Reflectance Confocal Microscopy in the Diagnosis of Non-Melanoma Skin Cancer and Benign Lesions Versus Normal Skin: A Blinded Prospective Trial

M Amjadi, B Coventry, J Greenwood AM

Citation

M Amjadi, B Coventry, J Greenwood AM. *Reflectance Confocal Microscopy in the Diagnosis of Non-Melanoma Skin Cancer and Benign Lesions Versus Normal Skin: A Blinded Prospective Trial*. The Internet Journal of Plastic Surgery. 2010 Volume 7 Number 2.

Abstract

Background Non-melanoma skin cancers (NMSC) are the most commonly diagnosed cancers in Australia[1]. Reflectance Confocal Microscopy (RCM) generates images comparable to histology. Past clinical trials on NMSC using RCM have shown promising results[2 3 4], but the role of RCM needs better definition. Objectives We aimed to compare RCM to excision biopsy histology in NMSC management in an Australian population to evaluate its diagnostic use.Materials and methodsPatients referred with difficult-to-diagnose skin lesions before excision were included. RCM images of each lesion were obtained prior to surgical intervention and were compared with the post-operative histological findings. Results A total of 137 patients were examined. Of 129 that were later histologically proven to be malignancies, 106 were diagnosed as 'malignant' by RCM. A further 23 were diagnosed as 'normal' by RCM (6 Basal Cell Carcenoma [BCC] and 17 Squamous Cell Carcenoma [SCC] on histology); demonstrating a false negative rate of 23/129 (17.83%) or a sensitivity of 82.17%. Of 8 histologically-proven, non-malignant lesions, RCM incorrectly attributed 'malignancy' in 2 cases, based on criteria defined during the study; giving a false positive rate of 2/8 (25%) or a specificity of 75%. Conclusion The results show that RCM can provide diagnostic information which is reliable for over 82% of clinically difficult-to-diagnose, but histologically proven NMSC. In addition, RCM might better define margins to perhaps reduce re-excision rates. As such, RCM can provide a particularly useful tool as an adjunct to clinical evaluation.

INTRODUCTION

Non-melanoma skin cancers are the most common cancers diagnosed in Australia[1] as in the majority of the western world[5]. The most common forms of NMSC are basal cell carcinoma and squamous cell carcinoma. In 2001, there were an estimated 256000 Australians treated for BCC and a further 118000 were treated for SCC[6]. These cancers are usually diagnosed and treated outside hospitals by general practitioners and dermatologists and in skin cancer clinics[2]; they are not legally notifiable and not routinely registered by all cancer registries. The continuing rise in the incidence of NMSC will translate into more surgical interventions. Although early or superficial NMSC can be effectively treated with topical agents[7 8]; the need for histological diagnosis, site of lesion, depth of lesion, histological subtype, and patient preference all necessitate some form of invasive procedure[9]. Surgical approaches include curettage and electro-desiccation, cryosurgery, surgical excision, and Mohs micrographic surgery (MMS). Surgical excision, curettage, and MMS are treatments that confer the advantage of histological evaluation.

Minimally invasive diagnostic tools have received increased attention for the diagnosis, screening and management of NMSC. Several modalities are commercially available; high frequency ultrasound, optical coherence tomography and RCM. These devices remain for the most part in limited use in tertiary referral centres and research facilities. RCM is reported to permit the morphologic differentiation of different skin tumours by detecting cellular and architectural patterns, comparable to routine histology. RCM is based on the reflectance, scattering and absorption of monochromatic light by cellular microstructures/inclusions (such as melanin and haemoglobin). By changing the depth at which the objective lens focuses in the z plane with respect to the skin, one can image any particular layer within the skin by scanning horizontally in two directions (y and x axis), and

sending these optical signals through interface software to reconstruct a thin horizontal image. One can create a series of these images that would stack vertically, reportedly from the stratum corneum to the upper papillary dermis. The resolution of these sections on the z axis is reported to be 2-5µm and therefore their thickness correlates closely with the axial thickness of excised histological sections, which should ease interpretation. Aside from the advantages that sequencing can provide; the image is displayed in real time and can be digitally recorded immediately.

A number of clinical trials on skin cancer have shown promising results for the differential-diagnosis of melanoma and NMSC using RCM with high sensitivity and specificity rates reported[2 3 4]. RCM has recently been evaluated for diagnosis of solar keratosis (SK) with sensitivity rates up to 97.7% with reference to the gold standard[10]. The most cited studies of RCM in the diagnosis of BCC have been conducted by a single group[11 12]. These papers reference a study[13] conducted in 2002, in which 8 lesions in 5 patients were used to create diagnostic criteria, develop sensitivity and specificity calculation to act as a basis of reference for subsequent studies by that group[2 14 15]. Bias limits the integrity of such studies, as well the 'statistical power' of a study conducted on 5 patients. Studies that have been performed outside of this group, which made use of the original group's results (both for determining power and for establishing diagnostic criteria), were not as extensive, nor as clear in demonstrating the benefit of RCM[4 16]. There is otherwise a paucity of studies on human skin performed by means of RCM in relation to NMSC. Several diagnostic morphologic features of skin tumours determined by in vivo confocal microscopy have been investigated previously, and there is hope that established criteria may help to improve its diagnostic accuracy. However, the numbers of studies and patients examined have been too small and the studies have been limited mainly to melanocytic skin tumours, resulting in insufficient experience of RCM in relation to sensitivity and specificity with various morphologic subtypes of NMSC.

One issue when dealing with non-pigmented skin malignancies is the determination of the horizontal extent of superficially-spreading carcinomas. The clinical margin often does not correspond to the histological margin, leading to incomplete excision of the lesion and the need for repeated surgery, causing inconvenience to the patient and increased cost to the health system. These clinical concerns have been the incentive behind this study. Our aim was to conduct a trial with clinical relevance to operating surgeons. Specifically, to establish whether confocal microscopy can be offered as a clinical diagnostic service to patients presenting with undiagnosed skin lesions or infiltrative lesions, after determining whether RCM can be used in place of tissue biopsy, or as a tool to facilitate marking of the margins of superficially infiltrative lesions preoperatively.

MATERIALS AND METHODS

One hundred and thirty seven patients with single lesions were recruited consecutively from April 2009 to May 2010. All patients had been referred for exisional biopsy of a non-pigmented skin lesion which had proved clinically difficult to diagnose by the referring surgeon or dermatologist. The study conformed to the Helsinki II declaration and was approved by Human Research Ethics Committees of Royal Adelaide Hospital (reference number 090301) and The Queen Elizabeth Hospital (reference number 2009127).

The equipment used is the commercially available near infrared confocal microscope (Vivascope 1500, Lucid, Inc., Henrietta, NY), equipped with a diode laser with peak emission at 830nm and a maximum power of 35mW. With this system, each image represents an effective 500 x 500µm field of view. The imaging depth in normal skin is 200-300µm; and the spatial resolution in the lateral dimension is 0.5-1.0µm. Images are oriented parallel to the surface and resolved in grey scale, with a lateral resolution of 0.5 to 1µm, and axial resolution of 3 to 5µm (which is comparable to routine histology)[17]. Three confocal fields consisting of 10 x 10 images of 0.5 x 0.5mm were taken at the level of epidermis, dermo-epidermal junction, and papillary dermis, at the centre of the lesion. A further three confocal fields at comparable levels were obtained of adjacent clinically normal skin (more than 2cm away from the clinical boarder of the lesion).

Sample size calculation for each RCM image group (clinically suspected SCC and clinically suspected BCC) was performed using the equation described by Simel and colleagues[18] from an expected low sensitivity and specificity of 75%: sample size was 52 lesions per group. All calculations were performed using SAS Version 9.2 (SAS Institute Inc., Cary, NC, USA). Predictors of tissue type (lesion or normal) were identified using exact conditional logistic regression models. $p<0.05$ was considered statistically significant. Predictors of malignancy were identified using exact logistic regression.

The sole operator of the confocal microscope underwent a training program with experienced operators within the Skin Engineering Laboratory of the Royal Adelaide Hospital. A preliminary study was carried out to determine the 'usability' of confocal microscope and its ability to visualise skin features, resulting in an award winning[19] publication[20]. The first 10 patients enrolled in the study familiarised the operator with the confocal microscope and determined the diagnostic criteria. Previously published work has indicated this number is required as the learning curve of confocal microscopy[1 3 4]. The time required for measurement was based on data generated subsequently. The participants were scanned on the day of, or the day before, their planned surgical procedure. The excision was carried out by a second operator (surgeon), and the histopathology was reviewed by a third operator (pathologist). The RCM operator, the surgeon, and the pathologist were all blinded to each others' findings or impressions.

All features visible, at each depth level, were recorded in order to create a database of the most prevalent features. The images were reviewed by a the operator. The features visible were tabulated for each layer of both normal and pathological skin examined. The presence of each feature was expressed in terms of a percentage. Diagnostic criteria, for both normal and pathological skin, were developed based on the prevalent features present in each skin type.

RESULTS

A total of 137 patients were examined with a mean age of 70.88 (range 43-93). Fifty-three percent were female. The majority of the lesions were on the trunk, upper limbs or face. Table 1 shows the anatomical distribution of the lesions

The epidermal field was found to be <30µm deep in 98% of cases. The first sighting of dermo-epidermal junction was found to be between 35 to 90µm in 97% of cases, and papillary dermal views were visible from 90µm in all cases. There were a limited number of features visible at each level. A clear distinction between features that were routinely present in normal skin and those routinely observed in the lesions was apparent. The visible features at each depth level of the clinical lesion comprising the diagnostic criteria for abnormality are outlined in Table 2. An example of normal dermis at dorsal forearm is included in Figure 1, and an abnormal skin image at papillary dermis at the same site in Figure 2. There was no significant distinction between RCM images of lesions that were later histologically diagnosed as BCC or SCC, and there was no identifiable difference between various subtypes of BCC. Unlike previous studies, no inter-cellular features were identifiable in any image, and therefore features such as 'basal cell polarity' or 'prominent nuclei' which are repeatedly mentioned in the literature to date were not included in our diagnostic criteria.

Scanning the total of six 10 x 10 fields in each patient took between 11 to 25 minutes depending on the site of the lesion. The adherent surface of the microscope is a circular area of 2.5cm in diameter. As such the device proved to be technically difficult to be used on the nose or ear.

The breakdown of the diagnostic features of lesions diagnosed by RCM as abnormal is outlined in Table 3. The findings and the results of sensitivity/specificity and positive predictive value (PPV) and negative predictive value (NPV) calculations are outlined in Table 4. Overall 106 of the total 129 malignant lesions were diagnosed as such by RCM; giving a sensitivity of 82.17% with a false negative rate of 17.83%. Of the 8 non-malignant lesions, two were attributed malignant status by RCM because they displayed criteria features of malignancy; giving a specificity of 75% with a false positive rate of 25%.

Predictors of tissue type (lesional or normal) were identified using exact conditional logistic regression models. Results are shown in Table 5. All the variables considered were highly significant predictors of tissue type in the exact conditional logistic regression models ($p < 0.0001$).

The total number of features (increased vascularity, increased vascular calibre, increased dermal peg calibre, not honeycomb pattern) contrasted with tissue type allowed further exploration of the diagnostic value of these features. The results are outlined in Table 6. Of 89 tissue samples with 3 or more features, all were from lesional tissue. In contrast if there were 0 features, there was 85% likelihood that it was normal tissue.

Predictors of malignancy were identified using exact logistic regression. Results are shown in Table 7. The total number of features (increased vascularity, increased vascular calibre, increased dermal peg calibre, not honeycomb pattern) was also contrasted with malignancy to further explore the diagnostic value of these features. Diagnostic values of features for malignancy, versus non-malignant lesions are outlined in Table 8. Of 105 lesions with 2 or more features, all were malignant.

DISCUSSION

Sampling for the study was clinically skewed because only those who were referred before excision of a difficult-to-diagnose lesion were included. These lesions are naturally problematic in clinical practice and any assistance in improving diagnosis is of considerable usefulness. There is insufficient evidence based on the results of this study to support the sole use of RCM to replace tissue biopsy for initial diagnosis of these skin lesions. There is evidence, however, to support RCM use peri-operatively, in conjunction with clinical judgement, to establish the cutaneous margins for BCC, especially when the clinical margin is indistinct. There were two superficially infiltrative BCC in the group; both were diagnosed as malignant with RCM. This is important because this BCC subtype is associated with the highest rate of incomplete excision (due to extension of the tumour beyond its clinically visible margins). The use of RCM in determining the margins for SCC is less clear-cut at present. However, during our studies we found that cutaneous margins were demonstrable for some lesions with poorly defined clinical margins using RCM and that it was very helpful for more accurate surgical margin placement. This aspect is currently being further evaluated.

Inter-observer reliability of RCM images was not evaluated in this study. All RCM images were evaluated by a single operator. There is previously published data regarding inter-observer reliability, evaluated in international multi-centre trials, indicating that, after a learning curve of 5 to 10 lesions, good inter-observer comparability can be expected[2].

The criteria for diagnosis established by this study do not distinguish between BCC and SCC or various BCC subtypes, but can be used for edge delineation in clinically difficult subtypes. The inherent selection bias of the study has resulted in a small number of non-malignant lesions within the sample, which limits validity of our specificity figures, although we tried to correct this by including a clinically normal skin site on each patient as the control.

These results indicate a very good PPV for malignant lesions, but poor NPV. The findings in this study do not support previous studies which report greater than 95% sensitivity and specificity in the diagnosis of BCC by RCM. If RCM is used peri-operatively and diagnoses malignancy; the diagnosis is likely to be correct. However, a ‘normal’ scan is not reliable of normality. The surgeon must default to the usual clinical indicators of histology and extent of the lesion.

The results with SCC are more disparate still than the existing literature. The reflectance index of keratin does not allow differentiation of any other structures within that field. As the construction of the image is dependant upon a software interpretation of various reflective indices of structures within the skin, a simple variation in the software can allow for an image to be constructed which automatically detracts keratin from the layer, and therefore allows for a clearer image to be constructed. A similar study using such modified software would be valuable in establishing the role of RCM in the diagnosis of SCC. Discussions with both hardware and software engineers for Lucid, reveal that software modification would be relatively easy to incorporate into future models. Until that time, the differentiation of SK from SCC, according to this study, is beyond the scope of the current RCM.

In summary, the results show that RCM can provide diagnostic information which is reliable for over 82% of clinically difficult-to-diagnose, but histologically proven NMSC. In addition, RCM might better define margins to perhaps reduce re-excision rates. As such, RCM can provide a particularly useful tool as an adjunct to clinical evaluation. At present however, it cannot be recommended as a replacement for tissue biopsy as the sole diagnostic tool.

References

1. AIHW & AACR 2004. Cancer in Australia 2001. AIHW cat. no. CAN 23. Canberra: AIHW (Cancer Series no. 28)
2. Nori S, Rius-Diaz F, Cuevas J et al. Sensitivity and specificity of reflectance-mode confocal microscopy for in vivo diagnosis of basal cell carcinoma: a multicenter study. J Am Acad Dermatol 2004; 51:923–30
3. Ulrich M, Maltusch A, Röwert-Huber J et al. Actinic keratoses: non-invasive diagnosis for field cancerisation. Br J Dermatol 2007; 156 (Suppl. 3):13–7
4. Gerger A, Koller S, Weger W et al. Sensitivity and specificity of confocal laser-scanning microscopy for in vivo diagnosis of malignant skin tumors. Cancer 2006; 107:193–200
5. Neville JA, Welch E, Leffell DJ. Management of non-melanoma skin cancer in 2007. Nat Clin Pract Oncol 2007;4:462–9
6. NCCI (National Cancer Control Initiative) 2003. The 2002 national non-melanoma skin cancer survey: a report by the NCCI Non-melanoma Skin Cancer Working Group. Ed. Staples MP. Melbourne: NCCI.
7. Goette, DK. Topical chemotherapy with 5-fluorouracil. A review. J Am Acad Dermatol 1981; 4:633
8. Love WE, Bernhard JD, Bordeaux JS. Topical imiquimod or fluorouracil therapy for basal and squamous cell carcinoma: a systematic review. Arch Dermatol 2009; 145:1431-8
9. Silverman MK, Kopf AW, Grin CM et al. Recurrence rates of treated basal cell carcinomas Part 1: Overview. J

Dermatol Surg Oncol 1991; 17:713-8
10. Ulrich M, Maltusch A, Ruis-Dias F et al. Clinical Applicability of in vivo Reflectance Confocal Microscopy for the Diagnosis of Actinic Keratoses. Dermatol Surg 2008; 34:610–619
11. Rajadhyaksha M, Grossman M, Esterowitz D et al. In vivo confocal scanning laser microscopy of human skin: melanin provides strong contrast. J Invest Dermatol 1995; 104:946-952
12. Rajadhyaksha M, González S, Zavislan JM et al. In vivo confocal scanning laser microscopy of human skin II: advances in instrumentation and comparison with histology. J Invest Dermatol. 1999; 113:293-303
13. González S, Tannous Z. Real-time, in vivo confocal reflectance microscopy of basal cell carcinoma. J Am Acad Dermatol 2002; 47:869-874
14. Goldgeier M, Fox C, Zavislan J et al. Noninvasive imaging, treatment, and microscopic confirmation of clearance of basal cell carcinoma. J Dermatol Surg 2003; 29:205-210
15. Nehal, KS, Gareau D, Rajadhyaksha M. Skin Imaging With Reflectance Confocal Microscopy. Semin Cutan Med Surg 2008; 27:37-43
16. Calzavara-Pinton P, Longo C, Venturini M et al. Reflectance Confocal Microscopy for In Vivo Skin Imaging. Photochemistry and Photobiology 2008; 84:1421–1430
17. Selkin B, Rajadhyaksha M, González S, Langley RG. In vivo confocal microscopy in dermatology. Dermatol Clin 2001; 19:369–77
18. Simel DL, Samsa GP, Matchar DB. Likelihood ratios with confidence: sample size estimation for diagnostic test studies. J Clin Epidemiol 1991; 44:763–70
19. Winner of Best Medical Presentation, Australia and New Zealand Burn Association Conference, Wellington, New Zealand, 22-25 September 2009
20. Greenwood J, Amjadi M, Dearman B, Mackie I. Real-Time Demonstration of Split Skin Graft Inosculation and Integra Dermal Matrix Neovascularization Using Confocal Laser Scanning Microscopy. Open Access Journal of Plastic and Reconstructive Surgery Eplasty 2009; 9:e33

Author Information

Mahyar Amjadi, BMBS, BDS, FRACDS
Royal Adelaide Hospital

Brendon J Coventry, MBBS, PhD, FRACS
Royal Adelaide Hospital

John E Greenwood AM, MBChB, MD, FRACS
Royal Adelaide Hospital