from inception until 28 June 2015. No language or other restrictions were used (Table 1). We assessed the diagnostic accuracy of punch biopsy for subtyping primary BCC and thus excluded lesions diagnosed by shave biopsy. Also, recurrent BCC lesions were excluded. BCCs were classified into aggressive BCCs (aBCC), such as micronodular and infiltrating, or nonaggressive BCCs (naBCC), such as superficial (sBCC) and nodular (nBCC). Mixed histological BCC subtypes (mBCC) were defined as the presence of more than one subtype within the same lesion.

True positives were defined as punch biopsy specimens correctly identifying aBCC, while true negatives were defined as punch biopsy specimens correctly identifying naBCC. False-positives were defined as naBCCs that were incorrectly classified as aBCC, and false-negatives were defined as aBCCs that were missed after analyzing histology of punch biopsy specimens.

Figure 1. Flowchart summarizing the selection process for studies concerning diagnostic accuracy of punch biopsy in subtyping primary BCC.
### Table 1. Database(s): Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) 1946 to Present Search Strategy: 2015-06-28

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<td><em>biopsy/ or exp &quot;biopsy, needle/ or (biop</em> or microbiop*).ti,ot,kf.</td>
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Is a punch biopsy reliable in subtyping basal cell carcinoma? A systematic review

Our study protocol has been registered in the PROSPERO database with the number CRD42014010550.

Eligible studies were screened by two independent reviewers (DK and AHW), and the references were also examined. Studies were included if (i) a punch biopsy was used to diagnose primary BCC; (ii) punch biopsy-proven primary BCCs were subsequently treated by conventional surgical excision (reference standard); (iii) pathology reports included the histopathological subtype of both the biopsy and excision specimen; and (iv) sensitivity and specificity were provided or a 2 x 2 contingency table could be constructed from the results. For all studies the following data were extracted: first author; year of publication; whether the study was prospective or retrospective; baseline patient characteristics; number of BCC lesions; size of punch biopsy; histopathological subtypes of punch-biopsy-proven BCCs and subtype of subsequent excision specimen; presence of a secondary BCC subtype within the same lesion (i.e. mBCC); procedures and BCC subtyping criteria used by (dermato)pathologists; and number of true-positives, true-negatives, false-positives and false-negatives. Included papers were assessed using the Quality Assessment of Diagnostic Studies tool. 5

In total, 960 potentially relevant titles were identified from the initial literature search. Of those, five studies with a total of 1285 punch-biopsy-proven primary BCC lesions were finally included in this systematic review (Table 2). 6–10 The most frequently diagnosed subtype was nBCC, in all five studies. The presence of a secondary subtype (mBCC) ranged from 19% to 74% in either biopsy or excision specimen. In most studies, more than one (dermato)pathologist performed histopathological evaluation. In two studies, the BCC subtyping criteria by Crowson 11 and Rippey 12 were used, whereas one study used the Dutch Dermatology Guidelines for BCC. 13 One of the five included studies was performed prospectively (Table 2). Wolberink et al. performed the only study with a low risk of patient selection bias (Table 3). 10 In none of the studies was the time interval between the index and reference test specified. This led to an unclear risk of bias of flow and timing in all studies. There were no applicability concerns with respect to patient selection, index test or reference standard.

In this systematic review, we found that a punch biopsy does not accurately reflect the BCC subtype in a substantial number of cases. Sensitivity ranged from 61% to 85% and specificity ranged from 79% to 88%. This is in line with the reported low accuracy of biopsy sampling in Mohs’ studies. 14 In contrast to our expectations, only a limited number of publications have investigated the reliability of punch biopsy in subtyping BCC. We were able to include only five studies. The presence of mBCCs was noted in 19–74% of cases in either the biopsy or excision specimen. This seems higher than in previous studies, which have reported an incidence of mixed subtypes in up to 49% of BCCs. 2,4,15

It is important to realize that the presence of multiple subtypes can negatively influence the accuracy of a punch biopsy, as only a small portion of a BCC lesion is obtained for histopathological evaluation. We strongly believe that sampling error (especially in larger lesions) is probably the most important explanation for the discordance between biopsy and excision. Understaging aggressive growth patterns in mBCC can easily result in insufficient treatment, excessive incomplete excisions, distress for patients, unnecessarily high costs and avoidable burden on the capacity of the hospital organization. We agree with Wolberink et al. 10 and Roozeboom et al. 8 that in selected cases, a punch biopsy can be omitted to confirm BCC diagnosis and divide between subtypes.
Table 2. Characteristics, diagnostic accuracy, overview of BCC subtypes and subtyping criteria of included studies

<table>
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<tr>
<th>Author</th>
<th>Year of publication (ref)</th>
<th>Study design</th>
<th>Age of patients, y</th>
<th># of BCC lesions</th>
<th># of BCC lesions per subtype diagnosed by excision specimen (%)</th>
<th>% of mixed type BCC</th>
<th>Assessment of histology specimen</th>
<th>References regarding subtyping criteria (ref)</th>
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<td>Kamyab-Hesari et al.</td>
<td>2014 (6)</td>
<td>CS Retro</td>
<td>Mean 64.8</td>
<td>333</td>
<td>61/333 (18%)</td>
<td>28 sBCC (8%), 118 nBCC (35%), 1 mnBCC, 20 iBCC, 14 mBCC and 90 aBCC (aBCC - 45%)</td>
<td>27% of excision specimen</td>
<td>One dermatopathologist blinded to patient data and histology</td>
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<td>Roozeboom et al.</td>
<td>2013 (7)</td>
<td>CS Retro</td>
<td>Mean 70.1</td>
<td>243</td>
<td>62/243 (26%)</td>
<td>38 sBCC (20%), 111 nBCC (46%), and 94 aBCC (34%)</td>
<td>74% either punch or excision specimen</td>
<td>Different pathologists</td>
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<td>Roozeboom et al.</td>
<td>2015 (8)</td>
<td>CS Prosp</td>
<td>Mean 68</td>
<td>152</td>
<td>85/152 (56%)</td>
<td>25 sBCC (22%), 79 nBCC (52%), and 48 aBCC (26%)</td>
<td>Unk</td>
<td>Two independent dermatopathologists blinded to clinical diagnosis</td>
</tr>
<tr>
<td>Russell et al.</td>
<td>1999 (9)</td>
<td>CS Retro</td>
<td>Unk</td>
<td>57</td>
<td>79/57 (55%)</td>
<td>10 sBCC (12%), 50 nBCC (58%) and 26 iBCC (30%)</td>
<td>Unk</td>
<td>One dermatopathologist blinded to patient data</td>
</tr>
<tr>
<td>Wolberink et al.</td>
<td>2013 (10)</td>
<td>CS Retro</td>
<td>Mean 65.6</td>
<td>500</td>
<td>72/500 (14%)</td>
<td>209 sBCC (42%), 150 nBCC (30%), and 98 iBCC (aBCC - 28%)</td>
<td>19% of biopsy- and 20% of excision specimen</td>
<td>Four independent dermatopathologists</td>
</tr>
</tbody>
</table>

Abbreviations: aBCC, aggressive BCC; CS Prosp, prospective case study; CS Retro, retrospective case study; FN, false negative i.e. aBCC missed by punch biopsy; FP, false positive i.e. nBCC misdiagnosed as aBCC by punch biopsy; iBCC, infiltrative basal cell carcinoma; mBCC, mixed type basal cell carcinoma; maBCC, metatypical BCC; mnBCC, micronodular BCC; moBCC, morpha BCC; nBCC, nodular basal cell carcinoma; sBCC, superficial basal cell carcinoma; Unk, unknown; y, year.
Table 3. Methodological quality assessments using QUADAS-2 tool

<table>
<thead>
<tr>
<th>Study (ref)</th>
<th>Risk of bias</th>
<th>Applicability concerns</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient selection</td>
<td>Index test</td>
</tr>
<tr>
<td>Kamyab-Heasari et al. (6)</td>
<td>Unclear</td>
<td>Low</td>
</tr>
<tr>
<td>Roozeboom et al. (7)</td>
<td>Unclear</td>
<td>Low</td>
</tr>
<tr>
<td>Roozeboom et al. (8)</td>
<td>Unclear</td>
<td>Low</td>
</tr>
<tr>
<td>Russel et al. (9)</td>
<td>Unclear</td>
<td>Low</td>
</tr>
<tr>
<td>Wolberink et al.(10)</td>
<td>Low</td>
<td>Low</td>
</tr>
</tbody>
</table>

Eliminating routine pretreatment biopsy could also reduce BCC treatment cost without compromising the quality of care.\cite{6} As shown in Table 2, different subtyping classifications have been used by the authors. We recommend an international consensus on BCC subtyping that should subsequently be used for future research. This consensus should focus on the most clinically relevant differentiation between sBCC, nBCC and aBCC as proposed by Rippey.\cite{12} Our findings are relevant to current standard care in diagnosing and adequately treating the increasing numbers of BCCs. Accurate subtyping of BCC remains imperative both in determining adequate preoperative surgical margins for nBCC and aBCC, and in increasing nonsurgical treatment success rates for sBCC. Our results indicate that aBCC is missed by a punch biopsy in up to 15% of cases. This means that a punch biopsy fails to diagnose an aBCC in up to one out of six BCCs.

More and more studies are investigating the diagnostic accuracy of clinical diagnosis, as well as upcoming noninvasive imaging modalities such as optical coherence tomography, raman spectroscopy and reflectance confocal microscopy (RCM) for BCCs.\cite{8,17,18} In addition, our research group is currently conducting a multicentre randomized controlled trial to compare punch biopsy with one-stop-shop RCM imaging in diagnosing and subtyping BCC.\cite{19} We caution on using a punch biopsy as the reference standard in subtyping BCC, both in daily practice and in future clinical research.

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None.

CONFLICTS OF INTEREST

None declared.
REFERENCES

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IN VIVO CONFOCAL MICROSCOPY OF BASAL CELL CARCINOMA: A SYSTEMATIC REVIEW OF DIAGNOSTIC ACCURACY
ABSTRACT

Basal cell carcinoma (BCC) is the most prevalent type of skin cancer. Histologic analysis of punch biopsy or direct excision specimen is used to confirm clinical diagnosis. In vivo reflectance confocal microscopy (RCM) is a non-invasive imaging modality that could facilitate early diagnosis and minimize unnecessary invasive procedures. We systematically reviewed diagnostic accuracy (sensitivity and specificity) of RCM in diagnosing primary BCCs to judge its usefulness. Eligible studies were reviewed for methodological quality using the QUADAS-2 tool. We used the bivariate random effects model to calculate summary estimates of sensitivity and specificity. Six studies met the selection criteria and were included for analysis. The meta-analysis showed a summary estimate of sensitivity 0.97 (95% CI, 0.90–0.99) and specificity 0.93 (95% CI, 0.88–0.96). All but one of the QUADAS-2 items showed a high or unclear risk of bias with regards to patient selection. RCM may be a promising diagnostic tool, but the limited number of available studies and potential risk of bias of included studies do not allow us to draw firm conclusions. Future accuracy studies should take these limitations into account.
INTRODUCTION
Basal cell carcinoma (BCC) is the most prevalent type of skin cancer predominantly affecting sun-exposed skin of fair-skinned people. Its increasing incidence is a major public health problem.1,2

Histological analysis of punch biopsy remains the gold standard to confirm clinical diagnosis of BCC and to differentiate between subtypes such as superficial (sBCC), nodular (nBCC), micronodular (mnBCC) and infiltrating (iBCC).3 Due to the increasing incidence of BCC and the ongoing development of new non-invasive therapies, other, preferably non-invasive diagnostic methods are needed.

For this purpose, multiple non-invasive imaging modalities have been developed, such as dermoscopy, high-frequency ultrasonography, optical coherence tomography, raman spectroscopy, terahertz pulsed imaging, positron emission tomography, high-resolution magnetic resonance imaging and reflectance confocal microscopy (RCM).4 Of those, only RCM has been proposed as a diagnostic tool with a high sensitivity and specificity by several clinical studies.5

RCM uses a confocal microscope to directly image a skin lesion at high resolution without the need for invasive biopsies.6 It has several potential advantages in comparison to conventional histology. The procedure itself is painless, relatively easy to learn and requires less processing while the resolution of the horizontal (en face) images are equivalent to routine histology.6 Furthermore, by using predefined RCM criteria for BCC, clinically suspected lesions can be evaluated in <10 min, thereby minimizing delay in the diagnostic process. Possible disadvantages include the purchase of a confocal microscope and limited penetration depth of this technique.

Various studies have reported on the diagnostic accuracy of RCM. While preparing a multicenter randomized controlled trial investigating RCM imaging in diagnosing BCC (www.clinicaltrials.gov Identifier: NCT02285790),7 we found that a systematic overview and quality assessment of RCM for diagnosing BCC was missing. Therefore, the objective of this review is to judge the usefulness of RCM in diagnosing primary BCC by summarizing diagnostic accuracy (sensitivity and specificity).

MATERIALS AND METHODS
This systematic review and meta-analysis was conducted according to the published standards for reviews of diagnostic accuracy studies8 and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines.9 Our study protocol has been registered in the PROSPERO database with number: CRD42014010379. Two of the authors, (DK and MS) independently performed all stages of study screening, data extraction and quality assessment. Disagreements were solved by discussion.

Study scope and definition of reference standard
We assessed diagnostic accuracy of RCM for in vivo diagnosis of primary BCC. The reference standard was defined as histopathological confirmation of BCC in either biopsy or excision specimen.
Search strategy
A medical librarian (author JL) searched the following data bases from inception till December 17th 2014: MEDLINE (OVID), EMBASE (OVID), the Cochrane Central Register of Controlled Trials (CENTRAL), complemented with a search of PubMed to find recent studies not yet available in OVID MEDLINE (command: “NOT MEDLINE[sb]”). The search strategy consisted of free-text words and Subject Headings (MeSH in MEDLINE) related to BCC and RCM. We did not apply language restrictions or methodological search filters. Animal studies were safely excluded by using double negation. The search included an iterative process to refine the search strategy through adding search terms as new relevant citations were identified (i.e. via reference and citation checking of relevant trials in Web of Science). We imported and deduplicated the bibliographic records retrieved using the Reference Manager® software (version 12.0).

The search strategy for MEDLINE is shown in Table 1.

Selection criteria
We screened titles and, if available, abstracts of the initially retrieved articles for their potential relevance. Studies were included if (i) RCM (Prototype and Vivascope 1000 or 1500; Lucid

Table 1. Search strategy for MEDLINE. Database(s): Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) 1946 to Present Search Strategy: 2014-12-16

<table>
<thead>
<tr>
<th>#</th>
<th>Searches</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>neoplasms, basal cell/ or exp carcinoma, basal cell/ or carcinoma, basosquamous/</td>
<td>16001</td>
</tr>
<tr>
<td>2</td>
<td>((basal cell or basocellular or baso-cellular or basosquamous or basaloid or basal squamous) adj4 (carcinom* or cancer* or neoplas* or malign* or epitheliom* or nev* or naev* or syndrome* or tumo?r*).tw,kf.</td>
<td>13586</td>
</tr>
<tr>
<td>3</td>
<td>(BCC or BCCs or BSCC or BS SCCs or sBCC or sBCCs or BCNS).tw,kf.</td>
<td>5191</td>
</tr>
<tr>
<td>4</td>
<td>basal?om*.tw,kf.</td>
<td>486</td>
</tr>
<tr>
<td>5</td>
<td>((Jacob* or rodent) adj ulcer*).tw,kf.</td>
<td>160</td>
</tr>
<tr>
<td>6</td>
<td>gorlin.tw,kf.</td>
<td>1043</td>
</tr>
<tr>
<td>7</td>
<td>(NMSC or NMSCs or non-ML).kf.</td>
<td>9</td>
</tr>
<tr>
<td>8</td>
<td>(non-melanoma or non-melanocytic or nonmelanom* or nonmelanocytic).tw,kf.</td>
<td>4335</td>
</tr>
<tr>
<td>9</td>
<td>or/1-8</td>
<td>25484</td>
</tr>
<tr>
<td>10</td>
<td>exp microscopy, confocal/</td>
<td>44894</td>
</tr>
<tr>
<td>11</td>
<td>confocal*.tw,kf.</td>
<td>53686</td>
</tr>
<tr>
<td>12</td>
<td>vivascope.tw,kf.</td>
<td>23</td>
</tr>
<tr>
<td>13</td>
<td>(CLSM or RCM or LCM or LSCM or RLSM).tw,kf.</td>
<td>6756</td>
</tr>
<tr>
<td>14</td>
<td>(laser adj2 micros*).tw,kf.</td>
<td>14568</td>
</tr>
<tr>
<td>15</td>
<td>(laser adj (scan* or imag*)).tw,kf.</td>
<td>15819</td>
</tr>
<tr>
<td>16</td>
<td>(reflectance adj3 microscop*).tw,kf.</td>
<td>718</td>
</tr>
<tr>
<td>17</td>
<td>non-invasive imag*.tw,kf.</td>
<td>2061</td>
</tr>
<tr>
<td>18</td>
<td>or/10-17</td>
<td>87449</td>
</tr>
<tr>
<td>19</td>
<td>9 and 18</td>
<td>187</td>
</tr>
<tr>
<td>20</td>
<td>animals/ not humans/</td>
<td>4004891</td>
</tr>
<tr>
<td>21</td>
<td>19 not 20</td>
<td>182</td>
</tr>
</tbody>
</table>
Technologies, Henrietta, NY, USA) was performed to diagnose BCC; (ii) clinically suspected lesions consisted of primary BCC, regardless of their histological subtype; (iii) histopathologic confirmation of BCC in either biopsy or excision specimen was used as reference standard and (iv) sensitivity and specificity were provided or a 2 x 2 contingency table could be constructed from the results.

Excluded were (i) reviews, editorials, opinions, ex vivo specimen studies or animal model studies; (ii) case reports with <10 BCC lesions studied to avoid small study effects; (iii) duplicate publications; (iv) full text that was not available after consulting medical libraries and/or contacting corresponding author. Reference lists of included studies were checked for additional relevant titles.

**Extraction of data and quality evaluation**

We extracted the following variables: (i) first author; (ii) year of publication; (iii) country; (iv) whether the study was prospective or retrospective; (v) baseline patient characteristics; (vi) number of BCC lesions and patients; (vii) histological subtypes of BCC lesions; (viii) reference standard used; (ix) type of RCM device; (x) RCM criteria used for diagnosing BCC; (xi) level of RCM training of the assessor; (xii) number of true and false positives and the true and false negatives.

Included papers were assessed using the Quality Assessment of Diagnostic Studies (QUADAS-2) tool. This instrument provides an overview of the quality of included studies on four key domains: patient selection, index test, reference standard and flow of patients (through the study and timing of the index test). Each domain is assessed for risk of bias, and the first three domains are also assessed for applicability concerns. In the assessment of the reference standard domain, both skin biopsy and surgical excision specimen were judged as having low risk of bias.

**Statistical analysis and meta-analysis**

We constructed two-by-two tables for each RCM-based diagnosis of BCC against histology from skin biopsy or surgical excision specimen as the reference standard. Study estimates of sensitivity, specificity and 95% confidence intervals were expressed in forest plots. We used the bivariate random-effects model for a meta-analysis of sensitivity and specificity and to create summary receiver operating characteristic (sROC) curves. The sROC curve depicts sensitivity versus specificity and provides information on the overall test performance across various thresholds.

A meta-analysis was performed if at least four studies evaluated BCCs with RCM imaging. In diagnostic test accuracy meta-analyses, heterogeneity is to be expected, mainly due to variation in test performance across thresholds. Therefore, investigating where heterogeneity may come from is considered to be more important than merely testing whether there is heterogeneity or not. We investigated sources of heterogeneity by subgroup analyses for the following variables: (i) study design; (ii) reference standard used; (iii) type of RCM device and (iv) level of RCM training of the assessor. For the subgroup analysis, at least four included studies with the same covariate were required. We used SAS (Version 9.3, SAS Institute, Cary, NC, USA) and Review Manager (Version 5.3; Nordic Cochrane Center, Copenhagen, Denmark) for the analyses.
RESULTS

Search results

A total of 312 potentially relevant titles were identified from the initial literature search. After scanning of all titles and abstracts by both reviewers independently, 57 were selected for full-text review (Fig. 1). Fifty-one studies were excluded as: no BCCs were investigated (n = 2), sensitivity and specificity could not be calculated (n = 15), diagnosed lesions were not primary BCCs (n = 4), or they were narrative review articles (n = 7), editorial articles (n = 2), ex vivo specimen studies (n = 4), case reports with <10 BCC lesions (n = 9) and conference abstracts (n = 8). Six of the eight conference abstracts were subsequently published.12–17 Contacting the authors of the remaining two conference abstracts did not yield potential relevant full text article(s).

Six studies with a total of 331 BCC lesions were finally included in the systematic review.17–22 Study characteristics are shown in Table 2. The male/female ratio and mean age could not be calculated, as it was not specified in all included studies. Most studies were at least partly conducted in Europe (Austria, Italy, Netherlands and Spain). Lucid Inc. (Lucid Technologies) was the manufacturer of the Vivascope 1000 and Vivascope 1500 devices. Of those, Vivascope 1500 is a newer and improved version of the Vivascope 1000. Nori et al. were the only one using a prototype version of the Vivascope 1000 at the Boston, USA location (Wellman Laboratories, Boston, MA, USA). In the two multicenter studies performed by Guitera et al. and Longo et al., data were gathered by different type of RCM devices depending on study location. RCM criteria for diagnosing BCC were

Figure 1. Flowchart summarizing the selection process for studies concerning diagnostic accuracy of RCM for primary BCC.
<table>
<thead>
<tr>
<th>Author</th>
<th>Year of publication (ref)</th>
<th>Country</th>
<th>Study design</th>
<th># patients (M/F)</th>
<th>Age of patients, y</th>
<th>Reference standard</th>
<th>RCM device</th>
<th>Training level</th>
<th>Sens (%)</th>
<th>Spec (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castro et al.</td>
<td>2014 (22)</td>
<td>Brazil &amp; USA</td>
<td>CS Prosp</td>
<td>32 (30/12)</td>
<td>Mean 65</td>
<td>Hist (biopsy)</td>
<td>Vivascope 1500</td>
<td>High</td>
<td>100</td>
<td>78</td>
<td>96</td>
<td>100</td>
</tr>
<tr>
<td>Gerger et al.</td>
<td>2006 (19)</td>
<td>Austria</td>
<td>CS Prosp</td>
<td>119 (62/57)</td>
<td>Unk</td>
<td>Clin &amp; Hist (excision)</td>
<td>Vivascope 1000</td>
<td>Low</td>
<td>96.7</td>
<td>96.7</td>
<td>96.7</td>
<td>96.7</td>
</tr>
<tr>
<td>Guitera et al.</td>
<td>2012 (20)</td>
<td>Australia &amp; Italy</td>
<td>CS Prosp</td>
<td>663 (354/309)</td>
<td>Median 53</td>
<td>Hist (excision)</td>
<td>Vivascope 1000 &amp; 1500</td>
<td>High</td>
<td>100</td>
<td>88.5</td>
<td>58.4</td>
<td>100</td>
</tr>
<tr>
<td>Longo et al.</td>
<td>2013 (17)</td>
<td>Italy</td>
<td>CS Retro</td>
<td>140 (64/76)</td>
<td>Mean 50</td>
<td>Hist (not specified)</td>
<td>Vivascope 1000 &amp; 1500</td>
<td>High</td>
<td>96.4</td>
<td>97.3</td>
<td>90</td>
<td>99.1</td>
</tr>
<tr>
<td>Nori et al.</td>
<td>2004 (18)</td>
<td>USA &amp; Spain</td>
<td>CS Retro</td>
<td>145 (Unk)</td>
<td>Unk</td>
<td>Clin &amp; Hist (biopsy)</td>
<td>Vivascope 1000 &amp; prototype Boston USA</td>
<td>Low</td>
<td>82.9</td>
<td>95.7</td>
<td>95.5</td>
<td>82.5</td>
</tr>
<tr>
<td>Peppelman et al.</td>
<td>2013 (21)</td>
<td>Netherlands</td>
<td>CS Prosp</td>
<td>27 (16/11)</td>
<td>Mean 66</td>
<td>Hist (biopsy)</td>
<td>Vivascope 1500 Not specified</td>
<td>95.4</td>
<td>78.6</td>
<td>93.2</td>
<td>84.6</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Clin; clinical judgement, CS Prosp; prospective case study, CS Retro; retrospective case study, F; female, Hist; histopathology, iBCC; infiltrative basal cell carcinoma, M; male, mBCC; mixed type basal cell carcinoma, nBCC; nodular basal cell carcinoma, NPV; negative predictive value, PPV; positive predictive value, RCM; reflectance confocal microscopy, sBCC; superficial basal cell carcinoma, Unk; unknown, y; year
not standardized. We found that the level of training of the assessors that performed the on-site evaluation of the RCM imaging varied and was not specified by Peppelman et al. An overview of RCM criteria for diagnosing BCC that were used in the included studies is shown in Table 3.

Quality assessment of study reports

Table 4 summarizes the results of the quality assessment. Of the six included studies, none had a case–control design and two were performed retrospectively. Longo et al. retrospectively included nodular lesions of which histopathological examination was available. In the other studies, selection process was poorly described. Guitera et al. was the only study with a low risk of patient selection bias. In their study, they used a test set and a training set in order to assess diagnostic accuracy of RCM for BCCs. Although the process of patient selection was clearly documented, it was not specified in which way both sets of RCM images were created.

Castro et al., Longo et al. and Peppelman et al. excluded lesions that were not evaluable with RCM (due to localization or presence of hyperkeratosis) or were diagnosed as non-specific. This may have led to an overestimation of specificity.

The conduct and interpretation of either index test or reference standard had a high or unclear risk in most of the studies (4/6). Ideally, histopathologic assessment of excision specimen should serve as the reference standard. This was only implemented by Guitera et al. Not all of the included skin lesions in Nori et al. and Gerger et al. were histopathological examined. Studies that diagnosed

Table 3. Overview of RCM criteria that were used by the included studies for diagnosing BCC

<table>
<thead>
<tr>
<th>Author</th>
<th>Year of publication (ref)</th>
<th>RCM criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castro et al.</td>
<td>2014 (22)</td>
<td>presence of inflammatory cells (IF), increased vascularization (IV), peripheral palisading (PP), thickened collagen bundles (TC), tumor nest surrounded by peritumoral dark spaces (TN)</td>
</tr>
<tr>
<td>Gerger et al.</td>
<td>2006 (19)</td>
<td>elongation of nuclei along same axis (EN), presence of inflammatory cells (IF), increased vascularization (IV), pleomorphism of overlying epidermis (PE)</td>
</tr>
<tr>
<td>Guitera et al.</td>
<td>2012 (20)</td>
<td>cauliflower architecture (CA), increased vascularization (IV), keratinocyte atypia with spongiosis (KA), non-visible papillae (NP), tumor nest surrounded by peritumoral dark spaces (TN)</td>
</tr>
<tr>
<td>Longo et al.</td>
<td>2013 (17)</td>
<td>bright filaments within tumor nest (BF), cauliflower architecture (CA), solar elastosis (SE), tumor nest surrounded by peritumoral dark spaces (TN)</td>
</tr>
<tr>
<td>Nori et al.</td>
<td>2004 (18)</td>
<td>elongation of nuclei along same axis (EN), presence of inflammatory cells (IF), increased vascularization (IV), pleomorphism of overlying epidermis (PE)</td>
</tr>
<tr>
<td>Peppelman et al.</td>
<td>2013 (21)</td>
<td>elongation of nuclei along same axis (EN), presence of inflammatory cells (IF), increased vascularization (IV), keratinocyte atypia with spongiosis (KA), leukocyte rolling (LR), peripheral palisading (PP), solar elastosis (SE), tumor nest surrounded by peritumoral dark spaces (TN)</td>
</tr>
</tbody>
</table>
lesions on purely clinical criteria were judged as having a high risk of bias, though it is understandable that it is not feasible to excise all included clinical benign lesions.

There were applicability concerns with regards to patient selection in half of the studies (3/6). In Gerger et al. patients with clinical suspected lesions for skin cancer (i.e. melanoma lesions) were also included in the analysis, not only clinically suspected BCC lesions. Therefore, it could have been easier to correctly diagnose a BCC. The same applied to Guitera et al. and Longo et al.

There were no applicability concerns with respect to the index test or reference standard. In Castro et al., Nori et al., Peppelman et al. and Gerger et al., the time interval between index and reference test was not specified.

### Diagnostic accuracy of reflectance confocal microscopy and meta-analysis

The distribution of sensitivity and specificity is presented in Fig. 2. Sensitivity ranged from 83% to 100% and specificity ranged from 78% to 97%. The positive predictive value (PPV) and negative predictive value (NPV) ranged from 58% to 97% and from 83% to 100% respectively. The meta-analysis results included six studies and showed a summary estimate of sensitivity 0.97 (95% CI, 0.90–0.99), and specificity 0.93 (95% CI, 0.88–0.96).17–22 (Fig. 3). There were several reasons to refrain from doing a meta-analysis. At the same time, the meta-analysis does give an overall impression of the estimated sensitivity and specificity for any confocal microscope in any situation. Furthermore, although the studies used different tools in different settings, their actual point estimates of sensitivity and specificity show what is called in diagnostic research a ‘threshold effect’: modalities with higher sensitivity also have a lower specificity.

We chose to report results of the meta-analysis but with its limitations and caution against variation and potential biases. These sources of bias include variation in (i) study design; (ii) reference standard used; (iii) type of RCM device and (iv) level of RCM training of the assessor. The summary ROC curve in Fig. 3 describes that effect, which accounts for most of the heterogeneity in these studies. In addition, by using a random effects method, between-study variation was taken into account. We were not able to statistically analyze sources of heterogeneity, as subgroups were too small (two or three studies per group).
IN VIVO CONFOCAL MICROSCOPY OF BASAL CELL CARCINOMA: A SYSTEMATIC REVIEW OF DIAGNOSTIC ACCURACY

Figure 2. Forest plots of sensitivity and specificity of RCM for diagnosing BCC. Squares represent sensitivity and specificity of one study, the black line its 95% confidence interval. FN, false negative; FP, false positive; TN, true negative; TP, true positive.

Figure 3. Summary ROC plot of sensitivity (Y-axis) vs. specificity (X-axis) of RCM for diagnosing BCC. Points represent sensitivity and specificity of one study. The size of the points is proportional to the study sample size. The solid line shows the summary ROC curve. The black point represents the summary estimate.

DISCUSSION

RCM has been proposed as an accurate tool for non-invasive real-time diagnosis of BCC. Our meta-analysis yielded a summary sensitivity of 97% (95% CI, 0.90–0.99) and a summary specificity of 93% (95% CI, 0.88–0.96). Despite of the seemingly high sensitivity and specificity, we caution that our meta-analysis was based on only six studies. Moreover, these studies were clinically heterogeneous and suffered from high risk of bias, meaning that there is still considerable uncertainty in the clinical applicability of these results. Furthermore, we were not able to statistically analyze sources of heterogeneity, as subgroups were too small.

In this systematic review, we compared diagnostic accuracy of RCM with conventional skin biopsy or surgical excision specimen for the diagnosis of primary BCC. Sensitivity ranged from 83%
to 100% and specificity ranged from 78% to 97%. This might be attributed to the different RCM criteria and RCM devices that have been used, as well as two different reference standards (biopsy vs. excision specimen) and various forms of bias. These potential sources of heterogeneity make it challenging to compare the accuracy of the included studies.

Relevance to clinical practice
Our findings may be relevant to the increasing number of patients suffering from BCC. By using a hypothetical cohort of 1000 people, absolute numbers of true positives, false positives, true negatives and false negatives can be estimated. Based on current literature, the expected prevalence of a primary BCC in the Netherlands is 1.4%. This means that 14 of the hypothetical 1000 people would have a primary BCC lesion. By using RCM as diagnostic tool with a sensitivity of 97% and a specificity of 93%, none of the 14 BCCs would be missed, and overtreatment would occur in 69 of 986 patients without BCC (Fig. 4). In vivo RCM might potentially become useful in clinical practice to non-invasively detect BCC. However, in order to be able to replace histologic assessment of skin biopsy specimen as current standard care, RCM should also be able to accurately distinguish between BCC subtypes. This is particularly relevant since BCC treatment requires different approaches according to histological growth patterns. Unfortunately, we were not able to generate sensitivity or specificity values for BCC subtypes. To date, only two studies have reported

Figure 4. Consequences of RCM imaging for diagnosing BCC in a hypothetical cohort of 1000 patients. The use of this test will, on average, mean that of 83 patients treated for BCC, 69 do not have a BCC, and of 917 patients that will not be treated for BCC, none should have been treated after all.
on RCM features correlating with superficial, nodular, micronodular and infiltrative growth patterns.\textsuperscript{21,24} Larger prospective studies are needed to confirm these results. If RCM would be able to correctly divide between subtypes, it might save time and avoid unpleasant invasive procedures for patients with BCC. In theory, this would also imply that for selected cases of superficial BCCs, both diagnosis and treatment could be non-invasively achieved.

**Strengths and limitations**

The strengths of our review include the use of published standards for reviews of diagnostic accuracy studies and the PRISMA guidelines, a comprehensive search strategy by an experienced clinical librarian, rigorous examination of the evidence by two independent reviewers for all stages of review process and quality assessment using QUADAS-2 tool.

Some limitations need to be carefully considered when interpreting the results. First, a relatively small number of studies were included in this study. Second, the predefined reference standard consisted of both punch biopsy specimen and excision specimen. Ideally, only histologic assessment of excision specimen should serve as the reference standard. Thirdly, selection of included patients was poorly described. Fourthly, adequate comparisons between accuracy of specific RCM criteria could not be made since different sets of RCM criteria were used. We recommend an international consensus on RCM criteria for diagnosing BCC that should subsequently be used for future research. Fifthly, both RCM devices as the training level of the assessors varied between studies. With regards to training level, we suggest to report the assessors’ RCM experience in years, number of lesions assessed and/or attended courses.

**Future research**

Future studies evaluating diagnostic accuracy of confocal microscopy in patients with BCC are expected. We recommend using study designs according to the STARD guidelines for reporting RCM diagnostic accuracy studies.\textsuperscript{25} Besides, histologic assessment of excision specimen should serve as the reference standard. We also recommend using the same RCM system with similar software and standardizing RCM imaging protocols in order to improve upcoming research in this area of expertise. Furthermore, the level of RCM training of the assessors should be similar to ensure comparable outcomes. Additional studies on the accuracy of RCM for BCC subtyping should be performed. As diagnostic technique for BCC, RCM could also be compared with other non-invasive techniques. Additionally, cost-effectiveness of \textit{in vivo} RCM imaging of BCC vs. current standard should be explored.

**CONCLUSION**

In conclusion, RCM may be a promising diagnostic tool, but the limited amount of available studies and potential risk of bias of included studies do not allow us to draw firm conclusions.

RCM imaging for diagnosing BCC should use standardized criteria and should be performed by trained people. High-quality prospective studies are needed to investigate diagnostic accuracy of RCM for diagnosing BCC using consecutive study design and standardized RCM criteria.
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CONFLICTS OF INTEREST

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TREATMENT OF BASAL CELL CARCINOMA USING A ONE-STOP-SHOP WITH REFLECTANCE CONFOCAL MICROSCOPY: STUDY DESIGN AND PROTOCOL OF A RANDOMIZED CONTROLLED MULTICENTER TRIAL
ABSTRACT

Background
Basal cell carcinoma (BCC) is the most common cancer diagnosed in white populations worldwide. The rising incidence of BCC is becoming a major worldwide public health problem. Therefore, there is a need for more efficient management.

Objective
The aim of this research is to assess the efficacy and safety of a one-stop-shop (OSS) concept, using real-time in vivo reflectance confocal microscopy (RCM) (Vivascope 1500; Lucid Technologies, Henrietta, NY, USA) as a diagnostic tool, prior to surgical management of new primary BCCs.

Methods
This is a prospective non-inferiority multicenter RCT designed to compare the “OSS concept using RCM” to current standards of care in diagnosing and treating clinically suspected BCC. Patients ≥ 18 years attending our outpatient clinic at the Department of Dermatology, Academic Medical Center, University of Amsterdam, and the Department of Dermatology, the Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital (Amsterdam, The Netherlands) with a clinically suspected new primary BCC lesion will be considered for enrollment using predefined inclusion and exclusion criteria, and will be randomly allocated to the experimental or control group. The main outcome parameter is the assessment of incomplete surgical excision margins on the final pathology report of confirmed BCC lesions (either by punch biopsy or RCM imaging). Other outcome measures include diagnostic accuracy (sensitivity and specificity) of RCM for diagnosing BCC and dividing between subtypes, and throughput time. Patient satisfaction data will be collected postoperatively after 3 months during routine follow-up.

Results
This research is investigator-initiated and received ethics approval. Patient recruitment started in February 2015, and we expect all study-related activities to be completed by fall 2015.

Conclusions
This RCT is the first to examine an OSS concept using RCM for diagnosing and treating clinically suspected BCC lesions. Results of this research are expected to have applications in evidence-based practice for the increasing number of patients suffering from BCC and possibly lead to a more efficient disease management strategy.

Trial Registration
### INTRODUCTION

#### Basal Cell Carcinoma

Basal cell carcinoma (BCC) is the most common cancer diagnosed in white populations worldwide. The rising incidence of BCC is becoming a major worldwide public health concern. Between 1973 and 2009, the European standardized rate quadrupled from 40 to 165 per 100,000 person-years for men and from 34 to 157 for women, most likely because of more intensive UV exposure. This is supported by previous published epidemiological literature indicating that ultraviolet radiation is an important risk factor for BCC, with a significant increase among outdoor workers. Despite the low mortality from BCC, multiple and recurring tumors confer a high morbidity and considerable burden for health care providers and health budgets. Although BCC does not seem to have a strong effect on patients’ quality of life, patients suffering from BCC are definitely interested in efficacy, low recurrence rates, and cosmetic outcomes of their treatment. Meanwhile, resources available at hospitals have not increased proportionally, and therefore, optimizing the effectiveness of present treatment modalities in daily dermatologic practice is necessary.

Clinical BCCs are characterized by small, translucent, or pearly papules, with raised telangiectatic edges. Most BCC occur in sun-exposed skin of the head and neck areas. Sensitivity and positive predictive value of the clinical diagnosis of BCC by dermatologists have been reported to be 95.4% and 85.9%, respectively. However, dividing between BCC subtypes is not always possible upon clinical assessment. To date, histological analysis of punch biopsy remains the gold standard to confirm the clinical diagnosis of BCCs and divide between the following subtypes: superficial (sBCC), nodular (nBCC), micro nodular (mnBCC) and infiltrating (iBCC). Of those, nBCC and sBCC have a less aggressive growth pattern in comparison to mnBCC and iBCC. Additionally, mixed type BCC (mBCC) can be defined as a combination of subtypes and is frequently composed of aggressive subtypes. Surgical excision remains the standard of treatment, with Mohs’ micrographic surgery typically utilized for high-risk lesions. Based upon the histological growth pattern, BCC are surgically removed with a margin of either 3 mm (nBCC and sBCC) or 5 mm (mnBCC and iBCC) in accordance with current Dutch guidelines.

#### Reflectance Confocal Microscopy

The use of real-time in vivo reflectance confocal microscopy (RCM) has proven successful to noninvasively diagnose BCC. Various studies have demonstrated that RCM is safe and accurate (sensitivity and specificity) to diagnose BCC. Reported sensitivity and specificity for RCM in diagnosing BCC range from 83%-100% and 79%-97%, respectively. Furthermore, Peppelman et al and Longo et al recently reported on RCM features that might divide between nodular, micronodular, superficial, and infiltrative subtypes of BCC.

#### One-Stop-Shop

In 2012, van der Geer et al reported on the feasibility of a one-stop-shop (OSS) concept for the treatment of skin cancer patients. One-stop-shop implies that on the day of the initial outpatient
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4.1

Aims and Objectives

The aim of our study is to assess the efficacy and safety of the OSS concept, using real-time in vivo RCM (Vivascope 1500; Lucid Technologies, Henrietta, NY, USA) as a diagnostic tool, prior to the surgical management of new primary BCC, of all subtypes, in the general population. We hypothesize that compared to current standards of care, the OSS concept using RCM will not result in a significant increase of incomplete surgical excision margins on the final pathology report of confirmed BCC lesions. It is further hypothesized that in this OSS concept, RCM will have acceptable diagnostic accuracy (sensitivity and specificity) for diagnosing BCC and dividing between subtypes, throughput time will not increase, and patient satisfaction will be higher for participating subjects.

METHODS

Recruitment, Screening, and Enrollment

Patients will be recruited from the outpatient clinics of the Department of Dermatology, Academic Medical Center, University of Amsterdam (AMC), and the Department of Dermatology, the Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital (AVL), tertiary reference centers. Consecutive patients with clinically suspected new primary BCC will be prospectively enrolled and randomly assigned to either the experimental (RCM-OSS) or control (standard care) group during times the study associates will be available. Clinical assessment will be performed by an experienced, board-certified dermatologist. Clinical and dermoscopy pictures of the BCC lesion will be taken by a medical photographer. Patients with multiple clinically suspected new primary BCC lesions will be included for only the lesion most suitable for conventional surgical treatment according to the following order: 1 chest, 2 extremities, and 3 head and neck area.

The inclusion criteria are the following:

1. patient with clinically suspected new primary BCC as assessed by an experienced board certified dermatologist, 2 age ≥18, 3 patient is willing and able to give written informed consent, 4 BCC lesion is suitable for conventional surgical excision under local anesthetics, and 5 BCC lesion has been present for at least 1 month.

2. The exclusion criteria are the following: 1 BCC lesion in a high-risk location of the face (H-zone and ears), 2 contra-indication for conventional surgical excision (primary surgical closure seems not achievable), 3 recurrent BCC lesion (BCC that has been previously unsuccessfully treated), 4 macroscopic ulcerating BCC lesions (not feasible for RCM analysis due to technical reasons), 5 patient with basal cell nevus syndrome, 6 patient treated with hedgehog inhibitor medication, 7 patient with a history of hypersensitivity and/or allergy to local anesthesia, 8 patient unavailable...
in the following 6 weeks (for example due to holidays or sports), and patient not able to understand the procedures involved.

The investigators will enroll subjects at both study locations (AMC and AVL). Included patients with clinically suspected new primary BCC lesions will be randomly allocated to the different diagnostic procedures. The investigators will obtain the patient’s consent. Each consecutive patient will be assigned a randomization number according to a computer-generated randomization list (ALEA) using random block sizes of 2, 4, 6, and 8 to ensure treatment concealment. Randomization will take place between the control and experimental group. This study will have an open label set-up. The patient and local investigator will not be blinded.

The randomization will be blinded. The pathologists analyzing the final excision specimen will be blinded to the patient’s history and to the results of RCM imaging. Whenever the histology of the punch biopsy is not required in the diagnostic process of the final excision specimen, the pathologist will also be blinded for those results. After initial RCM diagnosis by the study associates (DK and YE), two independent outcome assessors (M. Ulrich, Charite Berlin in Germany and C. Longo, Modena and Reggio Emilia in Italy) analyzing the RCM images will be blinded to the patient’s history and to the results of the final pathology report (reference standard).

We chose a cutoff of 95% as an acceptable radical BCC excision rate with standard care based on our experience. Using the Miettinen and Nurminen confidence interval around the risk difference, with two groups of 38 patients, we will have 80% power to assess noninferiority of the OSS concept with RCM over usual care, considering an expected radical BCC excision rate of 95% in both arms, a noninferiority limit (delta) of 15%, and a one-sided alpha of 0.05.

**Outcome Measures**

Incomplete surgical excision on the final pathology report of a routinely processed tissue specimen of confirmed BCC lesions (either by punch biopsy or RCM imaging) is the main outcome parameter. Assessment will be performed by an experienced board-certified pathologist. The number of incomplete excisions will be compared between the experimental and control group. Other assessments of included subjects with confirmed BCC lesions (either by punch biopsy or RCM imaging) will include the following:

1. Diagnostic accuracy (sensitivity and specificity) of the RCM for BCC diagnosing and subtyping will be separately analyzed by comparing RCM diagnosis and subtype with final pathology reports of the experimental group. This will be performed by using unidentifiable saved RCM images of all included lesions of the experimental group to analyze inter- and intra-rater variability in the interpretation of RCM imaging. The study associates (DK and YE) and two independent outcome assessors (MU and CL) will be blinded to the patient’s history and to the results of the final pathology report (reference standard).

2. Throughput time will be assessed by the study associates and compared between the experimental and control group.
3. Patient satisfaction will be assessed postoperatively 12 weeks after excision by using a standardized web-based questionnaire for patient reported outcomes in the management of skin diseases. An adjusted version of this web-based questionnaire has previously been published to assess patient satisfaction among patients suffering from psoriasis. The outcome of the questionnaire will be compared between the experimental and control group.

4. The frequency of and reasons for exclusions will be documented.

5. The frequency of interpretable, indeterminate, and intermediate tests will be documented.

6. Adverse events during the procedure will be documented.

**Study Procedures**

BCCs will be divided into 5 main subtypes based on the histopathological growth pattern of the final excision specimen: superficial (sBCC), nodular (nBCC), micronodular (mnBCC), infiltrating (iBCC), and basosquamous (bBCC). In the case of mixed-type diagnosis, defined as two or more single growth patterns, the histology will be classified into single subgroups determined by the most aggressive component of the pathological feature according to the descending gradation from bBCC, iBCC, mnBCC, nBCC, to sBCC. The most aggressive component will determine the excision margin (5 mm versus 3 mm).

After obtaining written informed consent, the screening will be completed. Patients with clinically suspected new primary BCC lesions will be randomly allocated to the following regimes:

1. **Experimental group (N=38):** Clinically suspected new primary BCC lesions will be diagnosed and divided into subtypes using RCM imaging (Vivascope 1500; Lucid Technologies, Henrietta, NY, USA) according to a standardized protocol (Table 1). After diagnosis, excision of BCC lesions with adequate margins will be performed on the same day at the Department of Dermatology according to the one-stop-shop concept. Clinically suspected primary BCCs that are not confirmed by RCM will also receive surgical treatment with a margin of 3 mm.

2. **Control group (N=38):** Clinically suspected new primary BCC lesions will be diagnosed and divided into subtypes according to current standards of care. A conventional 3 mm punch biopsy will be performed in the most elevated part of the lesion using local anesthetics (1% xylocaine/adrenaline). A biopsy specimen will be analyzed by a pathologist (within 2 weeks). After diagnosis, excision of the BCC lesions with adequate margins will be performed within the following 4 weeks according to current standards of care. Clinically suspected primary BCCs that are not confirmed by punch biopsy will also receive surgical treatment with a margin of 3 mm.

The study design incorporated five parts. First, screening took place. Second, intake involved the following steps: written informed consent, intake, randomization, and photo documentation. Third, allocation to the experimental or control group consisted of assessment of diagnosis and subtyping of clinically suspected new primary BCC, and assessment of surgical margins.
Fourth, surgical excision of the lesion took place: the excised surgical specimen was assessed by the pathologist and an assessment of throughput time was conducted. Finally, a routine 12-week postoperative control visit was conducted, involving an assessment of patient satisfaction using the web-based questionnaire.

**Data Analysis**

Data will be recorded on data entry forms and will be entered in a computer system for subsequent tabulation and statistical analysis. The data will be handled confidentially and anonymously. Furthermore, all information relevant to the treatment will be recorded in the electronic medical file.

All data will be collected and transferred to a Microsoft Excel database. The statistical analysis will be performed at the AMC using SPSS version 21.0. We will calculate the observed difference as the proportion of radical BCC excisions in the care-as-usual arm minus this proportion in the OSS with RCM arm, and calculate a one-sided 95% (or two-sided 90%) confidence interval for this difference using the Miettinen and Nurminen method. The inferiority hypothesis will be rejected when the upper limit of this confidence interval does not exceed 15%. Side effects will be described per item.

**RESULTS**

This is an investigator-initiated unfunded prospective open-label noninferiority randomized controlled multicenter trial. Development of the project commenced in fall 2012, and the study protocol has been approved by the ethics committee at the coordinating center (AMC, METC 2014_244) and by the local Institutional Review Board at the participating center (AVL) in fall 2014. This trial has also been registered publically at ClinicalTrials.gov (identification number: NCT02285790). Patient recruitment started in February 2015, and the expected date of completion is fall 2015.

The study is being conducted according to the principles of the Declaration of Helsinki (Fortaleza, Brazil, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO) and other relevant guidelines, regulations, and acts.

**DISCUSSION**

BCC is the most prevalent skin cancer, and its prevalence is increasing. Histological analysis of punch biopsy remains the gold standard to confirm a clinical diagnosis of BCC and dividing subtypes. However, due to the rising incidence of BCC, there is a need for more efficient, noninvasive methods of diagnosis. Incorporating RCM as a noninvasive diagnostic tool in a BCC OSS concept for lesions suitable for conventional surgical excision, in concordance with current Dutch guidelines, might reduce time between clinical diagnosis and treatment, administrative workload, and costs. Surgical treatment of BCC is generally performed under local anesthesia, which makes it suitable for an OSS approach.
Subjects participating in the study will be informed and will have to provide written informed consent prior to enrollment. Study participation will not result in additional follow-up visits other than clinically required 3 months postoperatively.

Real-time in vivo RCM uses a confocal microscope to noninvasively image a thin surface of the skin at high resolution directly without the need for invasive biopsies. The diagnostic procedure itself is painless and no side effects have been reported. Outcome measures involve routinely processed surgical specimens after excision, patient satisfaction, calculation of throughput time, and analyzing diagnostic accuracy of the RCM procedure in subtyping BCC lesions. The overall burden of the study is minimal. A possible inconvenience for participating patients in the experimental group is that specific features for BCC subtyping are still being established. Therefore, a potential side effect for those patients may be less accurate subtyping of BCCs resulting in less adequate surgical margins. At the same time, RCM imaging may be of additional value in scanning the complete lesion, which potentially could prevent missing a more aggressive part of a tumor in contrast to a biopsy.

Thus, there is a potential benefit for the participating subject, namely noninvasive confirmation of clinically suspected BCC lesions followed by direct surgical treatment. Considering the relatively quick and simple procedure, noninvasiveness of the diagnostic method, and the one-stop-shop concept of diagnosing and treating BCC at the same consultation, the balance between burden, possible side effects, and prospect for improvement might be very favorable.

This RCT is the first to examine an OSS concept using RCM for diagnosing and treating clinically suspected BCC lesions. Results of this research are expected to have applications in evidence-based practice for the increasing number of patients suffering from BCC, and possibly lead to a more efficient disease management strategy.

**CONFLICTS OF INTEREST**

None declared.
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ONE-STOP-SHOP WITH CONFOCAL MICROSCOPY IMAGING VERSUS STANDARD CARE FOR SURGICAL TREATMENT OF BASAL CELL CARCINOMA: AN OPEN LABEL, NON-INFERIORITY, RANDOMIZED CONTROLLED MULTICENTER TRIAL
SUMMARY

Background
Routine punch biopsies are considered as the standard care to diagnose and subtype basal cell carcinoma (BCC) when clinically suspected.

Objectives
We assessed the efficacy of a one-stop-shop concept using in vivo reflectance confocal microscopy imaging (RCM) as diagnostic tool versus standard care for surgical treatment in patients with clinically suspected BCC.

Methods
In this open-label, parallel-group, non-inferiority, randomized controlled multicenter trial we enrolled patients with clinically suspected BCC at two tertiary referral centers in Amsterdam, the Netherlands. Patients were randomly assigned to the RCM one-stop-shop (diagnosing and subtyping using RCM followed by direct surgical excision) or standard care (planned excision based upon the histological diagnosis and subtype of a punch biopsy). The primary outcome was the proportion of patients with tumour-free margins after surgical excision of BCC.

Results
Of the 95 included patients, 73 (77%) had a BCC histologically confirmed on surgical excision specimen. All (40/40, 100%) in the one-stop-shop group had tumor free margins. In the standard care group tumor free margins were found in all but two (31/33, 94%). The difference in the proportion of patients with tumor-free margins after BCC excision between the one-stop-shop group and the standard care group was -0.06 (90% confidence interval -0.17 to 0.01), establishing non-inferiority.

Conclusions
The proposed new treatment strategy seems suitable in facilitating early diagnosis and direct treatment for patients suffering from BCC, depending on factors such as availability of RCM, size and site of the lesion, patient preference and whether direct surgical excision is feasible.

Trial Registration
This trial is registered with the Netherlands Trial Register (NTR5305), and with ClinicalTrials.gov (NCT02285790)
INTRODUCTION

Basal cell carcinoma (BCC) is the most common type of skin cancer in white populations worldwide. Numerous studies have reported on the rising incidence of BCC causing a major burden on current health care systems.¹-⁴ Punch biopsies are considered as standard care not only to diagnose BCC when clinically suspected, but also to establish the histological subtype (superficial, nodular or aggressive) and subsequent excision margin.⁵ However, studies identified in our previous systematic review have reported that a punch biopsy does not accurately diagnose the most aggressive BCC subtype in a substantial number of cases; sensitivity ranged from 61% to 85% and specificity ranged from 79% to 88%.⁶ Besides non-invasive imaging devices have been developed that might revolutionize the diagnosis of the increasing numbers of skin cancer.⁷ Of those non-invasive imaging modalities, real-time in vivo reflectance confocal microscopy (RCM) has shown to be a promising technique for diagnosing and subtyping BCC.⁸

In 2012, van der Geer et al. presented a new disease management strategy for patients suffering from BCC consisting of a one-stop-shop concept with preoperative frozen section histology to confirm BCC diagnosis and divide in subtypes.⁹ One-stop-shop implies that on the day of the initial outpatient clinic consultation, diagnosis and treatment both take place.

We assessed the efficacy of a one-stop-shop concept using RCM as diagnostic tool versus standard care for surgical treatment in patients with clinically suspected BCC.

PATIENTS AND METHODS

Study design and participants

We performed this open-label, parallel-group, non-inferiority, randomized controlled multicenter trial at the Department of Dermatology, Academic Medical Center, University of Amsterdam (coordinating tertiary referral center), and the Department of Dermatology, the Netherlands Cancer Institute (participating tertiary referral center), in Amsterdam, the Netherlands. The study protocol has previously been published.¹⁰ Consecutive eligible patients of 18 years and older with a clinically suspected, primary, untreated BCC, regardless of subtype and present for at least one month were prospectively enrolled. We excluded patients with lesions not suitable for conventional surgical excision, lesions in a high-risk location of the face (H-zone and ears), lesions larger than 20mm, recurrent BCC, macroscopic ulcerating lesions, and those with basal cell nevus syndrome.

The study was conducted according to the principles of the Declaration of Helsinki (Fortaleza, Brazil, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO). The research protocol has been approved by ethics committees at both centers (NL50112.018.14). All participants gave written informed consent. The trial was registered with the Netherlands Trial Register (NTR5305), and with ClinicalTrials.gov (NCT02285790).

Randomization and masking

Patients were randomly allocated to the ‘one-stop-shop’ or ‘standard care’ treatment. Block randomization (1:1) was performed with random block sizes of 2, 4, 6, and 8. The allocation
sequence was generated through an online computer-based program developed by the Clinical Research Unit of the Academic Medical Center to ensure concealment of allocation. Participants and investigators were not masked for treatment. Masking of the pathologist assessing histology of punch biopsies and/or surgical excision specimen was only partly possible because a punch biopsy scar could be recognized during assessment of excision specimen.

**Procedures**

Initial clinical assessment, most times including dermoscopy, was performed by experienced board-certified dermatologists. The one-stop-shop group received RCM (VivaScope 1500®; CaliberID, Henrietta, NY, U.S.A.; MAVIG GmbH, München, Germany) to diagnose and subtype BCC followed by direct surgical excision. RCM was performed according to the previous published protocol to diagnose clinically suspected BCC and to divide in subtypes. Two clinicians performed RCM, DK at the Academic Medical Center and YE at the Netherlands Cancer Institute.

The standard care group received planned excision after a punch biopsy was performed. The routine 3mm punch biopsy was performed from the most elevated part of the lesion using infiltration anesthesia (2% xylocaine/adrenaline 1:80 000). Biopsy specimen were subsequently analyzed by a pathologist within two weeks. Surgical excision of the lesion with adequate margins was performed within the following four weeks after receiving the report of the punch biopsy. Excision specimens were analyzed by the same pathologist.

BCC were divided into superficial, nodular or aggressive subtypes (micronodular, infiltrating, or basosquamous). The most aggressive histological BCC subtype (as determined by either RCM in the one-stop-shop group or as shown by punch biopsy in the standard care group) determined the surgical excision margin (3mm: superficial and/or nodular versus 5mm: aggressive). An independent dermatologist or independent dermatology resident (supervised by a dermatologist) performed surgery under local anesthetics (2% xylocaine/adrenaline 1:80 000) followed by primary wound closure in both treatment groups.

Assessment of surgical excision specimen was performed by an experienced pathologist. Clinically suspected BCC that were not confirmed by either RCM or a punch biopsy were surgically treated with a 3mm excision margin.

**Outcomes**

The primary outcome was the proportion of patients with tumor-free margins on the final pathology report of routinely processed tissue specimen after surgical treatment of BCC.

Secondary outcomes were the proportion accurately diagnosed BCC by RCM and a punch biopsy including the most aggressive histological subtype, patients’ throughput time, patient satisfaction and adverse events.

The throughput time was defined by the total amount of time spent at our outpatient clinic for diagnosis and subsequent surgical treatment of the clinically suspected BCC, calculated from arrival at consultation until the end of surgical treatment.

Patients with histology proven BCC in surgical excision specimen were seen at three months’ follow-up. A clinical evaluation of the post-operative scar was performed by a dermatologist or
dermatology resident. Patient satisfaction was assessed using an adjusted version of a standardized questionnaire published by Van Cranenburgh et al. (appendix).\(^1\) This questionnaire consists of six questions and uses a five-point Likert scale (very satisfied, satisfied, not satisfied/not unsatisfied, unsatisfied, very unsatisfied) to assess the following domains: effectiveness, safety, convenience, information, doctor–patient communication, organization.

**Statistical analysis**

To test whether the RCM one-stop-shop concept was non-inferior to standard care for surgical treatment of BCC, a pre-specified non-inferiority margin of 15% was used. A sample size of 38 patients with histology proven BCC (excision specimen) per treatment group was needed to establish non-inferiority with a power of 80%, considering an expected tumor-free margin BCC excision rate of 95% in the standard care arm and a one-sided type I error of 5%. We recorded the characteristics of participants at baseline, summarized them for each treatment group with descriptive statistics and tested with the t-test, the \(\chi^2\) test or the Fisher’s exact test. We excluded from the analyses cases in which subsequent surgical excision of the clinically suspected BCC was not performed. Reasons for not performing surgical excision were recorded. For the primary outcome we constructed a score based on 90% confidence interval for the difference in proportions of patients with tumor-free margins after BCC excision between the one-stop-shop group and the standard care group using the PropCIs package in R for the Miettinen and Nurminen method.\(^1\)\(^2\)\(^3\)

The secondary outcomes groups were compared using the Wilcoxon rank sum test, the \(\chi^2\) test or the Fisher’s exact test. The statistical analysis was performed using Stata version 13 software.

**RESULTS**

Figure 1 shows the trial profile. Between February 3, 2015, and October 2, 2015, 100 patients with clinically suspected BCC were randomly assigned to treatment according to the RCM one-stop-shop (\(n=50\)) or standard care (\(n=50\)). The study period, including all follow-ups, ended in August 30, 2016. Baseline characteristics, except skin type, were not significantly different between the two groups (table 1).

95 patients underwent the assigned surgical treatment followed by primary wound closure and were included in the analyses (\(n=48\) in the one-stop-shop group and \(n=47\) in the standard care group). 73 (73/95, 77%) BCC were histologically confirmed on surgical excision specimen, 40 BCC in the one-stop-shop group and 33 in the standard care group (figure 1). Most of the BCC had a superficial or nodular subtype (table 2). Among the 22 other lesions, one melanoma, two squamous cell carcinomas, five Bowen’s diseases and 11 non-malignant lesions were found. In the remaining three lesions, no residual signs of BCC were found after a punch biopsy had been performed. In the one-stop-shop group all 40 (100%) patients suffering from BCC had tumor-free margins after surgical treatment. In the standard care group 31 of 33 (94%) patients suffering from BCC had tumor-free margins after surgical treatment. Of the two BCC with an incomplete surgical excision margin, one had a superficial subtype and the other one had a mixed subtype (superficial/nodular). In both patients with incomplete excisions, clinical follow-up was decided.
The difference in the proportion of patients with tumor-free margins after BCC excision between the one-stop-shop group and the standard care group was -0.06 (90% confidence interval -0.17 to 0.01). As the non-inferiority limit was 0.15, these data demonstrate that the RCM one-stop-shop care is non-inferior to standard care. The confidence limit contains zero, which means that it is not possible to interpret these data as providing support for the superiority of the RCM one-stop-shop care.

The time to perform RCM was recorded in 42 of the 48 clinically suspected lesions in the one-stop-shop group with a mean time of 13 minutes. In 38 of the 43 (88%) BCC diagnosed by RCM, surgical excision specimens confirmed the diagnosis (figure 1). The other 5 of 48 clinically suspected lesions tested negative for BCC presence by RCM. Of those, two BCC were later histologically confirmed by excision specimen, one ulcerating lesion and one lesion with

Figure 1. Flow chart. BCC = basal cell carcinoma; PDT = photodynamic therapy. * Two patients in the one-stop-shop group did not begin diagnosis and treatment. One refused directly after randomization and the other one could not participate due to technical malfunction of the confocal imaging device. ** Three patients in the standard care group did not receive subsequent surgical excision after the punch biopsy. In one patient the protocol was violated after histologic assessment of punch biopsy specimen showed actinic keratosis with no visible signs of the biopsied lesion on the day of surgery. Another patient with a histologically confirmed superficial BCC was mistakenly treated with photodynamic therapy instead of surgery. The last patient with a histologically confirmed BCC developed a large leiomyosarcoma on the same localization. Surgical excision of the BCC was cancelled and the patient was referred to an oncologic surgeon to treat the leiomyosarcoma.
a superficial crust. In 29 of the 38 (76%) correctly diagnosed BCC, RCM identified the most aggressive histological subtype.

In the standard care group (n=48) patients received the diagnosis of the punch biopsy within two weeks after the initial appointment. The time needed for the pathologist to establish the histological diagnosis and subtype of the punch biopsy was not recorded. In 31 of the 34 (91%) BCC diagnosed by a punch biopsy, surgical excision specimen confirmed the diagnosis (figure 1). The other 13 of 47 clinically suspected lesions tested negative for BCC presence by punch biopsy. Of those, two BCC were later histologically confirmed by excision specimen. In 28 of the 31 (90%) correctly diagnosed BCC, a punch biopsy identified the most aggressive histological subtype.

The mean throughput time for patients in the one-stop-shop group receiving direct surgical treatment (n=48) was 2 hours and 23 minutes. In the standard care group (n=47) the mean time that patients spent at our outpatient clinic during the initial visit for the punch biopsy was 1 hour and 15 minutes. The throughput time could not be calculated because the time spent at the hospital during the following visit for surgical treatment was not recorded.

Table 1. Tumour and patient characteristics separated by treatment group

<table>
<thead>
<tr>
<th></th>
<th>One-stop-shop</th>
<th>Standard care</th>
<th>p-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n= 50)</td>
<td>(n= 50)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>64 (39-88)</td>
<td>68 (41-92)</td>
<td>0.101</td>
</tr>
<tr>
<td>Men</td>
<td>31 (62%)</td>
<td>25 (50%)</td>
<td>0.227</td>
</tr>
<tr>
<td>Women</td>
<td>19 (38%)</td>
<td>25 (50%)</td>
<td></td>
</tr>
<tr>
<td>Fitzpatrick skin type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>8 (16%)</td>
<td>4 (8%)</td>
<td>0.037</td>
</tr>
<tr>
<td>II</td>
<td>32 (64%)</td>
<td>43 (86%)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>10 (20%)</td>
<td>3 (6%)</td>
<td></td>
</tr>
<tr>
<td>BCC in medical history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>34 (68%)</td>
<td>37 (74%)</td>
<td>0.610</td>
</tr>
<tr>
<td>no</td>
<td>15 (30%)</td>
<td>13 (26%)</td>
<td></td>
</tr>
<tr>
<td>Study site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Academic Medical Centre</td>
<td>37 (74%)</td>
<td>38 (76%)</td>
<td>0.817</td>
</tr>
<tr>
<td>Netherlands Cancer Institute</td>
<td>13 (26%)</td>
<td>12 (24%)</td>
<td></td>
</tr>
<tr>
<td>Immunocompromised * yes</td>
<td>4 (8%)</td>
<td>4 (8%)</td>
<td>1.000</td>
</tr>
<tr>
<td>no</td>
<td>46 (92%)</td>
<td>46 (92%)</td>
<td></td>
</tr>
<tr>
<td>Tumour diameter (mm)</td>
<td>8 (3-15)</td>
<td>8 (3-20)</td>
<td>0.803</td>
</tr>
<tr>
<td>Tumour location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head/neck</td>
<td>9 (18%)</td>
<td>12 (24%)</td>
<td>0.393</td>
</tr>
<tr>
<td>Trunk</td>
<td>32 (64%)</td>
<td>30 (60%)</td>
<td></td>
</tr>
<tr>
<td>Arm</td>
<td>4 (8%)</td>
<td>7 (14%)</td>
<td></td>
</tr>
<tr>
<td>Leg</td>
<td>5 (10%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
</tbody>
</table>

Continuous variables are expressed as mean (range) and categorical variables as n (%). BCC= basal cell carcinoma. *Patients who were taking immunosuppressive drugs such as oral steroids, methotrexate, ciclosporin for suppression of immunological disorder, or to prevent transplant rejection. **T-test for continuous variables; χ² or Fisher’s exact test for categorical variables.
All patients with a histology proven BCC on surgical excision specimen (*n*=73) were seen at three months’ post-operative follow-up. The mean follow-up time was 18 weeks in the one-stop-shop group (*n*=40) and 21 weeks in the standard care group (*n*=33). The average time between the initial visit and surgical treatment in the standard care group (*n*=33) was almost ten weeks (66 days). Patients in the one-stop-shop group rated treatment convenience significantly higher (five-point Likert scale mean 4·63) in comparison to patients in standard care group (five-point Likert scale mean 4·32) (*p*=0·031) (table 3).

Adverse reactions that were reported in this trial were four patients of the one-stop-shop group (*n*=48) with post-operative wound infections. In all cases the infection was successfully treated with oral antibiotics, without the need of hospitalization. One patient of the standard care group (*n*=50) using anti-coagulant medication developed an excessive post-operative bleeding requiring hospitalization for three days. She fully recovered. This was reported as the only serious adverse event.

### Table 2. Basal cell carcinoma subtypes separated by treatment groups

<table>
<thead>
<tr>
<th>BCC subtype distribution</th>
<th>One-stop-shop (<em>n</em>=40*)</th>
<th>Standard care (<em>n</em>=33*)</th>
<th>p-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superficial BCC</td>
<td>17 (43%)</td>
<td>14 (42%)</td>
<td>0.448</td>
</tr>
<tr>
<td>Nodular BCC</td>
<td>17 (43%)</td>
<td>17 (52%)</td>
<td></td>
</tr>
<tr>
<td>Aggressive BCC</td>
<td>6 (14%)</td>
<td>2 (6%)</td>
<td></td>
</tr>
</tbody>
</table>

Variables are expressed as n (%). BCC= basal-cell carcinoma. ^ This number represent the histologically confirmed basal-cell carcinoma based on surgical excision specimen. * Basal cell carcinoma subtype distribution according to the most aggressive subtype found at histology of surgical excision. ** Fisher’s exact test.

### Table 3. Patient satisfaction scores of patients with histology proven basal cell carcinoma in surgical excision specimen, separated by treatment groups

<table>
<thead>
<tr>
<th></th>
<th>One-stop-shop group (<em>n</em>= 40)</th>
<th>Standard care group (<em>n</em>=33)</th>
<th>p-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effectiveness</td>
<td>4-49 (median 5, range 2-5, n=37)</td>
<td>4-55 (median 5, range 3-5, n=31)</td>
<td>0.761</td>
</tr>
<tr>
<td>Safety</td>
<td>4-65 (median 5, range 3-5, n=37)</td>
<td>4-53 (median 5, range 4-5, n=32)</td>
<td>0.269</td>
</tr>
<tr>
<td>Convenience</td>
<td>4-63 (median 5, range 3-5, n=35)</td>
<td>4-32 (median 4, range 3-5, n=28)</td>
<td>0.031</td>
</tr>
<tr>
<td>Information</td>
<td>4-65 (median 5, range 2-5, n=37)</td>
<td>4-58 (median 5, range 2-5, n=33)</td>
<td>0.582</td>
</tr>
<tr>
<td>Doctor–patient communication</td>
<td>4-57 (median 5, range 2-5, n=37)</td>
<td>4-58 (median 5, range 3-5, n=31)</td>
<td>0.965</td>
</tr>
<tr>
<td>Organization</td>
<td>4-43 (median 5, range 1-5, n=37)</td>
<td>4-48 (median 5, range 3-5, n=33)</td>
<td>0.936</td>
</tr>
</tbody>
</table>

Mean assigned points to each domain ranging from 1 = ‘not satisfied at all’ to 5 = ‘very satisfied’. Convenience is significantly higher in one-stop-shop group in comparison to standard group (median 5 vs 4, respectively). ** Wilcoxon rank sum test.
DISCUSSION

Our findings show that a one-stop-shop concept using RCM was non-inferior to standard care in terms of tumor-free margins after surgical treatment of BCC. These results suggest that a RCM one-stop-shop concept could be considered in clinical dermatology practice as new treatment strategy for primary BCC, evidently depending on factors such as availability of RCM, size and site of the lesion, patient preference and whether direct surgical excision is feasible. This is the first randomized controlled trial that investigates the efficacy of a one-stop-shop concept using RCM skin imaging to diagnose and subtype primary BCC prior to surgical treatment. Current literature indicates that incomplete excision rate numbers after conventional surgical treatment of BCC may vary according to tumor localization, histologic subtype and training of the treating physician.\textsuperscript{14,15} We intentionally aimed at investigating primary BCC smaller than 20 mm at low-risk localization and predefined a 95% tumor free margin rate as acceptable. The exclusion of large BCC and/or high-risk localizations might have positively influenced the proportion of successfully treated patients in both treatment groups.

This study seems relevant to the rising number of BCC seen in clinical dermatology practice. Although BCC has a good prognosis, delay in diagnosis and appropriate treatment may result in extra public health costs.\textsuperscript{16} Non-invasive imaging techniques such as RCM can aid in earlier detection of BCC opposed to painful routine skin biopsies\textsuperscript{17}, even in BCC invisible to the naked eye.\textsuperscript{18} In addition to early diagnosis, another important advantage of the RCM one-stop-shop is direct treatment for patients suffering from BCC. This was supported by our results regarding patient satisfaction. Patients in the one-stop-shop group rated treatment convenience (mean 4.63) significantly higher compared to patients in standard care group (mean 4.32) ($p=0.031$). High patient satisfaction was also reported in the one-stop-shop performed by van der Geer et al. in 2012.\textsuperscript{9} However in their pilot study fresh frozen sections of 4 mm punch biopsies were used to diagnose 16 BCC and divide in subtypes. This resulted in a higher throughput time of 4 hours and 7 minutes compared to our mean throughput time of 2 hours and 23 minutes in the RCM one-stop-shop.

Strengths of this study are the prospective investigator-initiated pragmatic design, combining diagnosis and treatment to an entity and the randomization of these care strategies. By linking diagnostic testing to management actions we assessed how the introduction of RCM impacts current diagnostic pathway for clinically suspected BCC. Such a test-treatment pathway helps mapping out the ideal context in which RCM may be used opposed to standard care.\textsuperscript{19} Study limitations include the amount of excluded participants mostly due to BCC in high-risk areas of the face. The VivaScope 1500 device that was used in our study is not suitable for imaging these specific localizations. However by incorporating the more recently introduced VivaScope 3000\textsuperscript{®} flexible handheld version (VivaScope 3000\textsuperscript{®}; CaliberID, Henrietta, NY, U.S.A.; MAVIG GmbH, München, Germany), accessibility to the more concave and convex high-risk head and neck areas would be possible.\textsuperscript{20}

The misinterpretation of confocal images in our study may have been related to a relatively short RCM experience. Both investigators (DK and YE) had less than one year of experience prior...
to the start of the study. A higher level of RCM experience seems to result in higher diagnostic accuracy.21, 22 This was recently confirmed in a recent retrospective article that compared the inter-observer agreement of specific confocal skin cancer feature recognition between nine dermatologists with varying RCM.23

Unfortunately we were not able to calculate the throughput time in the standard care group as the time taken for surgical treatment on the second visit was not recorded. Another study limitation is the short post-operative follow-up time of three months. A longer clinical follow-up of at least one year would be needed to detect signs of BCC recurrence.

Lastly patient satisfaction was not assessed in the 22 patients without a histology confirmed BCC in surgical excision specimen.

In terms of external validity, performing a punch biopsy for clinically suspected BCC is recommended by international guidelines, while it may not be standard care for all practicing dermatologists.24, 25 In addition surgical treatment of superficial BCC may also not be in line with daily practice.26 However in our test-treatment pathway it was important to histologically confirm all types of BCC in surgical excision specimen. Furthermore, the pathologists assessing surgical excision specimen were not masked to the results of punch biopsy specimen. This is an important potential source of bias in favor of a punch biopsy as diagnostic tool.

In conclusion, this trial showed that a one-stop-shop concept with RCM was non-inferior to standard care in terms of tumor-free margins after surgical treatment of BCC. The proposed new treatment strategy seems suitable in facilitating early diagnosis and direct treatment for the rising number of patients suffering from BCC.

ACKNOWLEDGEMENT
We thank the patients who agreed to participate in this study and all involved employees of the Department of Dermatology of the Academic Medical Center, University of Amsterdam and the Department of Dermatology of the Netherlands Cancer Institute.

FUNDING
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CONFLICT OF INTEREST DISCLOSURES
None of the authors has any financial arrangements or potential conflicts of interest related to this article.
REFERENCES


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A. Wolkerstorfer MD, PhD 1, M. W. Bekkenk MD, PhD 1,7, M. A. de Rie MD, PhD 1,7

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6 Academic Medical Centre, Department of Pathology, Amsterdam, The Netherlands
7 VU Medical Centre, Department of Dermatology, Amsterdam, The Netherlands
DIAGNOSTIC ACCURACY OF CONFOCAL MICROSCOPY IMAGING VERSUS PUNCH BIOPSY FOR DIAGNOSING AND SUBTYPING BASAL CELL CARCINOMA
ABSTRACT

BACKGROUND

In vivo reflectance confocal microscopy (RCM) is a promising non-invasive skin imaging technique that could facilitate early diagnosis of basal cell carcinoma (BCC) instead of routine punch biopsies. However, the clinical value and utility of RCM versus a punch biopsy in diagnosing and subtyping BCC is unknown.

OBJECTIVE

To assess diagnostic accuracy of RCM versus punch biopsy for diagnosing and subtyping clinically suspected primary BCC.

METHODS

A prospective, consecutive cohort of 100 patients with clinically suspected BCC were included at two tertiary hospitals in Amsterdam, the Netherlands, between February 3, 2015 and October 2, 2015. Patients were randomized between two test-treatment pathways: diagnosing and subtyping using RCM imaging followed by direct surgical excision (RCM one-stop-shop) or planned excision based upon the histological diagnosis and subtype of punch biopsy (standard care). The primary outcome was the agreement between the index tests (RCM versus punch biopsy) and reference standard (excision specimen) in correctly diagnosing BCC. The secondary outcome the agreement between the index tests and reference standard in correctly identifying the most aggressive BCC subtypes.

RESULTS

Sensitivity to detect BCC was similar for RCM and punch biopsy (100% versus 93.94%), but a punch biopsy was more specific than RCM (79% versus 38%). RCM expert evaluation for diagnosing BCC had a sensitivity of 100% and a specificity of 75%. The agreement between RCM and excision specimen in identifying the most aggressive BCC subtype ranged from 50% to 85% versus 77% by a punch biopsy.

CONCLUSION

RCM and punch biopsy have comparable diagnostic accuracy to diagnose and subtype BCC depending on RCM experience. Although experienced RCM users could accurately diagnose BCC at a distance, we found an important difference in subtyping BCC. Future RCM studies need to focus on diagnostic accuracy, reliability and specific criteria to improve BCC subtype differentiation.
INTRODUCTION

Current international guidelines recommend a punch biopsy of clinically suspected basal cell carcinoma (BCC) to confirm clinical diagnosis and classify into histological subtypes (superficial, nodular, and aggressive) to ensure optimal treatment selection. Although considered as the most reliable diagnostic technique a punch biopsy fails to diagnose an aggressive subtype in up to one out of six BCC. Other obvious disadvantages of a punch biopsy are pain and discomfort for the patient, scarring, doctor’s delay in the diagnostic process and costs for the healthcare system.

Ideally, BCC diagnosing and subtyping should be performed non-invasively by a painless procedure leading to an immediate diagnosis and treatment. This could be particularly relevant to the growing use of topical treatments as nonsurgical first-line therapy for superficial BCC. As for nodular and aggressive BCC, surgical treatment with excision margins of respectively 3mm or 5mm remains the treatment of choice, reserving Mohs' micrographic surgery for primary BCC on high-risk facial areas (depending on tumour size) and recurrent or previously incompletely excised BCC in all facial areas.

Recently we performed a randomized controlled trial to assess the efficacy of a one-stop-shop concept with real time in vivo reflectance confocal microscopy imaging (RCM) versus standard care for surgical treatment of BCC. To determine the clinical value and utility of RCM versus a punch biopsy in diagnosing and subtyping BCC, a diagnostic analysis is needed. Especially since previous diagnostic testing of RCM and punch biopsy for BCC was not done in accordance with the Standards of Reporting of Diagnostic Accuracy (STARD).

The aim of this study was to assess diagnostic accuracy of RCM versus punch biopsy for diagnosing and subtyping clinically suspected primary BCC.

METHODS

Study design

Diagnostic accuracy data were prospectively collected alongside the multicenter non-inferiority clinical trial in which patients with clinically suspected primary BCC were randomized between two test-treatment pathways: diagnosing and subtyping using RCM followed by direct surgical excision (RCM one-stop-shop) or planned excision based upon the histological diagnosis and subtype of a punch biopsy (standard care). Full details of the clinical trial are given elsewhere.

Participants

Between February 3, 2015, and October 2, 2015, 100 patients presenting with clinically suspected BCC were consecutively included at the Department of Dermatology, Academic Medical Centre, University of Amsterdam (coordinating tertiary hospital), and the Department of Dermatology, the Netherlands Cancer Institute (participating tertiary hospital), in Amsterdam, the Netherlands. We included patients older than 18 years with previously untreated lesions, lesions suitable for conventional surgical excision and lesions present for at least one month. We excluded lesions on high-risk areas of the face (H-zone and ears), lesions larger than 20mm, recurrent BCC, lesions not
suitable for RCM (macroscopic ulceration or crust) and patients with basal cell nevus syndrome. Immunocompromised patients were not excluded.

Initial clinical assessment, most times including dermoscopy, was performed by experienced dermatologists. Patients with multiple clinically suspected new primary BCC were included for only one lesion being the most suitable for conventional surgical treatment according to the following order: (1) chest, (2) extremities, and (3) head and neck area.

**Test methods**

**RCM (index test 1)**

Patients allocated to the RCM one-stop-shop group prospectively received RCM (VivaScope 1500®; CaliberID, Henrietta, NY, U.S.A.; MAVIG GmbH, München, Germany) to diagnose and subtype BCC followed by direct surgical excision according to previously published protocol. DK performed RCM imaging including subsequent diagnosing of RCM cases at the Academic Medical Centre and YE did the same at the Netherlands Cancer Institute. At the time of RCM both assessors were masked to the results of surgical excision specimen but not to patients’ clinical history.

Prior to the study, DK and YE were trained in RCM and interpretation of the acquired images during a one week "Expert training in Confocal Laser Scanning Microscopy" course organized by MAVIG GmbH (distributor of the VivaScope® device) at the University of Modena in Italy. DK and YE had less than one year of RCM experience prior to the start of the study. After the trial was completed two independent international RCM experts (CL and MU) evaluated the RCM images for®, MAVIG GmbH). The experts were masked to the results of surgical excision specimen as well as patients’ clinical history. Both experts had more than 10 years RCM experience.

**Punch biopsy (index test 2)**

Patients allocated to the standard care group received planned excision after a punch biopsy was performed. The routine 3mm punch biopsy was performed from the most elevated part of the lesion using infiltration anesthesia (2% xylcaine/adrenaline 1:80 000). Biopsy specimens were subsequently analyzed by an experienced pathologist within 2 weeks. Surgical excision of the lesion with adequate margins was performed within the following four weeks after receiving the report of the punch biopsy. At the time of the punch biopsy the pathologists were masked to the results of surgical excision specimen but not to patients’ clinical history.

**Surgical excision (reference standard)**

Histopathologic confirmation of presence and subtype of BCC and inspection of resection margins with the use of hematoxylin and eosin stained sections taken from the excision specimen was defined as the reference standard.

An independent dermatologist or independent dermatology resident supervised by an independent dermatologist performed surgery under local anesthetics (2% xylcaine/adrenaline 1:80 000) followed by primary wound closure in both treatment groups. Clinically suspected BCC that were not confirmed by either RCM or punch biopsy were surgically treated with a 3mm excision margin. To prevent bias DK and YE did not perform the subsequent surgical procedures.
After formalin fixation and treatment of resection borders with ink, standard vertical section processing of the surgical excision specimen was used. Reporting of histopathological findings was performed by an experienced pathologist within 2 weeks after surgery. During assessment of the reference standard the pathologist was masked to the results of clinical assessment and RCM but not to the results of a punch biopsy and patients’ clinical history. In line with standard care, the pathologist re-evaluated the results of a punch biopsy during the assessment of the excision specimen in cases of doubt. Besides, a biopsy scar could be recognized in excision specimen.

Analysis
We recorded the following characteristics of participants and tumours at baseline and summarized them for each treatment group with descriptive statistics: age, gender, skin type, previous BCC, study site, immune status, tumour diameter and tumour localization Rippey’s classification was used for classifying BCC subtypes\(^\text{13}\). A distinction was made between superficial, nodular and aggressive (micronodular, infiltrating and basosquamous) growth patterns. In the case of mixed-type diagnosis, defined as two or more single growth patterns, the most aggressive component was used for analysis. Diagnoses of BCC and subtype by RCM versus punch biopsy were separately compared to surgical excision for all tumours. The primary outcome was the agreement between the index tests (RCM versus punch biopsy) and reference standard (excision specimen) in correctly diagnosing BCC. The secondary outcome the agreement between the index tests and reference standard in correctly identifying the most aggressive BCC subtypes. We excluded from the analyses cases in which RCM or a punch biopsy was indeterminate and cases in which subsequent surgical excision was not performed. Reasons for not performing surgical excision were recorded.

The number of true and false positives as well as true and false negatives were recorded. We established the sensitivity, specificity, positive and negative likelihood ratios, and predictive values for diagnosing BCC. For BCC subtyping concordant results were calculated as the proportion of tumours with the corresponding subtype diagnosis in RCM or punch biopsy compared to excision specimen. The statistical analysis was performed using SPSS version 21.0.

RESULTS
Participants
50 patients were randomized to RCM one-stop-shop (48 received index test and treatment) and 50 to standard care (47 received index test and treatment) (Fig. 1). Five patients were excluded, two patients in the RCM one-stop-shop group that did not receive imaging and three patients in the standard care group that did not receive surgical treatment (Fig. 1). Baseline characteristics are shown in table 1. In the RCM on-stop-shop group 40 BCC were confirmed by surgical excision specimen compared to 33 BCC in the standard care group. Most of the BCC had a superficial or nodular subtype. All patients in the RCM one-stop-shop group received surgical treatment directly after RCM at the same initial outpatient visit. The average time between the initial visit and surgical treatment in the standard care group was almost ten weeks (66 days).
Figure 1. Flow chart. BCC= basal cell carcinoma; PDT= photodynamic therapy. Two patients in the RCM one-stop-shop group did not begin diagnosis and treatment. One refused directly after randomization and the other one could not participate due to technical malfunction of the confocal imaging device. * Three patient in the standard care group did not receive subsequent surgical excision after the punch biopsy. In one patient the protocol was violated after histologic assessment of punch biopsy specimen showed actinic keratosis with no visible signs of the biopsied lesion on the day of surgery. Another patient with a histologically confirmed superficial BCC was mistakenly treated with photodynamic therapy instead of surgery. The last patient with a histologically confirmed BCC developed a large leiomyosarcoma on the same localization. Surgical excision of the BCC was cancelled and the patient was referred to an oncologic surgeon to treat the leiomyosarcoma. ** In the standard care group a punch biopsy identified three lesions as BCC while surgical excision specimen did not show (residual) histological signs of BCC. *** RCM incorrectly identified five lesions as BCC while surgical excision specimen diagnosed two non-malignant lesions, one actinic keratosis, one Bowen’s disease and one squamous cell carcinoma. **** In the RCM one-stop-shop group two histology proven BCC (excision specimen) cases were tested as inconclusive, one ulcerating lesion and one lesion with a superficial crust.

Test results
The RCM experts evaluated the images that were acquired at the Department of Dermatology, Academic Medical Centre, University of Amsterdam (coordinating tertiary hospital). CL evaluated 32/36 cases and MU evaluated 36/36 cases.

RCM versus punch biopsy for diagnosing BCC
Table 2 shows the agreement between specimens in correctly diagnosing BCC. Sensitivity to detect BCC was similar for RCM and punch biopsy (100% [90.75-100] versus 93.94% [79.77-99.26]), but a punch biopsy was more specific than RCM (79% [49.20-95.34] versus 38% [8.52-75.51]). The RCM expert evaluation for diagnosing BCC was the same for both readers with a sensitivity of 100% [85.75-100] and a specificity of 75% [34.91-96.81].
Table 1. Tumour and patient characteristics separated by treatment group

<table>
<thead>
<tr>
<th></th>
<th>One-stop-shop (n= 50)</th>
<th>Standard care (n= 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64 (39-88)</td>
<td>68 (41-92)</td>
</tr>
<tr>
<td>Sex</td>
<td>31 (62%) 25 (50%)</td>
<td>37 (74%) 37 (74%)</td>
</tr>
<tr>
<td>Fitzpatrick skin type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>8 (16%) 4 (8%)</td>
<td>43 (86%) 3 (6%)</td>
</tr>
<tr>
<td>II</td>
<td>32 (64%) 43 (86%)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>10 (20%) 3 (6%)</td>
<td></td>
</tr>
<tr>
<td>BCC in medical history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>34 (68%) 37 (74%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>15 (30%) 13 (26%)</td>
<td></td>
</tr>
<tr>
<td>Study site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Academic Medical Centre</td>
<td>37 (74%) 38 (76%)</td>
<td></td>
</tr>
<tr>
<td>Netherlands Cancer Institute</td>
<td>13 (26%) 12 (24%)</td>
<td></td>
</tr>
<tr>
<td>Immunocompromised *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4 (8%) 4 (8%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>46 (92%) 46 (92%)</td>
<td></td>
</tr>
<tr>
<td>Tumour diameter (mm)</td>
<td>8 (3-15) 8 (3-20)</td>
<td></td>
</tr>
<tr>
<td>Tumour location</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head/neck</td>
<td>9 (18%) 12 (24%)</td>
<td></td>
</tr>
<tr>
<td>Trunk</td>
<td>32 (64%) 30 (60%)</td>
<td></td>
</tr>
<tr>
<td>Arm</td>
<td>4 (8%) 7 (14%)</td>
<td></td>
</tr>
<tr>
<td>Leg</td>
<td>5 (10%) 1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Number of BCC</td>
<td>40 (80%) 33 (66%)</td>
<td></td>
</tr>
<tr>
<td>BCC subtype distribution ^</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superficial BCC</td>
<td>17 (43%) 14 (42%)</td>
<td></td>
</tr>
<tr>
<td>Nodular BCC</td>
<td>17 (43%) 17 (52%)</td>
<td></td>
</tr>
<tr>
<td>Aggressive BCC</td>
<td>6 (14%) 2 (6%)</td>
<td></td>
</tr>
</tbody>
</table>

Continuous variables are expressed as mean (range) and categorical variables as n (%). BCC= basal cell carcinoma. *Patients who were taking immunosuppressive drugs such as oral steroids, methotrexate, ciclosporin for suppression of immunological disorder, or to prevent transplant rejection. ^This number represent the histologically confirmed basal cell carcinoma based on surgical excision specimen. Basal cell carcinoma subtype distribution according to the most aggressive subtype found at histology of surgical excision.

RCM versus punch biopsy for subtyping BCC

Table 3 shows the agreement between RCM versus a punch biopsy compared to excision in correctly identifying the most aggressive BCC subtypes. The overall agreement was 68 % for RCM (26/38 concordant RCM cases) versus 77% for punch biopsy (24/31 concordant punch biopsy cases). The initial RCM assessment during the trial period led to overstaging of BCC subtype in 18% (7/38) versus 10% (3/31) by punch biopsy. Understaging of BCC subtype was seen in 13% of both RCM and a punch biopsy (5/38 versus 4/31).

The agreement between BCC subtype of the RCM experts and excision specimen ranged from 50% (12/24 concordant RCM cases diagnosed by CL) to 85% (23/27 concordant RCM cases diagnosed...
Table 2. Diagnostic performance of RCM versus a punch biopsy in diagnosing BCC compared to surgical excision

<table>
<thead>
<tr>
<th></th>
<th>Surgical excision</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BCC</td>
<td>No BCC</td>
<td>Total</td>
</tr>
<tr>
<td>RCM (DK/YE) trial period</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCC</td>
<td>38</td>
<td>5</td>
<td>43</td>
</tr>
<tr>
<td>No BCC</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>8</td>
<td>46</td>
</tr>
<tr>
<td>Punch biopsy trial period</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCC</td>
<td>31</td>
<td>3</td>
<td>34</td>
</tr>
<tr>
<td>No BCC</td>
<td>2</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>14</td>
<td>47</td>
</tr>
<tr>
<td>RCM expert (MU) after trial period</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCC</td>
<td>27</td>
<td>2</td>
<td>29</td>
</tr>
<tr>
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<td>0</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>8</td>
<td>35</td>
</tr>
<tr>
<td>RCM expert (CL) after trial period</td>
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</tr>
<tr>
<td>BCC</td>
<td>24</td>
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<tr>
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<tr>
<td>Total</td>
<td>24</td>
<td>8</td>
<td>32</td>
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</table>

BCC versus no BCC

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity % (n) [95% CI]</th>
<th>Specificity % (n) [95% CI]</th>
<th>Positive LR [95% CI]</th>
<th>Negative LR [95% CI]</th>
<th>PPV [95% CI]</th>
<th>NPV [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCM (DK/YE) trial period (n=46)</td>
<td>100% (38/38) [90.75-100]</td>
<td>37.50% (3/8) [8.52-75.51]</td>
<td>1.60 [0.94-2.74]</td>
<td>0 [81.63-92.86]</td>
<td>88.37% [79.77-99.26]</td>
<td>37.50% [8.52-75.51]</td>
</tr>
<tr>
<td>Punch biopsy trial period (n=47)</td>
<td>93.94% (31/33) [49.20-95.34]</td>
<td>78.57% (11/14) [1.60-12.00]</td>
<td>4.38 [0.02-0.30]</td>
<td>0.08 [76.32-98.14]</td>
<td>91.18% [76.32-98.14]</td>
<td>78.57% [1.60-12.00]</td>
</tr>
<tr>
<td>RCM expert (MU) after trial period (n=35)</td>
<td>100% (26/26) [87.23-100]</td>
<td>75% (6/8) [34.91-96.81]</td>
<td>4.00 [1.20-13.82]</td>
<td>0 [80.26-97.82]</td>
<td>92.31% [80.26-97.82]</td>
<td>75% [34.91-96.81]</td>
</tr>
<tr>
<td>RCM expert (CL) after trial period (n=32)</td>
<td>100% (24/24) [85.75-100]</td>
<td>75% (6/8) [34.91-96.81]</td>
<td>4.00 [1.20-13.82]</td>
<td>0 [78.32-97.55]</td>
<td>93.10% [78.32-97.55]</td>
<td>75% [34.91-96.81]</td>
</tr>
</tbody>
</table>

Bold numbers indicate concordant cases. Values in brackets are 95% confident intervals. BCC= basal cell carcinoma. LR= likelihood ratio. NPV= negative predictive value. PPV= positive predictive value. RCM=reflectance confocal microscopy imaging.

by MU) (Table 3). Overstaging of BCC subtype by RCM expert teleconsultation assessment after the trial period ranged from 11% (3/27) to 50% (12/24). Understaging of BCC subtype by RCM experts ranged from 0% to 4% (1/27).

There were no adverse events after performing RCM or punch biopsies. Adverse reactions after performing surgical excision included four patients of the RCM one-stop-shop group with post-operative wound infections. In all cases the infection was successfully treated with oral antibiotics, without the need of hospitalization. One patient of the standard care group using anti-coagulant medication developed an excessive post-operative bleeding requiring hospitalization for three days. She fully recovered. This was reported as the only serious adverse event.
DISCUSSION

Our findings show that for experienced users, RCM can have a similar diagnostic accuracy to diagnose and subtype clinically suspected BCC compared to a punch biopsy.

This is the first study that prospectively compared RCM with a punch biopsy for diagnosing and subtyping BCC. Previous RCM studies for diagnosing BCC showed varying high sensitivity and specificity values ranging from 85% to 97% and from 89% to 99%, respectively. However as reported by Que et al., most of these studies involved RCM experts with prior experience in RCM interpretation. Our result confirm the high sensitivity of RCM for diagnosing BCC (100%) but the specificity ranged from 38% (RCM users with less than one year experience) to 75% (RCM experts with more than 10 years of experience).

We caution that the range of specificities for confirming BCC diagnosis is large for both RCM (DK/YE 38% [8.52-75.51] vs CL/MU 75% [34.91-96.81]) and punch biopsy (79% [49.20-95.34]). A lower RCM specificity was also previously reported by Rao et al. that studied RCM users with varying levels of experience. Furthermore, Farnetani et al. also recently emphasized on the importance of the RCM learning curve and confirmed that diagnostic accuracy of RCM increases with experience.

Table 3. Diagnostic performance of RCM versus a punch biopsy in subtyping BCC compared to surgical excision

<table>
<thead>
<tr>
<th></th>
<th>Surgical excision</th>
<th></th>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sBCC</td>
<td>nBCC</td>
<td>aBCC</td>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCM (DK/YE) trial period</td>
<td>14</td>
<td>2</td>
<td>1</td>
<td>17</td>
<td></td>
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</tr>
<tr>
<td>sBCC</td>
<td>1</td>
<td>10</td>
<td>2</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>nBCC</td>
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<td>4</td>
<td>2</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
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<td>16</td>
<td>5</td>
<td>38</td>
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<tr>
<td>Punch biopsy trial period</td>
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<tr>
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<td>RCM expert (MU) after trial period</td>
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</tr>
<tr>
<td>nBCC</td>
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<td></td>
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</tr>
<tr>
<td>Total</td>
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<tr>
<td>RCM expert (CL) after trial period</td>
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<td>0</td>
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<tr>
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<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>nBCC</td>
<td>9</td>
<td>2</td>
<td>1</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>10</td>
<td>11</td>
<td>24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bold numbers indicate concordant cases. Values in brackets are 95% confident intervals. aBCC= aggressive basal cell carcinoma; BCC= basal cell carcinoma; nBCC= nodular basal cell carcinoma; RCM=reflectance confocal microscopy imaging; sBCC= superficial basal cell carcinoma.
The agreement between histological subtype on a punch biopsy and surgical excision specimen in our study was 77% (24/31 concordant cases). This seems consistent with previous studies. Interestingly, we found that RCM proved to be almost as reliable for accurately subtyping BCC (68%, 26/38 concordant cases). However we also found a large difference in subtyping BCC by RCM experts. This is an important finding that highlights the need for further training, guidelines and protocols for subtyping BCC using RCM.

With the growing number of patients suffering from BCC, new management strategies are needed. Non-invasive skin imaging could play a crucial role in improving BCC healthcare for both patients and clinicians. Previous diagnostic RCM studies have primarily focused on test accuracy (sensitivity and specificity for diagnosing BCC). However other aspects such as time between diagnosis and treatment, direct health effects of testing, costs of testing and patients’ emotional and behavioral responses to testing should also be taken into consideration. In our proposed RCM one-stop-shop we have assessed the efficacy of such a test-treatment pathway. The main advantages of using RCM include an immediate diagnosis and treatment for patients suffering from BCC opposed to painful skin biopsies with a doctor delay in the diagnostic process. Moreover in selected cases of superficial BCC, patients could benefit from a totally non-invasive disease management.

Study strengths include adherence to the STARD guidelines. Furthermore, we prevented sampling error in our reference standard by using final surgical excision specimen instead of a punch biopsy. In addition, we prevented heterogeneity of our results by using predefined RCM criteria and using the same VivaScope 1500® device at both participating centers. Although it may not be in line with daily practice to surgically treat superficial BCC, it was important in our study to histologically confirm all types of BCC and to prevent selection bias for specific BCC subtypes.

Limitations of our study include the limited sample size of aggressive BCC. Our study was primarily designed and powered to assess non-inferiority of a RCM one-stop-shop in terms of tumour-free margins after surgical treatment of BCC compared to standard care. Another important limitation that needs to be considered when interpreting the results is the potential bias in favor of the punch biopsy diagnosis. Although in line with current practice the pathologists were not blinded to the results of the punch biopsy during assessment of surgical excision specimen in the standard care group. Lastly, BCC on high-risk areas of the face were excluded due to technical limitations of the VivaScope 1500® device. This needs to be considered in terms of external validity. Nonetheless the potential value of RCM remains very high in the excluded patient population. Especially after the introduction of the VivaScope 3000® flexible handheld version (VivaScope 3000®; CaliberID, Henrietta, NY, U.S.A.; MAVIG GmbH, München, Germany), that permits imaging of the more concave and convex high-risk facial areas.

Based on our findings we believe that RCM could potentially replace a punch biopsy for diagnosing and subtyping selected BCC cases. Yet prior to doing so, it is mandatory to wait for the results of future and ongoing larger prospective clinical trials. Besides RCM, more and more studies are reporting on the added value of optical coherence tomography (OCT) for diagnosing and subtyping BCC. In addition, a first report on a combined RCM/OCT skin modality for ex vivo BCC detection has been published. This approach could potentially be of significant interest for
diagnosing and subtyping BCC in clinical practice as it combines the detailed features of RCM with the in-depth advantages of OCT. Finally, we underline that both routine histology as non-invasive skin imaging modalities such as RCM and OCT remain morphology based and thus subject to interpretation bias.

In conclusion, RCM and punch biopsy have comparable diagnostic accuracy to diagnose and subtype BCC depending on RCM experience. Although experienced RCM users could accurately diagnose BCC at a distance, we found an important difference in subtyping BCC. Future RCM studies need to focus on diagnostic accuracy, reliability and specific criteria to improve BCC subtype differentiation.

CONFLICTS OF INTEREST
We declare that we have no conflicts of interest.

ACKNOWLEDGEMENTS
We thank the patients who agreed to participate in this study. We thank all involved employees of the department of dermatology of the Academic Medical Centre, University of Amsterdam and the department of dermatology of the Netherlands Cancer Institute.

FINANCIAL DISCLOSURE AND PRODUCTS
The VivaScope 1500® device at the Department of Dermatology, Academic Medical Centre in Amsterdam, the Netherlands, was provided by MAVIG GmbH for the time of the study from February 2015 to September 2015.
REFERENCES


D.J. Kadouch MD †, *, A. van Haersma de With MSc †, *, Y. S. Elshot, MD †,‡, M. Peppelman, PhD ‡, M.W. Bekkenk MD, PhD †,¶,
A. Wolkerstorfer MD, PhD †, I. Eekhout, PhD §,*
C.A.C. Prinsen, PhD §, M. A. de Rie MD, PhD †,¶
INTER-RATER AND INTRA-RATER AGREEMENT OF CONFOCAL MICROSCOPY IMAGING IN DIAGNOSING AND SUBTYPING BASAL CELL CARCINOMA
ABSTRACT

Background
Reflectance confocal microscopy (RCM) imaging can be used to diagnose and subtype basal cell carcinoma (BCC) but relies on individual morphologic pattern recognition that might vary among users.

Objectives
We assessed the inter-rater and intra-rater agreement of RCM in correctly diagnosing and subtyping BCC.

Methods
In this prospective study we evaluated the inter-rater and intra-rater agreement of RCM on BCC presence and subtype among three raters with varying experience who independently assessed static images of 48 RCM cases twice with four weeks interval (T1 and T2). Histopathologic confirmation of presence and subtype of BCC from surgical excision specimen was defined as the reference standard.

Results
The inter-rater agreement of RCM for BCC presence showed an agreement of 82% at T1 and 84% at T2. The agreements for subtyping BCC were lower (52% for T1 and 47% for T2). The intra-rater agreement of RCM for BCC presence showed an observed agreement that varied from 79% to 92%. The observed agreements for subtyping varied from 56% to 71%.

Conclusions
In conclusion, our results show that RCM is reliable in correctly diagnosing BCC based on the assessment of static RCM images. RCM could potentially play an important role in BCC management if accurate subtyping will be achieved. Therefore future clinical studies on reliability and specific RCM features for BCC subtypes are required.
INTRODUCTION

The rising incidence of basal cell carcinoma (BCC) is causing a major burden on worldwide health care systems. With the increasing use of effective non-surgical therapies for superficial BCC, histological subtype (i.e. aggressiveness) becomes more important in determining the most suitable BCC treatment.

Current international guidelines recommend on performing a punch biopsy to confirm clinical diagnosis and divide between BCC subtypes. However non-invasive skin imaging techniques might be able to change the diagnostic pathway for patients suffering from BCC. Of those techniques, in vivo reflectance confocal microscopy (RCM) seems very promising as the procedure enables inspection of the whole lesion while the morphologic features are similar to routine histology. If RCM would be able to accurately diagnose and subtype BCC, not only the amount of painful invasive skin biopsies could be reduced but also the time-delay between diagnosis and treatment, administrative workload, and health care costs. Yet prior to replacement of routine punch biopsies a critical appraisal of the diagnostic RCM procedure is needed. An important risk of techniques such as RCM is that it relies on morphology based assessment. Therefore, it is subject to interpretation bias.

The purpose of this study was to determine the inter-rater and intra-rater agreement of RCM in correctly diagnosing and subtyping BCC based on static RCM images.

METHODS

Study design and patients

This reliability study evaluated inter-rater and intra-rater agreement using static images of 48 RCM cases among three raters (DK, YE, and MP). The series of images were prospectively derived from clinically suspected BCC that were included in our recent randomized controlled trial that was performed between February 3, 2015 and October 2, 2015. Consecutive eligible patients of 18 years and older with a clinically suspected, primary, untreated, BCC, regardless of subtype and present for at least one month were prospectively enrolled at the Department of Dermatology, Academic Medical Centre, University of Amsterdam (coordinating tertiary hospital), and the Department of Dermatology, the Netherlands Cancer Institute (participating tertiary hospital), in Amsterdam, the Netherlands. We excluded patients with lesions not suitable for conventional surgical excision, lesions in a high-risk location of the face (H-zone and ears), lesions larger than 20mm, recurrent BCC, macroscopic ulcerating lesions, and those with basal cell nevus syndrome. The study was conducted according to the principles of the Declaration of Helsinki (Fortaleza, Brazil, October 2013) and in accordance with the Dutch Medical Research Involving Human Subjects Act (WMO). The research protocol has been approved by ethics committees at both centers (reference number: NL50112.018.14). All participants gave written informed consent prior to their participation in the study.

Study procedures

All clinically suspected BCC were surgically excised directly after RCM imaging. Histopathologic confirmation of presence and subtype of BCC with the use of hematoxylin and eosin stained sections...
taken from the excision specimen was defined as the reference standard. No punch biopsies were performed on the RCM cases.

RCM imaging was performed according to a standardized protocol to diagnose clinically suspected primary BCC and to divide between subtypes during the trial period. DK performed RCM imaging at the Academic Medical Center and YE at the Netherlands Cancer Institute. The VivaScope 1500® (VivaScope 1500® CaliberID, Henrietta, NY, U.S.A.; MAVIG GmbH, München, Germany) was used to acquire confocal images at both participating study centers. Each case for evaluation had horizontal optical RCM images at different levels of the skin, including one image at the granular spinous layer of the epidermis, one image at the basal layer of the epidermal and dermal-epidermal junction, and one image at the superficial dermis. BCC were divided into superficial, nodular and aggressive subtypes (i.e. micronodular, infiltrating or basosquamous). Three raters (DK, YE and MP) independently reviewed all images of RCM de-identified cases twice, with a 4-week time interval. At the time of the study raters had between 1-5 years of experience with RCM.

**Inter-rater and intra-rater agreement**

Inter-rater agreement was defined as the extent to which the interpretation of the selected RCM images are the same for repeated measurement by different persons on the same occasion. Intra-rater agreement was defined as the extent to which the interpretation of the selected RCM images are the same for repeated measurement by the same persons on different occasions. Raters reviewed the RCM images only, without any information on clinical data, clinical photos or dermoscopic pictures. They were also blinded to their own previous interpretation and to each other’s interpretation. Before the first and second assessment, RCM cases were shuffled and recoded to prevent identification by a computer-based system (GraphPad Software, La Jolla, CA). Diagnoses were recorded on standardized study forms including: BCC presence (yes or no); BCC subtype (superficial, nodular, aggressive or any combination). In addition, raters scored the images as easy, moderate or difficult to diagnose.

**Statistical analysis**

For assessing inter- and intra-rater agreement on BCC presence and BCC subtype, the percentages observed agreement (i.e. \((a + d)/(a + b + c + d)\)) and specific agreement (i.e. positive agreement (PA): \(PA = 2a/[2a + b + c]\); or negative agreement (NA): \(NA = 2d/[2d + b + c]\)) were calculated. The proportion of specific agreement distinguishes agreement on positive or negative scores. To obtain an agreement parameter for three raters, all pairwise 2 x 2 tables (i.e. \(m(m-1)/2\)) were summed and the b and c cells were averaged. Herewith, placement of the b and c cell values remains arbitrary. Subsequently, the observed agreement and specific agreement were calculated. We predefined an observed and/or specific agreement of more than 80% to be acceptable. In addition, the 95% confidence intervals were obtained by bootstrap resampling.
RESULTS

In total, 288 RCM assessments were analyzed; 48 RCM cases were reviewed two times by 3 raters. The reference standard of the 48 RCM cases revealed 40 BCC (83%), two actinic keratosis (4%), two Bowen's disease (4%), one squamous cell carcinoma (SCC) (2.0%), one nevus (2%), one solar lentigo (2%) and two other non-malignant inflammatory lesions (4%). Of the BCC, 17 (35%) had a superficial subtype, 17 (35%) had a nodular subtype and 6 (13%) had an aggressive subtype. Reference standard and BCC subtypes are summarized in table 1.

Description of RCM diagnosis at both reviewing sessions

At the first rating session (T1) the three raters diagnosed ‘BCC presence’ correctly (equally to reference standard) in 34-39 out of 40 (mean 89%, range 85-98%) compared to 34-36 at the second

Table 1. Tumour and patient characteristics of the 48 RCM cases

<table>
<thead>
<tr>
<th></th>
<th>RCM cases, n=48 (%)</th>
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<tbody>
<tr>
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<td>64 (39-84)</td>
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<tr>
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<td></td>
</tr>
<tr>
<td>Men</td>
<td>30 (62%)</td>
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<tr>
<td>Women</td>
<td>18 (38%)</td>
</tr>
<tr>
<td>Fitzpatrick skin type</td>
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<tr>
<td>I</td>
<td>8 (17%)</td>
</tr>
<tr>
<td>II</td>
<td>30 (63%)</td>
</tr>
<tr>
<td>III</td>
<td>10 (20%)</td>
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<td>32 (67%)</td>
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<tr>
<td>Yes</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>No</td>
<td>46 (96%)</td>
</tr>
<tr>
<td>Tumour diameter (mm)</td>
<td>8 (3-15)</td>
</tr>
<tr>
<td>Tumour location</td>
<td></td>
</tr>
<tr>
<td>Head/neck</td>
<td>8 (17%)</td>
</tr>
<tr>
<td>Trunk</td>
<td>31 (65%)</td>
</tr>
<tr>
<td>Arm</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>Leg</td>
<td>5 (10%)</td>
</tr>
<tr>
<td>Number of BCC</td>
<td>40 (80%)</td>
</tr>
<tr>
<td>BCC subtype distribution ^</td>
<td></td>
</tr>
<tr>
<td>Superficial BCC</td>
<td>17 (43%)</td>
</tr>
<tr>
<td>Nodular BCC</td>
<td>17 (43%)</td>
</tr>
<tr>
<td>Aggressive BCC</td>
<td>6 (14%)</td>
</tr>
</tbody>
</table>

Continuous variables are expressed as mean (range) and categorical variables as n (%). BCC=basal cell carcinoma. *Patients who were taking immunosuppressive drugs such as oral steroids, methotrexate, ciclosporin for suppression of immunological disorder, or to prevent transplant rejection. ^This number represent the histologically confirmed basal cell carcinoma based on surgical excision specimen. Basal cell carcinoma subtype distribution according to the most aggressive subtype found at histology of surgical excision.
rating session (T2) (mean 88%, range 85-92%) (Table 2). Of the correctly diagnosed BCCs, raters accurately diagnosed an aggressive subtype at T1 in 43-59% compared to 46-64% at T2. At T1 the raters scored 14-27% of the RCM cases as ‘difficult to diagnose’ compared to 13-40% at T2.

**Inter-rater agreement**

With three raters we created three 2 x 2 tables (m (m-1) /2 = 3x2 / 2 = 3), representing the agreement between the raters: 1 vs 2; 1 vs 3; 2 vs 3. For BCC presence, calculating the proportions of observed agreement for the summed table with averaged b and c cells results in an observed agreement of 82% at T1 (95% CI = 74% - 92%) and 85% at T2 95% (CI = 76% - 92%)). The observed agreements for BCC subtype were lower (52% (95% CI = 42% - 63%) for T1 and 47% (95% CI = 35% - 58%) for T2).

The specific agreements on a positive score for BCC presence were high at both reviewing sessions 89% (95% CI = 82% - 94%) for T1 and 90% (95% CI = 84% - 95%) for T2) but the specific agreements on a negative score were lower (54% (95% CI = 30% - 71%) for T1 and 66% (95% CI = 40% - 82%) for T2). The specific agreements for BCC subtyping were also lower.

**Intra-rater agreement**

The observed agreements within the raters for BCC presence were 79% (95% CI = 67% - 90%) for rater DK, 92% (95% CI = 83% - 98%) for rater YE and 88% (95% CI = 77% - 96%) for rater MP. For BCC subtyping, the observed agreements within the raters were 56% (95% CI = 42% - 69%) for rater DK, 71% (95% CI = 58% - 83%) for rater YE and 57% (95% CI = 44% - 70%) for rater MP.

**DISCUSSION**

In this study, the inter-rater and intra-rater agreement of RCM in correctly diagnosing and subtyping BCC was assessed based on static RCM images. Our results show that RCM is reliable in correctly diagnosing BCC. The observed inter-rater agreements for BCC presence were higher than 80% in both reviewing sessions. The observed intra-rater agreement of the three raters for BCC presence ranged from 79% to 92%. This confirms previous findings on the usefulness of RCM in accurately diagnosing BCC.

<table>
<thead>
<tr>
<th>Raters</th>
<th>RCM experience (years)</th>
<th>BCC present, n=40 (%)</th>
<th>Correct BCC subtype (%)</th>
<th>Difficult to diagnose RCM images (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>DK at T1</td>
<td>1</td>
<td>34 (85)</td>
<td>17 out of 34 (50)</td>
<td>6 out of 43 (14)</td>
</tr>
<tr>
<td>YE at T1</td>
<td>2</td>
<td>39 (98)</td>
<td>23 out of 39 (59)</td>
<td>6 out of 35 (17)</td>
</tr>
<tr>
<td>MP at T1</td>
<td>5</td>
<td>35 (88)</td>
<td>15 out of 35 (43)</td>
<td>12 out of 45 (27)</td>
</tr>
<tr>
<td>DK at T2</td>
<td>1</td>
<td>35 (88)</td>
<td>16 out of 35 (46)</td>
<td>6 out of 47 (13)</td>
</tr>
<tr>
<td>YE at T2</td>
<td>2</td>
<td>36 (92)</td>
<td>26 out of 36 (64)</td>
<td>10 out of 47 (21)</td>
</tr>
<tr>
<td>MP at T2</td>
<td>5</td>
<td>34 (85)</td>
<td>18 out of 34 (53)</td>
<td>19 out of 47 (40)</td>
</tr>
</tbody>
</table>

BCC= basal cell carcinoma. *This item was not recorded by the raters in all 48 RCM cases at both reviewing sessions.
diagnosing BCC. As for subtyping BCC, we found that inter- and intra-rater agreements were lower than 80%. The lower agreements for subtyping BCC seem consistent with the results of our recent diagnostic accuracy study. Thus far only two other studies have previously reported on subtype specific in vivo RCM features. The challenges for RCM users to accurately divide between BCC subtypes might be explained by absence of studies that have reported on the reliability of individual subtype specific RCM features.

This is the first prospective study that investigates the inter-rater and intra-rater agreement of RCM in correctly diagnosing and subtyping BCC. Farnetani et al. also previously reported on reproducibility of RCM feature recognition and accuracy of diagnosing skin cancer. In their retrospective web-based study, Cohen’s kappa was used to test the inter-observer reproducibility of recognition of previously published RCM descriptors for melanoma and BCC. In line with their findings we found RCM to be reliable in diagnosing BCC. However, Farnetani et al. did not report on the reliability of RCM in dividing BCC into subtypes. Besides, the use of Cohen’s kappa is less informative for clinicians as it is considered to be a measure of reliability and not a measure of agreement. In clinical practice we are interested in inter-rater and/or intra-rater agreement. Following the methods of De Vet and colleagues, we therefore decided to calculate the proportion of observed and specific agreement instead of Cohen’s kappa. We believe that this is one of the strengths of our study. Another study strength is the use of de-identified static RCM images to prevent interpretation bias of the raters as a result of clinical information.

Limitations of our study include a selection bias of RCM cases. The series of images were derived from our recent randomized controlled trial that excluded lesions not suitable for conventional surgical excision, lesions in a high-risk location of the face (H-zone and ears), lesions larger than 20mm, recurrent BCC, macroscopic ulcerating lesions, and lesions of patients with basal cell nevus syndrome. In addition two different researchers (DK and YE) performed RCM imaging during the study period leading to a potential source of bias in acquiring the series of RCM images.

In terms of external validity, it is important to emphasize that our study results are based on the interpretation of static RCM images that were acquired with the VivaScope 1500®. There is an important difference in diagnosing and subtyping clinically suspected BCC using real time in vivo RCM combined with clinical information and dermoscopy compared to the blinded static RCM images that were assessed in our study. As demonstrated by Borsari et al. RCM should ideally be used as an add-on tool to clinical inspection and dermoscopy in order to increase accuracy in the diagnosis of skin cancer. Therefore future research should be aimed at investigating the reliability of real-time RCM as it is expected to further improve RCM’s inter-rater and intra-rater agreement for diagnosing and subtyping BCC.

RCM could potentially play an important role in the management of BCC if accurate subtyping will be achieved. We recommend on achieving international consensus on specific RCM features for subtyping BCC based on the results of large prospective clinical trials. For example currently ongoing randomized controlled multicenter trial in Nijmegen, The Netherlands, that has been designed to investigate whether in vivo RCM can correctly identify the subtype of BCC. Furthermore by using the more recent introduced flexible handheld VivaScope 3000® RCM (VivaScope 3000®; CaliberID, Henrietta, NY, U.S.A.; MAVIG GmbH, München, Germany) clinically suspected BCC can
be evaluated even faster. Previous studies already confirmed that the VivaScope 3000® is suitable in diagnosing BCC, including lesions on the more concave and convex high-risk head and neck areas.\textsuperscript{22, 23} It would be valuable to compare the reliability of the wide probe VivaScope 1500® with the VivaScope 3000® for accurately subtyping BCC.

In conclusion, our results show that RCM is reliable in correctly diagnosing BCC based on the assessment of static RCM images. RCM could potentially play an important role in BCC management if accurate subtyping will be achieved. Therefore future clinical studies on reliability and specific RCM features for BCC subtypes are required.

**FUNDING**

None.

**CONFLICT OF INTEREST DISCLOSURES**

None of the authors has any financial arrangements or potential conflicts of interest related to this article.
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CONFOCAL MICROSCOPY IMAGING OF BASAL CELL CARCINOMA

The rising incidence of BCC is causing a major burden on worldwide health care systems. With the increasing use of effective non-surgical therapies for superficial BCC, the histological subtype (i.e. aggressiveness) becomes more important to determine the most suitable BCC treatment. As for nodular and aggressive BCC subtypes, surgical excision remains needed to achieve tumor clearance. According to current international guidelines a punch biopsy should be performed to confirm clinical diagnosis of BCC and divide between subtypes. While considered standard care, results of our systematic review showed that a punch biopsy fails to identify an aggressive subtype in one out of six BCCs. Consequently, misidentification of an aggressive BCC subtype can easily result in insufficient treatment, distress for patients, as well as unnecessary high costs and avoidable burden on the capacity of the hospital organization.

Ideally BCC diagnosing and subtyping should be performed non-invasively by a painless procedure leading to an immediate diagnosis and treatment. In the light of this matter, various non-invasive skin imaging devices have been developed in the last decades. Of those, RCM has been proposed as the most promising diagnostic tool for diagnosing BCC. RCM uses a confocal microscope to directly image a skin lesion at high resolution and is most frequently used as an additional diagnostic tool for suspected lesions that are difficult to assess by physical examination and dermoscopy. In comparison to conventional histology of a punch biopsy, the procedure is painless and requires approximately 10 minutes. RCM can be applied to examine both melanoma and non-melanoma skin cancer. In addition to the diagnostic workflow, RCM can also be used to monitor treatment response such as the effect of imiquimod in lentigo maligna and the response of topical treatments in BCC. However, RCM has several potential drawbacks, such as the limited penetration depth in the skin (up to the upper dermis) and the expensive purchase of the RCM device. Furthermore, RCM image interpretation strongly corresponds with RCM user experience as confirmed by the inter- and intra-rater reliability findings in this thesis.

Since the introduction in dermatology, in vivo RCM has become a welcome addition to the diagnostic workflow of skin cancer. RCM is being used worldwide and commercially available devices include the VivaScope 1500 and VivaScope 3000 (CaliberID, Henrietta, NY, U.S.A.; MAVIG GmbH, München, Germany). Because of the high costs of the RCM device, implementation in daily dermatology practice predominantly depends on reimbursement by each country’s health care system. As of 2016 RCM imaging is being reimbursed for dermatologists in the U.S.A. In The Netherlands, RCM has not yet been integrated in clinical dermatological guidelines due to the lack of high quality prospective studies.

In this thesis we describe the design of a randomized controlled multicenter trial to assess the efficacy of a one-stop-shop concept using RCM as diagnostic tool for surgical management of basal cell carcinoma. By linking diagnostic testing to management actions, we assessed how the implementation of RCM impacts the current diagnostic pathway for clinically suspected BCC. The results of the randomized controlled trial that was performed, support the added value of RCM in a clinical setting for diagnosing BCC. Based on the findings in this thesis RCM could potentially

7  
GENERAL DISCUSSION
replace a punch biopsy for diagnosing and subtyping in selected BCC cases depending on RCM user experience.

**FUTURE CONSIDERATIONS**

It is eminent that non-invasive skin imaging devices have found their way into the field of clinical dermatological oncology. Procedures such as RCM are more patient friendly and could help in reducing the time between clinical diagnosis and treatment, administrative workload, and even health care costs. However, prior to replacement of routine punch biopsies, clinicians using RCM should be able to more accurately distinguish between BCC subtypes. A higher level of consensus is needed between RCM users. Therefore, future studies need to focus on diagnostic accuracy, reliability and specific criteria to improve BCC subtype differentiation. Furthermore cost-effectiveness of RCM versus standard care should also be explored.

In addition to RCM more studies are reporting on the added value of optical coherence tomography (OCT). It would be valuable to compare RCM with OCT in diagnosing and subtyping BCC. In daily practice both techniques can be used on equivocal clinically suspected lesions combining detailed features of RCM with the in-depth advantages of OCT.

While skin imaging is revolutionizing dermatology, the current pitfall of RCM, OCT and even routine histology is that these diagnostic tools rely on morphology based assessment. Therefore, all are subject to interpretation bias. To overcome this diagnostic obstacle, impressive advancements are being made on automated algorithms that can recognize patterns of specific skin cancers.

All together a new innovative era has started for dermatologists. Hopefully, this thesis contributes in further raising the awareness of the potential role of non-invasive skin cancer imaging, both for clinicians and health care policy makers.
SUMMARY & SAMENVATTING
SUMMARY

In this thesis, incorporating reflectance confocal microscopy (RCM) into a new workflow for the management of basal cell carcinoma (BCC), is being discussed and evaluated. The aim of this thesis was to assess the efficacy of a one-stop-shop concept, using real-time in vivo RCM as a diagnostic tool, for surgical management of BCC. One-stop-shop implies that diagnosis and treatment both take place on the day of initial outpatient clinic consultation.

The general introduction in Chapter 1 describes the rising incidence of skin cancer. BCC is the most common form of all skin cancer and causes a major burden on both patients and current healthcare systems. Also, an introduction on non-invasive skin cancer imaging in the dermatological practice is given.

Current management of BCC relies on the histopathological subtype. Although a punch biopsy is routinely performed to confirm the diagnosis of clinically suspected BCC, its reliability in identifying the most aggressive subtype has been questioned. Therefore we performed a systematic review.

In Chapter 2, electronic databases were searched for articles on this topic. The reference standard was defined as the outcome of histopathological analysis of surgical excision specimen. The quality of included papers was evaluated using the Quality Assessment of Diagnostic Studies tool to assess the risk of bias. Five articles, with a total of 1285 punch-biopsy-proven primary BCCs, met the inclusion criteria. We found that, although only a limited number of publications have investigated this topic, a punch biopsy missed an aggressive BCC subtype in up to 15% of cases. This means that a punch biopsy fails to identify an aggressive BCC in up to one out of six BCCs. We cautioned on using a punch biopsy as standard care in subtyping BCC, both in daily clinical practice and in future clinical research.

As an alternative to routine biopsies for BCC, various non-invasive skin imaging devices have been developed in the past few decades. Of those, RCM has been proposed as a promising non-invasive technique for diagnosing BCC by several clinical studies. However, we found that a systematic overview and quality assessment of those articles was missing. Therefore we performed a systematic review and meta-analysis that is described in Chapter 3. The reference standard was defined as histopathological confirmation of BCC in either biopsy or excision specimen. Electronic databases were searched for relevant articles. The quality of included papers was evaluated using the Quality Assessment of Diagnostic Studies tool to assess the risk of bias. Six studies, with a total of 331 histopathological confirmed BCCs, met the inclusion criteria. We found that sensitivity ranged from 83% to 100% and specificity ranged from 78% to 97%. Despite of the seemingly high sensitivity and specificity, we cautioned that our analysis was based on only six heterogeneous studies that suffered from high risk of bias. As future diagnostic accuracy studies on RCM for BCC were to be expected, we recommended using the Standards for Reporting Diagnostic accuracy studies (STARD) guidelines. In addition, we advised on using histopathological assessment of surgical excision specimen as reference standard.

Chapter 4.1 covers the design of our randomized controlled trial. While RCM had been shown to be promising, high-quality prospective studies were needed to investigate the clinical value and utility of RCM in diagnosing and subtyping BCC. Our study was designed as an open-label, non-inferiority, multicenter clinical trial to examine a one-stop-shop concept using RCM as a diagnostic
tool for surgical management of clinically suspected primary BCC. Consecutive eligible patients of 18 years and older with a clinically suspected, primary, untreated, BCC, regardless of subtype and present for at least one month were prospectively enrolled. After enrollment and written informed consent, patients were randomly assigned (1:1) to the RCM one-stop-shop (diagnosing and subtyping using RCM followed by direct surgical excision) or standard care (planned excision based upon the histological diagnosis and subtype of a punch biopsy). The primary outcome was the proportion of patients with tumor-free margins on the final pathology report of routinely processed tissue specimen after surgical treatment of BCC. Secondary outcomes were the proportion of accurately diagnosed BCC by RCM and a punch biopsy including the most aggressive histological subtype, patients’ throughput time, patient satisfaction and adverse events.

Between February 3rd and October 2nd of 2015 the randomized controlled trial was performed at the Department of Dermatology, Academic Medical Center, University of Amsterdam (coordinating tertiary referral center), and the Department of Dermatology, The Netherlands Cancer Institute (participating tertiary referral center), in Amsterdam, The Netherlands. The results are reported in Chapter 4.2. We found that 73 (77%) of the 95 included patients had a BCC that was histologically confirmed on surgical excision specimen. All (40/40, 100%) in the one-stop-shop group had tumor free margins. In the standard care group tumor free margins were found in all but two (31/33, 94%). Our findings showed that a one-stop-shop concept using RCM was non-inferior to standard care in terms of tumor-free margins after surgical treatment of BCC. We concluded that a one-stop-shop using RCM seems suitable in facilitating early diagnosis and direct treatment for patients suffering from BCC.

In Chapter 5 we performed a diagnostic analysis to assess the accuracy of RCM versus a punch biopsy in diagnosing and subtyping BCC. Our findings showed that for experienced users, RCM had a similar diagnostic accuracy to diagnose and subtype clinically suspected BCC compared to a punch biopsy. Based on these findings RCM could potentially replace a punch biopsy for diagnosing and subtyping selected BCC cases. Yet prior to doing so, results of future and ongoing larger prospective clinical trials, focusing on diagnostic accuracy, reliability and specific criteria, are needed to improve BCC subtype differentiation.

As RCM also relies on individual morphologic pattern recognition that might vary among users, a critical appraisal of the diagnostic procedure was needed in addition to the diagnostic analysis. In Chapter 6 the inter-rater and intra-rater agreement of RCM in correctly diagnosing and subtyping BCC based on static RCM images was determined. In this prospective reliability study the presence and subtype of BCC was evaluated among three raters with varying experience. The raters independently assessed static images of 48 RCM cases twice with four weeks interval. We found that the inter-rater and intra-rater agreement of RCM was reliable in correctly diagnosing BCC and less reliable in subtyping BCC based on static RCM images. RCM could potentially play an important role in BCC management if accurate subtyping will be achieved. Therefore future clinical studies on reliability and specific RCM features for BCC subtypes are required.
SAMENVATTING

In dit proefschrift wordt de klinische toepasbaarheid van niet-invasieve reflectie confocale microscopie (RCM) in een nieuwe werkwijze voor de behandeling van het basaalcelcarcinoom (BCC) besproken en beoordeeld. Het doel van dit proefschrift was om de effectiviteit van een zogenaamde ‘one-stop-shop’, gebruikmakend van in vivo RCM as diagnostisch hulpmiddel, voor de chirurgische behandeling van BCCs te evalueren. Een ‘one-stop-shop’ houdt in dat diagnose en behandeling op dezelfde dag van het initiële consult plaatsvinden.

De algemene introductie in Hoofdstuk 1 beschrijft de stijgende incidentie van huidkanker. BCC is de meest voorkomende vorm van huidkanker en leidt tot een toenemende last voor zowel patient als huidige zorgsystemen. Daarnaast wordt ook een introductie gegeven over niet-invasieve beeldvormende technieken van de huid voor de dagelijkse praktijk.

De huidige behandeling van BCC berust op het histopathologische subtype. Hoewel een punch biopt routinematig wordt uitgevoerd om de diagnose van klinisch suspecte BCC te bevestigen, is de betrouwbaarheid bij het identificeren van het meest agressieve subtype betwijfelijk. Daarom hebben we een systematische review uitgevoerd. In Hoofdstuk 2 werd in elektronische databanken gezocht naar artikelen over dit onderwerp. De referentiestandaard werd gedefinieerd als de uitkomst van histopathologische analyse van chirurgisch excisie weefsel. De kwaliteit van alle gevonden studies werd geëvalueerd met behulp van de ‘Quality Assessment of Diagnostic Studies’ (QUADAS) tool om het risico op bias te beoordelen. Vijf artikelen, met in totaal 1285 punch-biopt-bewezen primaire BCCs, voldeden aan de inclusiecriteria. Alhoewel een beperkt aantal publicaties dit onderwerp heeft onderzocht, vonden wij dat een punch biopt een agressief BCC-subtype kan missen in ongeveer 15% van de gevallen. Dit heeft tot gevolg dat een punch biopt in één op de zes BCCs het agressieve subtype niet goed identificeert. Op basis van deze resultaten hebben wij gewaarschuwd over het gebruik van routinematige punch biopten voor het subtyperen van BCC, zowel in de dagelijkse klinische praktijk als in toekomstig klinisch onderzoek.

Als alternatief voor routinematige biopten voor BCC, zijn er in de afgelopen decennia verschillende niet-invasieve beeldvormende technieken ontwikkeld om suspecte huidaardes te beoordelen. Van deze technieken hebben meerdere klinische studies RCM als veelbelovend beschouwd voor het diagnosticeren van BCC. Er ontbrak echter een systematisch overzicht en kwaliteitsbeoordeling van deze klinische studies. Daarom hebben we een systematische review en meta-analyse uitgevoerd die in Hoofdstuk 3 wordt beschreven. De referentiestandaard werd gedefinieerd als de uitkomst van histopathologische analyse van een punch biopt of chirurgisch excisie weefsel. Elektronische databanken werden gezocht naar relevante artikelen. De kwaliteit van de geïncludeerde studies werd geëvalueerd met behulp van de QUADAS tool om het risico op bias te beoordelen. Zes studies, met in totaal 331 histopathologische bevestigde BCC’s, voldeden aan de inclusiecriteria. We vonden dat de sensitiviteit varieerde van 83% tot 100% en de specificiteit van 78% tot 97%. Ondanks de schijnbaar hoge sensitiviteit en specificiteit, hebben we erop gewezen dat onze analyse gebaseerd was op slechts zes heterogene studies met een hoog risico op bias. Aangezien toekomstig diagnostische accurate onderzoek naar de rol van RCM voor BCC te verwachten was, hebben wij aanbevolen om de ‘Standards for Reporting Diagnostic accuracy
studies’ (STARD) richtlijnen te handhaven. Daarnaast adviseerden we om histopathologische beoordeling van chirurgisch excisie weefsel als referentiestandaard te gebruiken in plaats van het punch biopt.

**Hoofdstuk 4.1** behandelt het ontwerp en de opzet van onze eigen klinische gerandomiseerde gecontroleerde studie. Terwijl RCM veelbelovend leek voor het diagnosticeren en subtyperen van BCC, waren studies van hoge kwaliteit nodig om de klinische waarde en toepasbaarheid van RCM verder te onderzoeken. Onze studie was ontworpen als een open-label, niet-inferioriteit, multicenter klinische trial om het ‘one-stop-shop’ concept met behulp van RCM als diagnostisch hulpmiddel te onderzoeken voor de chirurgisch behandeling van klinisch suspecte primaire BCCs. Opeenvolgende patiënten van 18 jaar en ouder met een klinisch suspecte, primaire, onbehandelde, BCC, ongeacht subtype en aanwezig voor ten minste een maand, kwamen in aanmerking voor deelname. Na schriftelijke toestemming werden patiënten willekeurig toegewezen (1: 1) aan de ‘RCM one-stop-shop’ (diagnose en subtypering met behulp van RCM gevolgd door directe chirurgische excisie) of ‘standaardzorg’ (geplande excisie op basis van de histologische diagnose en subtype van een punch biopt). Onze primaire uitkomst was het percentage patiënten met tumorvrije marges conform het pathologieverslag na conventionele chirurgische behandeling van BCC. Secundaire uitkomsten waren het percentage BCCs die goed werden gediagnosticeerd door RCM dan wel het punch biopt inclusief het meest agressieve histologische subtype, de doorlooptijd van patiënten, patiënt tevredenheid en mogelijke complicaties. In de periode van 3 februari tot en met 2 oktober 2015 werd deze studie uitgevoerd op de afdeling Dermatologie van het Academisch Medisch Centrum van de Universiteit van Amsterdam (coördinerend centrum) en de afdeling Dermatologie van het Nederlands Kanker Instituut (NKI) in Amsterdam, Nederland. De studieresultaten zijn beschreven in **Hoofdstuk 4.2**. We vonden dat in 73 (77%) van de 95 geïncludeerde patiënten het klinisch suspect BCC histologisch werd bevestigd in chirurgisch excisie weefsel. Alle (40/40, 100%) patiënten in de ‘RCM one-stop-shop’ groep hadden tumorvrije marges. In de ‘standaardzorg’ groep werden tumorvrije marges gevonden bijna alle patiënten (31/33, 94%). Uit onze bevindingen bleek dat het ‘RCM one-stop-shop’ concept niet-inferieur was aan de ‘standaardzorg’ in termen van tumorvrije marges na chirurgische behandeling van BCC. Wij concludeerden dat een ‘RCM one-stop-shop’ geschikt lijkt voor het faciliteren van vroege diagnose en directe behandeling voor patiënten met BCC.

In **Hoofdstuk 5** hebben we een diagnostische analyse uitgevoerd om de nauwkeurigheid van RCM ten opzichte van het punch biopt te beoordelen bij het diagnosticeren en subtyperen van BCC. Onze bevindingen toonden aan dat voor ervaren gebruikers, RCM een vergelijkbare diagnostische nauwkeurigheid had om klinisch suspecte BCCs te diagnosticeren en te subtyperen in vergelijking met een punch biopt. Op basis van deze bevindingen concludeerden wij dat RCM mogelijk een punch biopt zou kunnen vervangen voor het diagnosticeren en subtyperen van een geselecteerd aantal BCCs. Echter voordat RCM een punch biopt daadwerkelijk kan vervangen, moeten gebruikers beter in staat zijn om met behulp van RCM het BCC subtype te bepalen. Hiervoor zijn resultaten van toekomstige prospectieve klinische studies nodig die zich richten op diagnostische nauwkeurigheid, betrouwbaarheid en BCC subtype afhankelijke RCM criteria. Aangezien RCM ook afhankelijk is van individuele morfologische patronen en herkenning die onder gebruikers kan variëren,
was een kritische beoordeling van de diagnostische procedure ook vereist. In Hoofdstuk 6 werd de inter-beoordelaar en intra-beoordelaar overeenkomst van RCM bepaald voor het correct diagnosticeren en subtyperen van BCC op basis van statische RCM beelden. In deze prospectieve betrouwbaarheid studie werd de diagnose en subtype van BCC onder drie beoordelaars met variërende RCM ervaring geëvalueerd. De beoordelaars beoordeelden onafhankelijk de statische RCM beelden van 48 casus, twee keer, met vier weken interval. We vonden dat de inter-beoordelaar en intra-beoordelaar overeenkomst van RCM betrouwbaar was bij voor het correct diagnosticeren van BCC en minder betrouwbaar voor het subtyperen van BCC op basis van statische RCM beelden. RCM zou in potentie een belangrijke rol kunnen spelen in de zorg rondom BCC als het juiste subtype accuraat herkend zou kunnen worden. Hiervoor zijn toekomstige klinische studies nodig die verder onderzoek doen naar de betrouwbaarheid en specifieke BCC subtype afhankelijke RCM criteria.
PHD PORTFOLIO
ABOUT THE AUTHOR
DANKWOORD
**PHD PORTFOLIO**

Name: D.J. Kadouch  
PhD period: October 2012 – October 2017  
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Co-promotores: Dr. A. Wolkerstorfer, Dr. M.W. Bekkenk  
Institution: University of Amsterdam, Academic Medical Center, Department of Dermatology

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<tr>
<td>Seminars, workshops and master classes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weekly department seminars, AMC</td>
<td>2012-2017</td>
<td>280</td>
</tr>
<tr>
<td>Clinical research meeting every two months</td>
<td>2012-2017</td>
<td>140</td>
</tr>
<tr>
<td>Histo-confocal course, Munich, Germany</td>
<td>2012</td>
<td>30</td>
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<tr>
<td>Confocal expert course, Modena, Italy</td>
<td>2014</td>
<td>40</td>
</tr>
<tr>
<td>Skin surgery evidence based update, Nottingham, United Kingdom</td>
<td>2015</td>
<td>10</td>
</tr>
<tr>
<td>Ex vivo confocal course, Barcelona, Spain</td>
<td>2015</td>
<td>30</td>
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<tr>
<td>EADV Review</td>
<td>2015</td>
<td>30</td>
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<tr>
<td>(Inter)national presentations</td>
<td></td>
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<tr>
<td>One-stop-shop with confocal imaging versus standard care for basal cell carcinoma; a randomized controlled trial, International Confocal Working Group (ICG), Copenhagen, Denmark</td>
<td>2015</td>
<td>20</td>
</tr>
<tr>
<td>One-stop-shop with confocal imaging versus standard care for basal cell carcinoma; a randomized controlled trial, Dutch Society of Experimental Dermatology (NVED), Lunteren, The Netherlands</td>
<td>2016</td>
<td>20</td>
</tr>
<tr>
<td>Case of a deep mycosis, Dutch Society of Dermatology and Venereology (NVDV), Amsterdam, The Netherlands</td>
<td>2016</td>
<td>20</td>
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<tr>
<td>One-stop-shop with confocal imaging versus standard care for basal cell carcinoma; a randomized controlled trial, International Society of Dermatologic Surgery (ISDS), Amsterdam, The Netherlands</td>
<td>2016</td>
<td>20</td>
</tr>
<tr>
<td>(Inter)national conferences</td>
<td></td>
<td></td>
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<tr>
<td>European Academy of Dermatology and Venereology (EADV), Amsterdam, The Netherlands</td>
<td>2014</td>
<td>30</td>
</tr>
<tr>
<td>International Society of Dermatologic Surgery (ISDS), Jerusalem, Israel</td>
<td>2014</td>
<td>30</td>
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<tr>
<td>European Academy of Dermatology and Venereology (EADV), Copenhagen, Denmark</td>
<td>2015</td>
<td>30</td>
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<tr>
<td>Dutch Society of Experimental Dermatology (NVED), Lunteren, The Netherlands</td>
<td>2016</td>
<td>30</td>
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<tr>
<td>International Society of Dermatologic Surgery (ISDS), Amsterdam, The Netherlands</td>
<td>2016</td>
<td>30</td>
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<tr>
<td>European Academy of Dermatology and Venereology (EADV), Vienna, Austria</td>
<td>2016</td>
<td>30</td>
</tr>
</tbody>
</table>
PhD PORTFOLIO (continued)

<table>
<thead>
<tr>
<th>Year</th>
<th>Workload (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>American Academy of Dermatology (AAD), Orlando, Florida, USA</td>
<td>2017</td>
</tr>
<tr>
<td>Dermatologendagen, Papendal, The Netherlands</td>
<td>2017</td>
</tr>
</tbody>
</table>

2. Teaching

Mentoring
Anne van Haersma de With, UvA student: reliability of confocal microscopy imaging for diagnosing and subtyping basal cell carcinoma 2015-2017 40

Supervising
Bob de Vries, UvA student, bachelor thesis: diagnostic accuracy of a punch biopsy for subtyping basal cell carcinoma; a systematic review 2015 40

3. Parameters of Esteem

Other
Peer reviewer for several international scientific medical journals 2014-present
ABOUT THE AUTHOR

Daniel Kadouch was born on June 5th, 1982, in Senlis, France. He grew up in Amsterdam, The Netherlands. After graduation at the JSG Maimonides (2001) he studied Biomedical Sciences at the VU University in Amsterdam. Prior to completing his Bachelor of Science (2005) he went on to study Medicine at the VU University Medical Center in 2004. Highlights during his study were an internship at the Department of Plastic and Reconstructive Surgery of the Sourasky Medical Center in Tel Aviv, Israel under supervision of Dr. Eyal Gur and a research internship at the Laboratory for Bioregenerative Medicine and Surgery (LBMS) of Weill Cornell Medical College in New York City, New York, USA, under supervision of Dr. Jason Spector. During his research internship in New York City, Daniel was the recipient of the New York Presbyterian’s Division of Plastic Surgery Basic Science Research Award. His research projects at the LBMS resulted in his first international scientific publications.

After receiving his medical degree in 2010, Daniel worked as a resident at the Department of Plastic and Reconstructive Surgery and the Department of General Surgery of the OLVG Hospital in Amsterdam. During this period Daniel was involved in different research projects and became interested in Dermatology.

In 2012 Daniel started his Dermatology residency at the Department of Dermatology of the Academic Medical Center in Amsterdam (AMC), under supervision of Dr. J.R. Mekkes. Fascinated by research, he simultaneously began his PhD at the Department of Dermatology of the AMC under supervision of Prof. Dr. M.A. de Rie, Dr. A. Wolkerstorfer and Dr. M.W. Bekkenk. The results of his PhD research are described in this thesis and were presented at several national and international scientific conferences.

Daniel completed his Dermatology residency in 2017, after which he started his career as Dermatologist at Centrum Oosterwal in Alkmaar, The Netherlands.
DANKWOORD

En dan tot slot het meest gelezen onderdeel van een proefschrift. In de afgelopen vijf jaar heb ik veel hulp, steun en inspiratie mogen ontvangen. Mijn dank gaat uit naar iedereen die heeft bijgedragen aan de totstandkoming van dit proefschrift, op welke manier dan ook. Graag wil ik ook alle patiënten bedanken voor hun deelname aan onze studie.

Om te beginnen mijn gezin

Allerliefste Evelyne, men zal vast niet geloven dat ik zeer sporadisch de meest onmogelijke persoon ter wereld kan zijn maar jij weet wel beter. Ik kan je niet genoeg bedanken voor de liefde, het geduld en de betrokkenheid.

Mes amours de ma vie, merci ! Vous êtes une inspiration magique. Maman et moi vous aimes infiniment.

Prof. dr. M.A. de Rie, mijn promotor

Beste Menno, kort na jouw aantreden als afdelingshoofd in 2012 sloten wij met elkaar een ‘package-deal’; het opzetten en uitvoeren van promotieonderzoek in combinatie met mijn opleiding tot dermatoloog. Inmiddels blijkt het een geslaagd plan te zijn geweest. Bedankt voor het vertrouwen maar ook de bijzonder prettige sturing en begeleiding gedurende het promotietraject. Ondanks jouw drukke agenda reageerde jij meestal als eerste met correcties op mijn voorlopige manuscript versies. Ik had me het niet beter kunnen wensen!

Dr. A. Wolkerstorfer en Dr. M.W. Bekkenk, mijn co-promotoren

Beste Albert en Marcel, samen met Menno vormden jullie mijn thesis ‘dreamteam’. Ondanks dat onze thesis besprekingen niet veel langer dan 15 minuten duurden hebben we veel voor elkaar gekregen.

Albert, ik herinner mij nog goed onze eerste research bespreking in de bus tijdens het derma-ski weekend in Oostenrijk. Jouw enthousiasme over onderzoek was direct aanstekelijk. Sindsdien hebben we veel ad hoc overleg momenten gehad waarbij jij altijd de tijd en rust nam om uitgebreid lopende zaken te bespreken. Bedankt voor jouw ideeën, inspiratie en adviezen. Ik blijf ten alle tijden bereikbaar voor de speciale voedingsadviezen.

Marcel, tijdens ons ‘evidence based medicine’ tweedaagse in Nottingham was jij akkoord om mij, samen met Albert, als co-promotor te begeleiden. Aan mij de eer om jouw eerste promovendus te zijn. Bedankt voor de vriendschap die is ontstaan de afgelopen jaren. Ik kijk ernaar uit om onze toekomstplannen tot een succes te maken!
**Co-auteurs**

Beste Phyllis, graag zou ik jou in het bijzonder willen bedanken. Jouw scherpe blik op de systematische review, studie design en het daaropvolgend manuscript was cruciaal. Van jouw kritische feedback op het onderzoek heb ik veel geleerd, maar ook van de fijne gesprekken daarbuiten.

Beste Markus, jij hebt mij geïntroduceerd in de wereld van de confocale microscopie tijdens de ‘histo-confocal’ cursus in München. Ik heb veel bewondering voor jouw passie van de klinische dermatologie en de histopathologie. Bedankt voor de leerzame jaren in het AMC.

Beste Allard, zonder jouw pathologie expertise waren onze studies niet mogelijk geweest. Bedankt voor het laagdrempelig overleg en de tijd die jij hebt vrijgemaakt om mee te werken aan ons onderzoek.

Beste Mariska, een diagnostisch accuratesse onderzoek zonder jouw methodologische deskundigheid kan ik mij niet voorstellen. Tijdens ons overleg kon jij altijd een goede balans houden tussen klinische relevantie versus de methodologie van het onderzoek. Bedankt voor jouw tijd en kritische kijk op de manuscripten.

Beste Jacqueline, bedankt voor jouw bijdrage aan de systematische reviews.

Beste Marianne, Biljana en Yannick, dankzij jullie hebben we een multicenter studie kunnen opzetten. Dank voor de fijne samenwerking. Yannick, veel succes met het voortzetten van de confocale onderzoeksplannen en uiteraard de opleiding tot dermatoloog. Dear Kasia, thank you for helping us out with the study statistics. I enjoyed working together with you.

Beste Anna, tijdens jouw coschap dermatologie besloot je om ons te helpen met het onderzoek. Bedankt voor jouw inzet en succes met het doorzetten van jouw klinische en research plannen binnen de dermatologie!

Beste Malou, Sanna en Iris, bedankt voor jullie hulp bij de opzet en uitwerking van de laatste studie. Malou succes met je confocale studie in Nijmegen, ik ben erg benieuwd naar de uitkomsten en hoop in de toekomst vaker met je te kunnen samenwerken.

**Promotiecommissie**

Prof. dr. C.J.M. van Noesel, Prof. dr. C.M.A.M. van der Horst, Prof. dr. R. Hoekzema, Prof. dr. A.J.M. Balm, Dr. N.W.J. Kelleners-Smeets, Dr. S. González Rodríguez, leden van de promotiecommissie – ik dank u allen voor uw deelname aan de commissie en voor de kritische beoordeling van mijn
proefschrift. Dear Salvador González, thank you for critically reviewing my thesis manuscript and for travelling from abroad to be present as a member of the doctoral examination board.

Collega’s van de afdeling dermatologie van het AMC
Op de eerste plaats wil ik hier Dr. J.R. Mekkes bedanken, mijn opleider. Beste Jan, bedankt voor de fantastische opleidingstijd waar (bijna) alles mogelijk was. Lieve doktersassistenten, verpleegkundige, (oud-) aios, overige stafleden, en andere collega’s, ontzettend bedankt voor jullie steun, begrip en interesse. Mariska, Carmen en Bernice, bedankt voor de altijd aanwezige ondersteuning, zelfs toen ik in het buitenland verbleef kon dankzij jullie alles te allen tijde geregeld worden.

Paranimfen
Jonathan, niet zomaar een paranymf, maar ook mijn broer, vriend en zelfs collega dermatoloog. Zoveel als wij op elkaar lijken zoveel verschillen wij ook van elkaar. De ene ‘Kadouchian’ is de ander niet! Hoe bijzonder dat wij dan toch binnen de dermatologie dezelfde interesses en ambities delen. Bedankt voor jouw continue support, strategisch overleg, advies en broederschap. Ik kijk uit naar meer broeder ‘quality-time’ tijdens alle buitenlandse congressen die wij samen zullen ‘moeten’ bezoeken.

Shai, ook niet zomaar een paranymf. Onze vriendschap gaat terug naar de eerste brugklas van de middelbare school. Sindsdien hebben we samen een bijzondere band opgebouwd die onvoorwaardelijk is en het meeste lijkt op broederschap. Tijdens onze tienerjaren kon ik dankzij jou altijd Amsterdam Zuidoost verruilen voor Amstelveen. We hebben in de afgelopen 20+ jaar veel bijzondere momenten met elkaar gedeeld, zowel de mooiste als de meest verdrietige life-events. Het toeval wou dat wij beiden arts zijn geworden, en op dezelfde dag onze specialisatie in het AMC hebben afgerond. Ik verheug me erop om ook deze nieuwe fase in ons leven met elkaar te delen. Dank voor alles.

Vrienden
Mannen (ook jij Josh), dank voor de vriendschap en de daaraan gekoppelde fantastische heren diners. Dat we deze traditie lang mogen blijven voortzetten met elkaar!

Mijn familie en schoonfamilie
Bedankt voor jullie interesse en betrokkenheid bij mijn werkzaamheden. Broertjes, wij hebben iets unieks met zijn vijven, waarbij de ene Kadouch net iets knapper is dan de ander.

Lieve Mama, bedankt voor jouw onvoorwaardelijke liefde. Vanuit een wetenschappelijk oogpunt, waarbij statistiek leidend is, heb jij als alleenstaande moeder met vijf jongens in Amsterdam Zuidoost een statistische onwaarschijnlijkheid bereikt. Wij zijn als zoons bevoorrecht met jou als moeder, en onze kinderen met jou als super oma. Laten we onwijs genieten van deze bijzondere tijd samen!
Through the looking glass: confocal microscopy imaging of basal cell carcinoma

Daniel J. Kadouch