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ORIGINAL ARTICLE

Diagnostic accuracy of confocal microscopy imaging vs. punch biopsy for diagnosing and subtyping basal cell carcinoma

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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 vs. a punch biopsy in diagnosing and subtyping BCC is unknown.

Objective To assess diagnostic accuracy of RCM vs. 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 3 February 2015 and 2 October 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 vs. punch biopsy) and reference standard (excision specimen) in correctly diagnosing BCC. The secondary outcome was 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% vs. 93.94%), but a punch biopsy was more specific than RCM (79% vs. 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% vs. 77% by a punch biopsy.

Conclusion Reflectance confocal microscopy 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.

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Conflicts of interest

We declare that we have no conflict of interests.

Financial disclosure and products

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.[1,2] Although considered as the most reliable diagnostic technique, a

punch biopsy fails to diagnose an aggressive subtype in up to one of six BCCs.[3] 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 non-surgical first-line therapy for superficial BCC.[4] As for nodular and aggressive BCC, surgical treatment with excision margins of, respectively, 3 or 5 mm 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.[5–8]

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) vs. standard care for surgical treatment of BCC.[9] To determine the clinical value and utility of RCM vs. a punch biopsy in diagnosing and subtyping BCC, a diagnostic analysis is needed. Besides, previous diagnostic testing of RCM and punch biopsy for BCC was not done in accordance with the Standards of Reporting of Diagnostic Accuracy (STARD).[3,10,11]

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

Methods

Study design
Diagnostic accuracy data were prospectively collected alongside the multicentre 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.[12]

Participants
Between 3 February 2015 and 2 October 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 1 month. We excluded lesions on high-risk areas of the face (H-zone and ears), lesions larger than 20 mm, 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: (i) chest, (ii) extremities, and (iii) 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, USA; MAVIG GmbH, München, Germany) to diagnose and subtype BCC followed by direct surgical excision according to previously published protocol.[12] 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 1 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 <1 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 BCC presence and subtype through a secured online teleconsultation platform designed to share RCM cases (VivaNet®, MAVIG, GmbH).[13] 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 3-mm punch biopsy was performed from the most elevated part of the lesion using infiltration anaesthesia (2% xylocaine/adrenaline 1 : 80 000). Biopsy specimens were subsequently analysed by an experienced pathologist within 2 weeks. Surgical excision of the lesion with adequate margins was performed within the following 4 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) Histopathological confirmation of presence and subtype of BCC and inspection of resection margins with the use of haematoxylin and eosin

stained sections taken from the excision specimen was defined as the reference standard.

An independent dermatologist or independent dermatology resident supervised by a dermatologist performed surgery under local anaesthetics (2% xylocaine/adrenaline 1 : 80 000) followed by primary wound closure in both treatment groups. Clinically suspected BCCs that were not confirmed by either RCM or punch biopsy were surgically treated with a 3-mm 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.[14] 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 vs. punch biopsy were separately compared to surgical excision for all tumours. The primary outcome was the agreement between the index tests (RCM vs. punch biopsy) and reference standard (excision specimen) in correctly diagnosing BCC. The secondary outcome was 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 was 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 (SPSS Inc., Chicago, IL, USA).

Results

Participants

A total of 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 who did not receive imaging and three patients in the standard care group who did not receive surgical treatment (Fig. 1).

Baseline characteristics are shown in Table 1. In the RCM on-stop-shop group, 40 BCCs were confirmed by surgical excision specimen compared to 33 BCCs 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 10 weeks (66 days).

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 vs. 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] vs. 93.94% [79.77–99.26]), but a punch biopsy was more specific than RCM (79% [49.20–95.34] vs. 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].

RCM vs. punch biopsy for subtyping BCC Table 3 shows the agreement between RCM vs. 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) vs. 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) vs. 10% (3/31) by punch biopsy. Understaging of BCC subtype was seen in 13% of both RCM and a punch biopsy (5/38 vs. 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 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
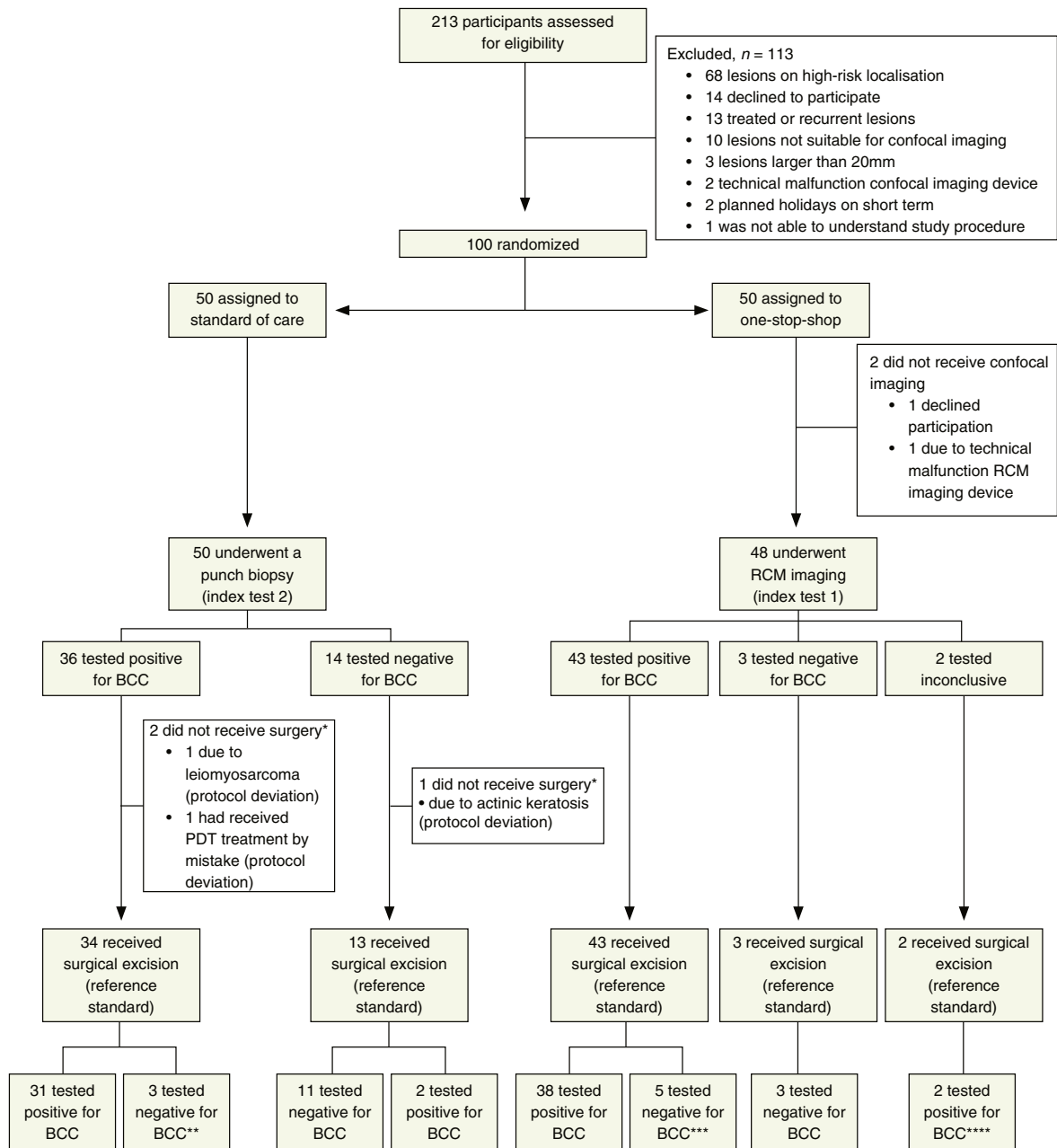
Figure 1 Flow chart. BCC, basal cell carcinoma; PDT, photodynamic therapy. Two patients in the RCM one-stop-shop group did not begin diagnosis and treatment. One refused directly after randomization, and the other one could not participate due to technical malfunction of the confocal imaging device. *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 histological 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.

Table 1 Tumour and patient characteristics separated by treatment group

	One-stop-shop (n = 50)	Standard of care (n = 50)
Age (years)	64 (39–88)	68 (41–92)
Sex		
Men	31 (62%)	25 (50%)
Women	19 (38%)	25 (50%)
Fitzpatrick skin type		
I	8 (16%)	4 (8%)
II	32 (64%)	43 (86%)
III	10 (20%)	3 (6%)
BCC in medical history		
Yes	34 (68%)	37 (74%)
No	15 (30%)	13 (26%)
Study site		
Academic Medical Centre	37 (74%)	38 (76%)
Netherlands Cancer Institute	13 (26%)	12 (24%)
Immunocompromised†		
Yes	4 (8%)	4 (8%)
No	46 (92%)	46 (92%)
Tumour diameter (mm)	8 (3–15)	8 (3–20)
Tumour location		
Head/neck	9 (18%)	12 (24%)
Trunk	32 (64%)	30 (60%)
Arm	4 (8%)	7 (14%)
Leg	5 (10%)	1 (2%)
Number of BCC	40 (80%)	33 (66%)
BCC subtype distribution‡		
Superficial BCC	17 (43%)	14 (42%)
Nodular BCC	17 (43%)	17 (52%)
Aggressive BCC	6 (14%)	2 (6%)

†Patients who were taking immunosuppressive drugs such as oral steroids, methotrexate, ciclosporin for suppression of immunological disorder, or to prevent transplant rejection.
‡This number represents 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.
Continuous variables are expressed as mean (range) and categorical variables as n (%).
BCC, basal cell carcinoma.

included four patients of the RCM one-stop-shop group with postoperative 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 anticoagulant medication developed an excessive postoperative bleeding requiring hospitalization for 3 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.[15] However as reported by Que et al., most of these studies involved RCM experts with prior experience in RCM interpretation.[15] Our results confirm the high sensitivity of RCM for diagnosing BCC (100%), but the specificity ranged from 38% (RCM users with <1 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 a punch biopsy (79% [49.20–95.34]). A lower RCM specificity was previously reported by Rao et al.[16] that studied RCM users with varying levels of experience. Furthermore, Farnetani et al.[17] also recently emphasized on the importance of the RCM learning curve and confirmed that diagnostic accuracy of RCM increases with experience.

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 between 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.[18] Non-invasive skin imaging could play a crucial role in improving BCC health care for both patients and clinicians.[19] 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 behavioural responses to testing should also be taken into consideration.[20,21] 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.[22]

Study strengths include adherence to the STARD guidelines.[10] Furthermore, we prevented sampling error using final surgical excision specimen as our reference standard instead of a punch biopsy. We also prevented heterogeneity of our results using predefined RCM criteria and using the same VivaScope 1500® device at both participating centres. 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.

Table 2 Diagnostic performance of RCM vs. a punch biopsy in diagnosing BCC compared to surgical excision

RCM (DK/YE) trial period	Surgical excision		Total
	BCC	No BCC	
BCC	**38**	5	43
No BCC	0	3	3
Total	38	8	46
Punch biopsy trial period	**Surgical excision**		**Total**
	BCC	No BCC	
BCC	**31**	3	34
No BCC	2	11	13
Total	33	14	47
RCM expert (MU) after trial period	**Surgical excision**		Total
	BCC	No BCC	
BCC	**27**	2	29
No BCC	0	6	6
Total	27	8	35
RCM expert (CL) after trial period	**Surgical excision**		Total
	BCC	No BCC	
BCC	**24**	2	26
No BCC	0	6	6
Total	24	8	32

BCC vs. no BCC	Sensitivity % (n) [95% CI]	Specificity % (n) [95% CI]	Positive LR [95% CI]	Negative LR [95% CI]	PPV [95% CI]	NPV [95% CI]
RCM (DK/YE) trial period (n = 46)	100% (38/38) [90.75–100]	37.50% (3/8) [8.52–75.51]	1.60 [0.94–2.74]	0	88.37% [81.63–92.86]	100%
Punch biopsy trial period (n = 47)	93.94% (31/33) [79.77–99.26]	78.57% (11/14) [49.20–95.34]	4.38 [1.60–12.00]	0.08 [0.02–0.30]	91.18% [76.32–98.14]	84.62% [54.55–98.08]
RCM expert (MU) after trial period (n = 35)	100% (26/26) [87.23–100]	75% (6/8) [34.91–96.81]	4.00 [1.20–13.82]	0	93.10% [80.26–97.82]	100%
RCM expert (CL) after trial period (n = 32)	100% (24/24) [85.75–100]	75% (6/8) [34.91–96.81]	4.00 [1.20–13.82]	0	92.31% [78.32–97.55]	100%

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.

Limitations of our study include the limited sample size of aggressive BCC. Our randomized controlled trial 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.[12] Another important limitation that needs to be considered when interpreting the results is the potential bias in favour 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, BCCs 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 since the introduction of the VivaScope 3000® flexible hand-held version (VivaScope 3000®; CaliberID; MAVIG GmbH), that permits imaging of the more concave and convex high-risk facial areas.[23]

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.[24] Besides RCM, more and more studies are reporting on the added value of optical coherence tomography (OCT) for diagnosing and subtyping BCC.[25–28] In addition, a first report on a combined RCM/OCT skin modality for *ex vivo* BCC detection has been published.[29] 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.

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

RCM (DK/YE) trial period	Surgical excision			Total
	sBCC	nBCC	aBCC	
sBCC	**14**	2	1	17
nBCC	1	**10**	2	13
aBCC	2	4	**2**	8
Total	17	16	5	38
Punch biopsy trial period	**Surgical excision**			**Total**
	sBCC	nBCC	aBCC	
sBCC	**13**	4	0	17
nBCC	0	**9**	0	9
aBCC	0	3	**2**	5
Total	13	16	2	31
RCM expert (MU) after trial period	**Surgical excision**			**Total**
	sBCC	nBCC	aBCC	
sBCC	**13**	1	0	14
nBCC	1	**9**	0	10
aBCC	1	1	**1**	3
Total	15	11	1	26
RCM expert (CL) after trial period	**Surgical excision**			**Total**
	sBCC	nBCC	aBCC	
sBCC	**3**	0	0	18
nBCC	1	**8**	0	10
aBCC	9	2	**1**	6
Total	13	10	1	24

Bold numbers indicate concordant cases.
aBCC, aggressive basal cell carcinoma; BCC, basal cell carcinoma; nBCC, nodular basal cell carcinoma; RCM, reflectance confocal microscopy imaging; sBCC, superficial basal cell carcinoma.

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.

Acknowledgements

References

1 Trakatelli M, Morton C, Nagore E et al. Update of the European guidelines for basal cell carcinoma management. Eur J Dermatol 2014; 24: 312–329.
2 Telfer NR, Colver GB, Morton CA, British Association of D. Guidelines for the management of basal cell carcinoma. Br J Dermatol 2008; 159: 35–48.
3 Kadouch DJ, van Haersma de With A, Limpens J et al. Is a punch biopsy reliable in subtyping basal cell carcinoma? A systematic review Br J Dermatol 2016; 175: 401–403.
4 Arits AH, Mosterd K, Essers BA et al. Photodynamic therapy versus topical imiquimod versus topical fluorouracil for treatment of superficial basal-cell carcinoma: a single blind, non-inferiority, randomised controlled trial. Lancet Oncol 2013; 14: 647–654.
5 Bath-Hextall FJ, Perkins W, Bong J, Williams HC. Interventions for basal cell carcinoma of the skin. Cochrane Database Syst Rev 2007; (1): CD003412.
6 Kauvar AN, Cronin T Jr, Roenigk R, Hruza G, Bennett R. American Society for Dermatologic S. Consensus for nonmelanoma skin cancer treatment: basal cell carcinoma, including a cost analysis of treatment methods. Dermatol Surg 2015; 41: 550–571.
7 Flohil SC, van Dorst AM, Nijsten T, Martino Neumann HA, Munte K. Mohs micrographic surgery for basal cell carcinomas: appropriateness of 'Rotterdam' criteria and predictive factors for three or more stages. J Eur Acad Dermatol Venereol 2013; 27: 1228–1235.
8 Dutch Society for Dermatology and Venereology. Evidence-based guideline for treatment of basal cell carcinoma. http://www.nvdv.nl/wp-content/uploads/2014/08/20160725-eindversie-richtlijn-BCC-2015.pdf2015. Accessed on 17 April 2017.
9 Kadouch DJ, Elshot YS, Zupan-Kajcovski B et al. 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. Br J Dermato 2017. DOI: 10.1111/bjd.15559.
10 Bossuyt PM, Reitsma JB, Bruns DE et al. STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. BMJ 2015; 351: h5527.
11 Kadouch DJ, Schram ME, Leeflang MM, Limpens J, Spuls PI, de Rie MA. In vivo confocal microscopy of basal cell carcinoma: a systematic review of diagnostic accuracy. J Eur Acad Dermatol Venereol 2015; 29: 1890–1897.
12 Kadouch DJ, Wolkerstorfer A, Elshot Y et al. Treatment of Basal Cell Carcinoma Using a One-Stop-Shop With Reflectance Confocal Microscopy: Study Design and Protocol of a Randomized Controlled Multicenter Trial. JMIR Res Protoc 2015; 4: e109.
13 Longo C, Hemmer P, Pellacani G. Tele-reflectance confocal microscopy. In: Soyer HP, Binder M, Smith AC, Wurm EMT, eds. Telemedicine in Dermatology. Springer Verlag Berlin Heidelberg, New York, NY, 2012: 73–77.
14 Rippey JJ. Why classify basal cell carcinomas? Histopathology 1998; 32: 393–398.
15 Que SK, Grant-Kels JM, Longo C, Pellacani G. Basics of confocal microscopy and the complexity of diagnosing skin tumors: new imaging tools in clinical practice, diagnostic workflows, cost-estimate, and new trends. Dermatol Clin 2016; 34: 367–375.
16 Rao BK, Mateus R, Wassef C, Pellacani G. In vivo confocal microscopy in clinical practice: comparison of bedside diagnostic accuracy of a trained physician and distant diagnosis of an expert reader. J Am Acad Dermatol 2013; 69: e295–e300.
17 Farnetani F, Scope A, Braun RP et al. Skin cancer diagnosis with reflectance confocal microscopy: reproducibility of feature recognition and accuracy of diagnosis. JAMA Dermatol 2015; 151: 1075–1080.
18 van der Geer S, Reijers HA, van Tuijl HF, de Vries H, Krekels GA. Need for a new skin cancer management strategy. Arch Dermatol 2010; 146: 332–336.
19 Que SK. Research techniques made simple: noninvasive imaging technologies for the delineation of basal cell carcinomas. J Invest Dermatol 2016; 136: e33–e38.
20 Gopalakrishna G, Langendam MW, Scholten RJ, Bossuyt PM, Leeflang MM. Defining the clinical pathway in cochrane diagnostic test accuracy reviews. BMC Med Res Methodol 2016; 16: 153.
21 Ferrante di Ruffano L, Hyde CJ, McCaffery KJ, Bossuyt PM, Deeks JJ. Assessing the value of diagnostic tests: a framework for designing and evaluating trials. BMJ 2012; 344: e686.

22 Manubens E, Barreiro A, Bennassar A *et al.* Fast evaluation and monitoring of ingenol mebutate treatment of multiple basal cell carcinomas by in vivo hand-held reflectance confocal microscopy. *J Eur Acad Dermatol Venereol* 2017; **31**: e284–e286.

23 Cinotti E, Jaffelin C, Charriere V *et al.* Sensitivity of handheld reflectance confocal microscopy for the diagnosis of basal cell carcinoma: a series of 344 histologically proven lesions. *J Am Acad Dermatol* 2015; **73**: 319–320.

24 Peppelman M, Nguyen KP, Alkemade HA *et al.* Diagnosis of basal cell carcinoma by reflectance confocal microscopy: study design and protocol of a randomized controlled multicenter trial. *JMIR Res Protoc* 2016; **5**: e114.

25 Olsen J, Themstrup L, De Carvalho N, Mogensen M, Pellacani G, Jemec GB. Diagnostic accuracy of optical coherence tomography in actinic keratosis and basal cell carcinoma. *Photodiagnosis Photodyn Ther* 2016; **16**: 44–49.

26 Ulrich M, von Braunmuehl T, Kurzen H *et al.* The sensitivity and specificity of optical coherence tomography for the assisted diagnosis of non-pigmented basal cell carcinoma: an observational study. *Br J Dermatol* 2015; **173**: 428–435.

27 Meekings A, Utz S, Ulrich M *et al.* Differentiation of basal cell carcinoma subtypes in multi-beam swept source optical coherence tomography (MSS-OCT). *J Drugs Dermatol* 2016; **15**: 545–550.

28 Cheng HM, Lo S, Scolyer R, Meekings A, Carlos G, Guitera P. Accuracy of optical coherence tomography for the diagnosis of superficial basal cell carcinoma: a prospective, consecutive, cohort study of 168 cases. *Br J Dermatol* 2016; **175**: 1290–1300.

29 Iftimia N, Peterson G, Chang EW, Maguluri G, Fox W, Rajadhyaksha M. Combined reflectance confocal microscopy-optical coherence tomography for delineation of basal cell carcinoma margins: an ex vivo study. *J Biomed Opt* 2016; **21**: 16006.