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The use of Reflectance Confocal Microscopy to diagnose Basal Cell Carcinoma in the United Kingdom: A Prospective Observational Trial at a Single Centre

Running head: Diagnostic Accuracy of Reflectance Confocal Microscopy for diagnosis of BCC

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Patient consent: Written patient consent for publication was obtained.

What is already known about this topic?

- Dermoscopy increases the diagnostic accuracy of basal cell carcinoma (BCC) but this has not been quantified in the UK population
- Reflectance Confocal Microscopy (RCM) is an emerging imaging modality that has been shown in to be beneficial in the non-invasive diagnosis of BCC.

What does this study add?

- First UK prospective study quantifying diagnostic accuracy of Dermoscopy and RCM in BCC.
- Support RCM use in UK for BCC diagnosis as it increases diagnostic accuracy.

- Accuracy of RCM is greater in dermoscopically pigmented BCC.
- High diagnostic accuracy of BCC with RCM is rapidly achieved.
- RCM plus Dermoscopy have higher accuracy than Dermoscopy alone.

Abstract

Background

Previous work with Reflectance Confocal Microscopy (RCM) imaging has shown high sensitivity and specificity for Basal Cell Carcinoma (BCC), but to date there have been few studies on a UK cohort.

Objectives

The study hypothesised that RCM could be used prospectively to accurately diagnose BCC in a private UK secondary care, single clinician setting. The study assessed the potential for RCM to be used as a routine diagnostic procedure.

Methods

522 lesions were recruited prospectively where BCC featured in the differential diagnosis after clinical examination. 78 were subsequently excluded. Imaging used the arm-mounted confocal microscope unless access was restricted and required the handheld probe. The likelihood of BCC was scored for each modality, each diagnosis building on the last. Histology was assessed by a single blinded histopathologist [JJ].

Results

444 lesions from 326 patients were included in the analysis, including 327 BCCs. Median maximum diameter was 6 mm. The sensitivity and specificity for BCC was 69.42% (64.11% to 74.37%) and 52.99% (43.55% to 62.28%) for clinical examination alone; 91.77% (88.25% to 94.51%) and 41.03% (32.02% to 50.50%) plus dermoscopy; 98.78% (96.91% to 99.67%) and 85.47% (77.76% to 91.30%) plus RCM. For RCM PPV was 95.01% (92.14% to 97.07%) and NPV was 96.15% (90.44% to 98.94%). Area under the curve increased from 0.61 to 0.66 to 0.92 as modalities were added.

Conclusion

This study demonstrates that RCM can, reliably and quickly, diagnose BCC, and that the addition of RCM to dermoscopy permits higher diagnostic accuracy for BCC in the UK. The specificity and sensitivity of the RCM diagnosis did not alter significantly with experience, reflecting the ease and speed of acquiring the skill.

Clinical Trial Registration: NCT03509415

Introduction

Non-melanoma skin cancer (NMSC) in fair-skinned populations has become a significant financial burden for healthcare providers worldwide. The incidence of Basal Cell Carcinoma (BCC) is increasing worldwide, with the UK experiencing an increase of 81% from 1999 to 2010.[1,2]

The majority of BCCs are diagnosed by dermoscopy then histopathology, and either treated with non-invasive modalities, such as imiquimod, or by curette and cautery, laser ablation, or photodynamic therapy.[3–5] However, higher risk BCC may require a more aggressive surgical excision or Mohs surgery. The latter is a very successful treatment for high-risk primary or recurrent BCC as incomplete excision increases the risk of recurrence.

Nice guidelines recommend a diagnostic biopsy resulting in significant delays before the result is known.[6] Treatment of high risk BCCs may be delayed further due to the need to discuss them in multidisciplinary team (MDT) meetings as recommended by NICE.[7,8] Despite low mortality, these tumours may become invasive and cause higher morbidity.[9]

Reflectance Confocal Microscopy (RCM) is already used routinely to assess BCC in Europe[10] and the USA,[11] but is not standard practise in the UK. Currently 320 centres in the EU are using RCM for clinical dermatology, plus another 60 cosmetic centres, while there are currently only 4 clinical centres in the UK. Whilst RCM has been shown to improve patient care internationally,[12–16] applicability to the UK population remains unproven. The UK has greater reliance on screening for BCC by GPs rather than by Dermatologist. BCCs are often pink in predominantly Caucasian populations, while more pigmented lesions are typical in Mediterranean and darker skinned populations. BCCs in the latter are often easier to identify by RCM due to the presence of hyper-reflective tumour nests; non-pigmented BCCs feature more challenging hypo-reflective tumour nests. NICE, therefore recommended further UK research in order to verify that results from southern European countries could be replicated in the UK.[17]

Use of RCM prior to excision and histological assessment could reduce delays, lower costs, and decrease the use of unnecessary invasive biopsies, and may replace histological examination entirely in some cases. Reported sensitivity and specificity for RCM in diagnosing BCC range from 83-100% and 79-97%.[16,18,19]

This study prospectively aimed to

(1) Assess the specificity, sensitivity, positive, and negative predictive values of RCM to diagnose BCC in the UK population;
(2) Use the ROC probability curve to compare the performance of the 3 modalities - clinical examination, clinical examination combined with dermoscopy and the RCM examination;
(3) Assess the impact of increasing RCM experience on enhancing diagnostic accuracy of RCM, using the ROC probability curve.

The histopathological diagnosis was used as gold standard in all analyses.

Methods

Design

The prospective blinded observation trial ran from March 2017 to December 2019 with recruitment from a single private clinic setting (The Skin Care Network, Barnet, London) in the UK. The lack of UK centres using RCM precluded a multi-centre approach, so the study was designed in accordance with international guidelines and practices [10,19] so that results could be read in conjunction with international data.

Training

The principal investigator (HS) and the nurses assisting in the image acquisition attended an RCM teaching program at the University of Modena and Reggio Emilia, Modena, Italy, consisting of a 3-day basic training course on the use of RCM, followed by 3 days of practice and the controlled evaluation of 100 cases on an on-line platform.

Ethics and Governance

Prior to initiating this study, the protocol obtained Ethical Committee approval (Wales REC 7 17/WA/0045; 218989) and was registered with Clinicaltrials.gov, reference NCT03509415. Eligible patients were approached during their initial consultation where the presumptive diagnosis of BCC was made, informed about the study and asked if they would consent.

Patient data was anonymised and managed in accordance with the General Data Protection Regulation, Caldicott Guardian requirements, the Research Governance Framework for Health and Social Care, and Research Ethics Committee approval.

Study data is stored at within the Bluespier (Droitwich, Worcestershire, WR9 7ER) electronic patient management system computer system under normal arrangements for patient confidentiality.

Sample size calculation

A pre-study evaluation of the power of the study was undertaken by Quantics Biostatistics (Edinburgh. Report Number: 0088, HS-MAV-003). Specificity was the basis for the sample size calculation.

The sample size was determined assuming that lesions within a patient are independent. The numbers of lesions required to provide a lower confidence limit for a specificity no more than 3% lower than the estimate was calculated for a range of assumptions (**Table S1)**.

As pathology was unknown at the point where patients were recruited, lesions were added to the study until 100 true negative lesions had been recruited.

Traditional histopathology was used as the gold standard for all lesions. Pre-trial statistical analysis determined that for the RCM to be concluded as having a specificity equal to that of the biopsy, the lower limit of the 95% confidence interval must not be less than 94%. Similarly, for sensitivity the lower limit of the 95% confidence must not be less than 92%.

Study population

Patients 18 years or older who had lesions where BCC was in the differential diagnosis following clinical and dermoscopic evaluation were recruited prospectively and sequentially; lesions totalled 522.

Seventy-eight (78) lesions were subsequently excluded from the database, either due to technical difficulties with the imaging, failure to have an RCM scan, failure to obtain histological diagnosis, or the patient electively choosing treatment in the NHS.

Study workflow

Clinical, dermoscopic, and RCM examinations were conducted sequentially by the principal investigator (HS) prior to a histological diagnosis. At each stage the lesion was scored from 0-3 (0 = not BCC, 1 = possibly BCC, 2 = probably BCC, 3 = BCC). A clinical score of at least 1 was an inclusion criteria for the study. Once a patient was included in the trial exclusion only occurred where it was not possible to obtain histology or RCM, or due to patient not attending follow-up.

Dermoscopy used the two-step algorithm method[20] using both polarised and non-polarised immersion (using alcohol) contact dermoscopy (DermLite DL4, 3Gen, San Juan Capistrano, CA, USA) and 20x magnification. Polarised and non-polarised dermoscopic images were taken with an iPad 3 fitted with a 3Gen iPad adaptor.

RCM (VivaScope 1500 or 3000, Gen.3 or Gen 4, VivaScope GmbH, Munich, Germany) was performed at three different skin levels (superficial epidermal layers, dermo-epidermal junction (DEJ) and papillary dermis). A minimum of three mosaics were obtained per lesion. Mosaic was the full size of lesion or maximum capture size available in the case of large lesions. The handheld 3000 system was used also especially where access was an issue, for example on the nose. The two-step diagnostic method has previously been described.[5,19,21] Using this method, positive criteria include polarized elongated features in the superficial layer, linear telangiectasia-like horizontal vessels, basaloid cord and nodules, and epidermal shadow. Negative features are disarray of the honeycomb epidermal layer, papillae that are ''non-visible'', and cerebriform nests.[19]

All RCM images were assessed by the principal investigator prior to biopsy (HS) who generated a confocal report and graded the confidence of diagnosis. Digital still confocal images were captured, anonymised and stored with dedicated software SmartVivaNet confocal imaging platform (VivaScope GmbH, Munich, Germany).

The histopathological diagnosis was made by a dermatopathologist (JEJ) using conventional haematoxylin and eosin stained sections.

Statistical analysis was performed using Microsoft Excel, MedCalc's online statistics calculator[22], and easyROC.[23] Histopathological diagnosis was converted into a numerical value where non-BCC = 0 and BCC = 1. Based on diagnosis of BCC vs. other diagnoses, sensitivity, specificity, PPV, NPV, accuracy, and likelihood ratios were all calculated.

Results

444 lesions were identified in 326 patients. 244 patients had 1 lesion, 58 had 2 lesions, 14 had 3 lesions, 5 had 4 lesions, 3 had 5 lesions, and 1 had 7 lesions.

Median age was 71; median maximum diameter 6 mm, range 4-9 mm. 269 of the 444 lesions (60.4%) occurred in males. **Table 1** details the location, clinical findings, and dermoscopic features of the 444 lesions. The commonest location was the head and neck accounting for 56.3% (250) of total lesions. Clinically, the "pink lesion" was the most common occurring in 365 lesions (82%); dermoscopically, the "pink white" was the most common finding occurring in 367 lesions (82.5%).

Table 2 shows the scoring for the BCC diagnostic confidence in the 3 examination modalities – clinical examination, clinical examination combined with dermoscopy and the RCM diagnosis. The highest diagnostic confidence score was obtained in score 3 (very high) in the RCM examination (71%).

The sensitivity, specificity, TP (true positive), TN (true negative), FN (false negative), FP (false positive), NPV (negative predictive value) and PPV (positive predictive value) for the 3 examination modalities using a score threshold of 2 are detailed in **table 3**. The corresponding ROC probability curve is shown in **figure 1,** and the statistics are shown in **figure 2**. Table 4 shows counts for diagnostic decision at each threshold and for each modality, with performance at alternative thresholds is shown in Figure 3.

Of the 444 lesions examined, 327 lesions (73.7%) were confirmed histologically as BCC. The histological subtypes are detailed in **table 5**, nodular BCC was most common (133 lesions, 40.7%).

The diagnoses in the remaining 117 lesions (**table 6**) covered a wide range of diagnoses, most commonly actinic keratosis/Bowen's disease occurring in 31.6% (37 lesions).

False Negative cases

Of the 4 false negative, in the first the images obtained were poor as a result of superficial erosion of the overlying skin which is a known limitation of RCM that in eroded or ulcerated lesions image quality is poor and RCM is not the investigation of choice,[24] however retrospectively dark silhouettes were visible in one stack but not sufficient to confidently diagnose a BCC; in the second case there was a collision between a seborrheic wart and a BCC, whilst the BCC was clearly visible it was missed due to investigator inexperience. In the third case which occurred early in the study a single tumour island was clearly visible. In the fourth case, tumour islands were also clearly visible but were misread due to reader inexperience.

False Positive cases

Of the 17 false positive results on the first RCM reading (U-RCM): 3 were actinic keratoses and 2 were sebaceomas, where the basaloid islands were mistaken for tumour islands of BCC as the bright aggregates of ovoid cells with bright refractile granular cytoplasm, previously reported as sebocytes[25] were visible in both cases. 2 were squamous cell carcinoma (SCC) where the hypo-reflective tumour islands of SCC were mis-read as BCC, 4 were inflammation, 3 solar lentigo or lichenoid keratoses. Finally a naevus, a schwannoma, and a sarcoma were mis-diagnosed.

Discussion

We believe this is the first UK-based prospective observational study of the efficiency benefits of BCC diagnosis with RCM. RCM was used to acquire and accurately diagnose BCCs in 5-10 minutes, allowing the procedure to be incorporated into the workflow of a normal clinic.

The 98.78% (96.91% to 99.67%) sensitivity of this study is similar to the 93% to 100% range seen previously, [16,19,26–28] but only 3 previous studies were prospective.[19,29] Specificity was 85.47% (77.76% to 91.30%).

Median maximum lesion diameter was only 6 mm, and 400 of the 444 lesions (90%) were non-pigmented, in contrast to Japan (less than 25%[30]) but similar to Italy (90%[31]). This might be explained by the demographics of the population: a large Caucasian Jewish community with skin types II-III. Clinically the presence of pigmentation within the BCC considerably eased the diagnosis of BCC.

Surprisingly, hairpin and polka dot vessels both showed a higher sensitivity than the dermoscopically specific arborising serpentine vessels, possibly as the latter tend to occur in 48-81% of BCC, but are most specific for superficial BCC.[32,33] Despite the relatively small size of the study, 2 completely amelanotic superficial spreading malignant melanomas were correctly identified by RCM (true negatives).

This study has confirmed that Dermoscopy significantly improves diagnostic accuracy for BCC over clinical examination with non-pigmented lesions in a UK population. This mirrors the findings of a large prospective multicentre study recently published from Italy.[16] It further shows that the addition of RCM enables another significant improvement of similar magnitude. NICE guidelines for pigmented lesion states "Dermoscopy performed by suitably trained specialists is more sensitive and more specific in classifying skin lesions than clinical examination with the naked eye. It lessens the chance of missing a diagnosis of melanoma and reduces the number of unnecessary surgical procedures to remove benign lesions" [7].

This study utilised both the handheld and arm mounted confocal microscopes, with lesion selection not restricted to areas where imaging was more straightforward. Handheld confocal has previously been shown to have a slightly lower sensitivity than the arm mounted unit,[27,29] but we found that the faster and higher quality Gen4 arm mounted microscope largely obviates the need for the handheld device, the exception being where anatomical access was an issue, such as the nasolabial fold.

A downside of examining the images prospectively at the patient's bedside was the tendency for positive biases to influence the reading of the RCM images based on the clinicians clinical/dermoscopic evaluation and this was a factor in over diagnosing BCC particularly early in the study when the experience of reading confocal images was limited. To reduce the clinicians' workload it was found image acquisition could be undertaken by suitable trained nurses or technicians using the VivaScope 1500, but not the handheld VivaScope 3000 potentially enabling initial patient screening, confocal diagnosis and stratification into appropriate treatment pathways within a single outpatient visit without the need for multiple visits. When RCM image capture is so delegated, it should then be read by suitably trained dermatologists in conjunction with the clinical and dermoscopic images. Incorporation of RCM into patient screening prior to diagnosis not only speeds up diagnosis but also reduces the risk of inappropriate treatment options for amelanotic malignant melanoma.

Arguments against the incorporation of RCM into the routine management of BCC in the UK are three-fold.

First, cost. RCM has been shown to be cost effective in both melanocytic[34] and general skin oncology[28] because it reduces unnecessary biopsies.

Second, required skill and training. This study demonstrated that a 3-day RCM training course is sufficient to competently read confocal images, demonstrating a rate of knowledge acquisition similar to Dermoscopy.

Thirdly, in this study 1.7% of these "obvious" lesions were amelanotic melanomas, demonstrating that a strong case should be made for a diagnostic procedure before definitive treatment however confident clinicians may feel in their diagnosis.

Qualitative observations about the use of RCM in the diagnosis of BCC

- In hairy areas such as the beard, scalp or eyebrows, differentiating hair follicles from the dark silhouettes of tumour nests was sometimes difficult.
- When definitive diagnosis was difficult with the handheld device, switching to the VivaScope 1500 facilitated rapid diagnosis.
- Appropriately trained technician or nurses were quickly able to take and read confocal images using the VivaScope 1500, freeing up physician time.
- Image acquisition using the handheld VivaScope 3000 had to be undertaken by the reading physician as interpretation was a dynamic process.
- Pigmented BCCs were much easier to identify due to the presence of highly refractive dendritic melanocytes within tumour nests.
- Differentiation of non-pigmented superficial BCC from amelanotic melanoma was straightforward.
- Using a combination of RCM and Dermoscopy, tumour typing could be performed but further studies are required to confirm significance.

Conclusion

For a new technology to be incorporated into the clinical workflow it must demonstrate that it is quicker than the existing methodology, has acceptable sensitivity and specificity, an appropriate NNT, and has an acceptable cost/benefit ratio when compared to standard practice. It must be acceptable to patients, and clinicians must be able to train to an appropriate standard in a reasonably short period of time. This study has shown that with only 6 months of RCM use and a basic training course, sensitivities of 99% and specificities of 85% are achievable, and the immediacy of results is appreciated by patients who do not have to wait for the outcome of a biopsy.

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Figure legends

Figure 1. Diagnostic statistics for each modality. Error bars show 95% confidence limits for sensitivity, specificity, accuracy, and predictive values.

Figure 2. ROC Curve for each diagnostic modality

Figure 3. Diagnostic performance at alternative diagnostic thresholds. Error bars show 95% confidence limits.

Table 1: Clinical characteristics of the 444 lesions

Variables		**N**	**%**
Age (median; IQR)		71	64-79
Max diameter (median; IQR)		6	4-9
Sex	**M**	270	60.7
	F	175	39.3
Location	**H&N**	250	56.2
	Trunk anterior	46	10.3
	Trunk posterior	52	11.7
	Upper limbs	56	12.6
	Lower limbs	41	9.2
Histology	**other**	117	26.3
	BCC	327	73.7
Clinical	**Pink lesion**	365	82.0%
	White Lesion	36	8.1%
	Pigmented lesion	51	11.5%

Dermoscopy	**Scale**	111	24.9
	White-white	65	14.6
	Blue Ovoid nest	33	7.4
	Multiple blue-grey globules	27	6.1
	Spoke wheel pigment	25	5.6
	Specks of brown and grey pigment	93	20.9
	Pigment Network	2	0.4
	Hairpin vessels	23	5.2
	Serpentine Vessels in focus	331	74.4
	Polka dot vessels	21	4.7
	Pink white	367	82.5%
	Erosions	177	39.8
	Shiny white structures	248	55.7
Total		**444**	**100**

Table 2: Scoring of the BCC diagnostic confidence in the three examination modalities

Examination Modalities		**BCC diagnostic confidence**				
		Not BCC	**Possibly BCC**	**Probably BCC**	**BCC**	**Total**
Clinical	**N**	0	162	215	67	444
	%	0.0	36.5	48.4	15.1	100
Clinical plus Dermoscopy	**N**	1	74	175	194	444
	%	0.2	16.7	39.4	43.7	100
RCM	**N**	94	10	23	317	444
	%	21.2	2.3	5.2	71.4	100

Table 3: Statistical data for the three examination modalities

	Clinical		**Clinical + Dermoscopic**		**Clinical + Dermoscopic + RCM**	
Statistic	Value	95% CI	Value	95% CI	Value	95% CI
Sensitivity	69.42%	64.11% to 74.37%	91.74%	88.21% to 94.49%	98.78%	96.90% to 99.67%
Specificity	52.99%	43.55% to 62.28%	41.03%	32.02% to 50.50%	85.47%	77.76% to 91.30%
Positive Predictive Value	80.50%	75.38% to 84.96%	81.30%	76.94% to 85.15%	95.00%	92.12% to 97.06%

Negative Predictive Value	38.27%	30.76% to 46.22%	64.00%	52.09% to 74.77%	96.15%	90.44% to 98.94%
Accuracy	65.09%	60.45% to 69.52%	78.38%	74.25% to 82.12%	95.27%	92.86% to 97.05%
Positive Likelihood Ratio	1.48	1.20 to 1.81	1.56	1.33 to 1.82	6.80	4.38 to 10.55
Negative Likelihood Ratio	0.58	0.46 to 0.73	0.20	0.13 to 0.31	0.01	0.01 to 0.04
Disease prevalence	73.65%	69.29% to 77.69%	73.65%	69.29% to 77.69%	73.65%	69.29% to 77.69%
Number Needed to Diagnose	4.46	2.73 to 13.06	3.05	2.22 to 4.94	1.19	1.10 to 1.34
Number needed to Predict	0.95		1.32		0.17	

Table 4: Counts for diagnostic decision at each threshold and for each modality.

The main analysis used a threshold of 2 for each modality.

Modality	**Threshold**	**TP**	**FP**	**TN**	**FN**
Clinical	0	328	117	0	0
Clinical	1	327	117	0	1
Clinical	2	227	55	62	101
Clinical	3	63	4	113	265
Dermoscopy	0	328	117	0	0
Dermoscopy	1	328	116	1	0
Dermoscopy	2	301	69	48	27
Dermoscopy	3	283	11	106	145
RCM	0	328	117	0	0
RCM	1	326	25	92	2
RCM	2	324	17	100	4
RCM	3	304	14	103	24

Table 5: Details of the histological subtypes of BCC

BCC histo-type	**No**	**%**
BCC not specified	3	0.9%
BCC infiltrative	57	17.4%

BCC infiltrative/moprheic	1	0.3%
BCC micronodular	15	4.6%
BCC nodular	133	40.7%
BCC nodulocystic	1	0.3%
BCC superficial	117	35.8%
Total	**327**	**100.0%**

1

2 Table 6: Details of the histological diagnoses of the remaining 117 lesions

Other lesions histology	No	%
AK/Bowen	37	31.6%
Dermatofibroma/Scar	3	2.6%
Eccrine benign	2	1.7%
Fibrous papule of nose	2	1.7%
Inflammation	16	13.7%
Lichenoid keratosis	7	6.0%
Molluscum contagiosum	2	1.7%
Naevus	14	12.0%
Neurofibroma	2	1.7%
Sarcoma	1	0.9%
SCC	11	9.4%
Schwannoma	2	1.7%
Sebaceous benign	7	6.0%
SL/SK	9	7.7%
Melanoma SSM	2	1.7%
Total	**117**	**100.0%**

3

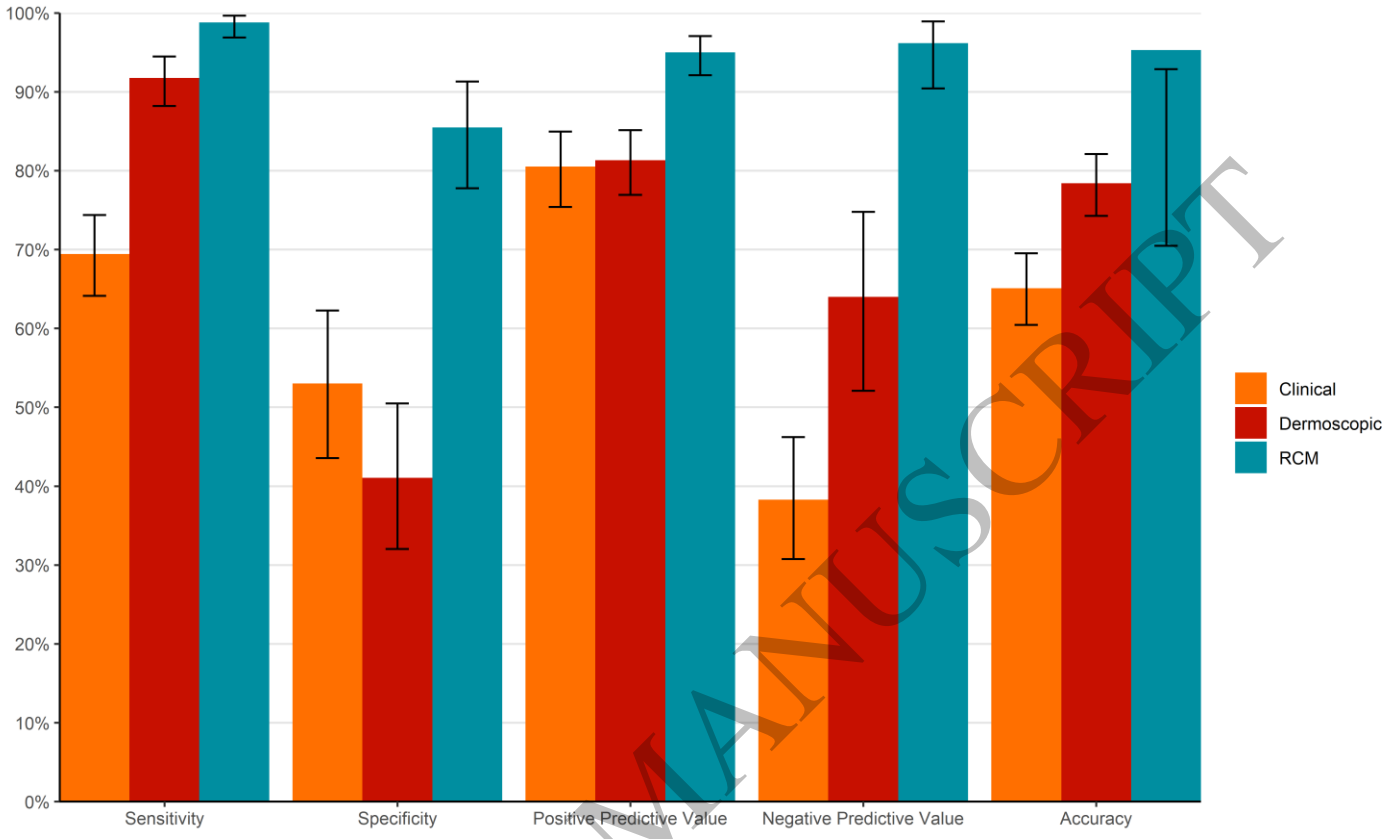

Figure 1
190x114 mm (x DPI)

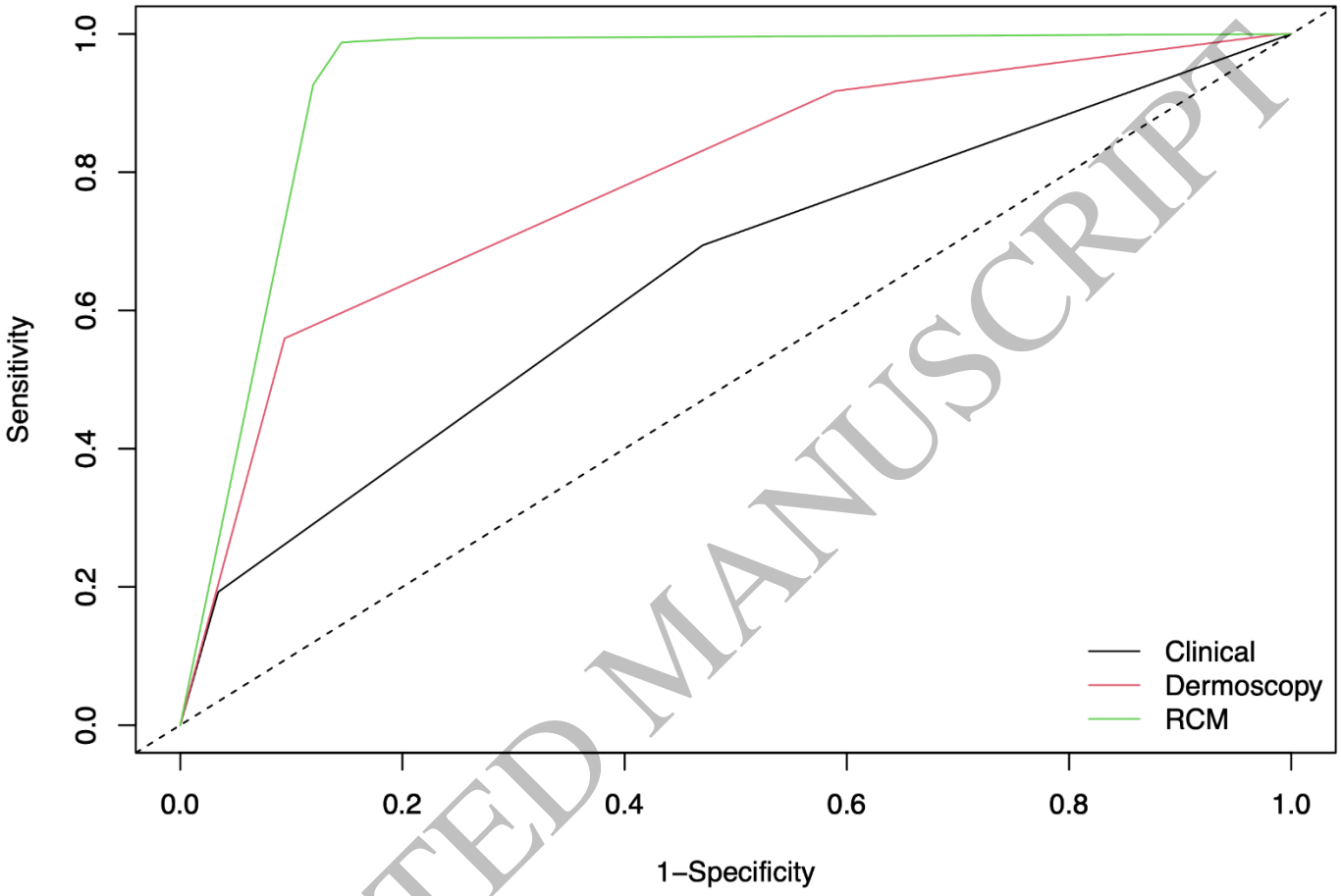

Figure 2
190x142 mm (x DPI)

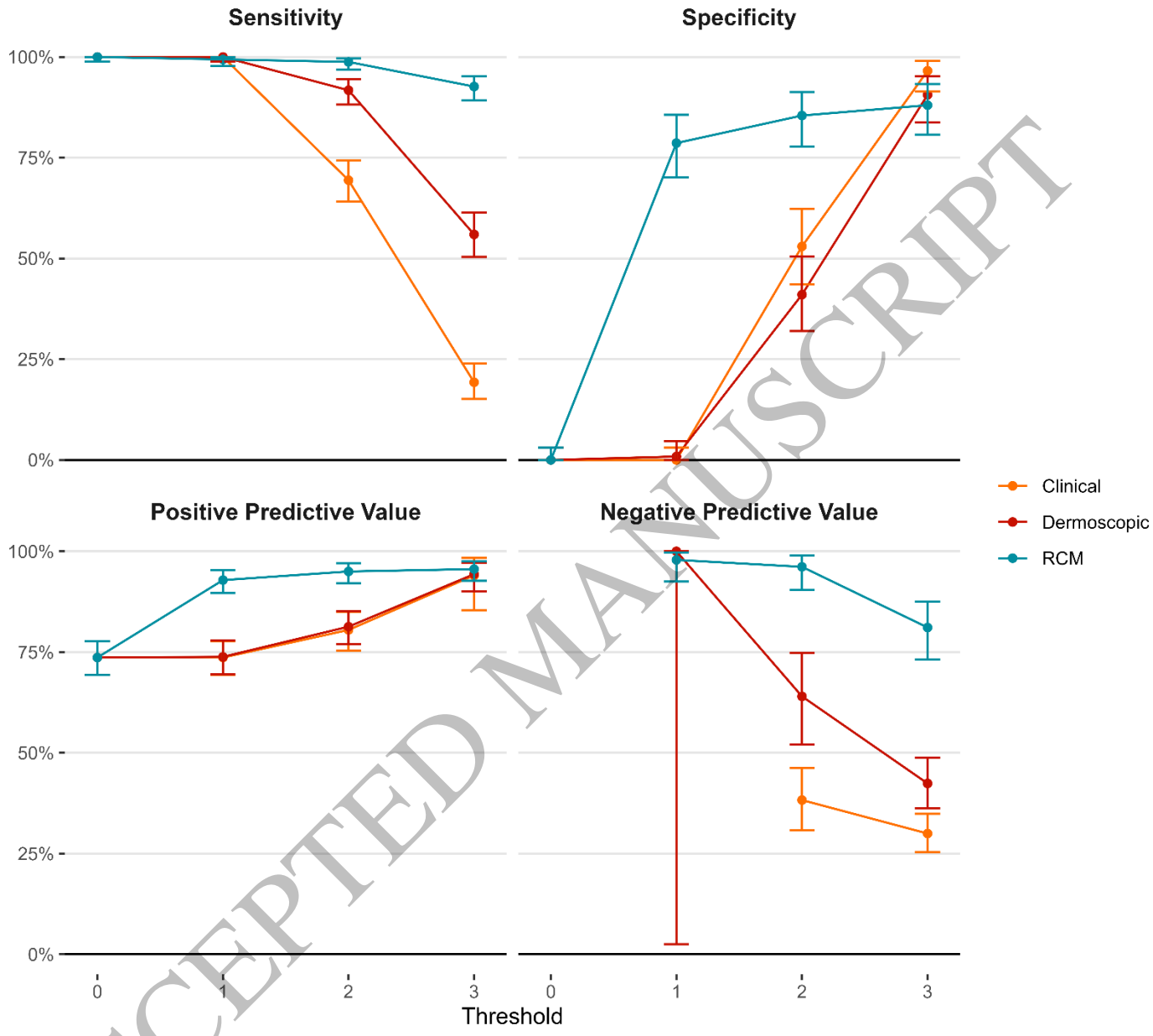

Figure 3
190x166 mm (x DPI)