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Title: Dermoscopy and reflectance confocal microscopy for basal cell carcinoma diagnosis and diagnosis prediction score: a prospective and multicenter study on 1005 lesions.

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73 **Capsule summary**

- 74 • **(First bullet)** Diagnostic accuracy of reflectance confocal microscopy for basal cell carcinoma is
- 75 largely established by retrospective studies
- 76 • **(Second bullet)** Our prospectively designed study demonstrates that adjunctive handheld
- 77 reflectance confocal microscopy in clinical practice increases BCC diagnostic accuracy for
- 78 clinically suspicious lesions

79

80 **Abstract**

81
82 **Background:** Basal cell carcinoma is usually diagnosed by clinical and dermoscopy examination,
83 but diagnostic accuracy may be suboptimal. Reflectance confocal microscopy (RCM) imaging
84 increases skin cancer diagnostic accuracy.

85 **Objective:** To evaluate additional benefit in diagnostic accuracy of handheld RCM in a prospective
86 controlled clinical setting

87 **Methods:** A prospective, multicenter study in 3 skin cancer reference centers in Italy enrolling
88 consecutive lesions with clinical-dermoscopic suspicion of basal cell carcinoma. Clinicaltrials.gov;
89 NCT04789421

90 **Results:** A total of 1005 lesions were included, of which 474 histopathologically confirmed versus
91 531 diagnosed by clinical-dermoscopic-RCM correlation, confirmed with 2 years of follow up.
92 Specifically, 740 were confirmed basal cell carcinomas. Sensitivity and specificity for dermoscopy
93 alone was 93.2% (CI 95% 91.2 - 94.9) and 51.7% (CI 95% 45.5 - 57.9); PPV was 84.4 (CI 95%
94 81.7 – 86.8) and NPV 73.3 (66.3 - 79.5). Adjunctive reflectance confocal microscopy reported
95 higher rates: 97.8 (CI 95% 96.5 - 98.8) sensitivity and 86.8 (CI 95% 82.1 - 90.6) specificity, with
96 PPV of 95.4 (CI 95% 93.6 – 96.8) and NPV 93.5 (89.7 - 96.2).

97 **Limitations:** study conducted in one single Country

98 **Conclusion:** Adjunctive handheld RCM assessment of lesions clinically suspicious for basal cell
99 carcinoma permits higher diagnostic accuracy with minimal false negative lesions.

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103 **Introduction**

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105 Basal-cell carcinomas (BCC) make up 80% of all skin cancers and are the most common malignant
106 disease among white populations. Incidence is increasing worldwide, estimated to be as high as
107 10% per year.¹ Diagnosis is usually obtained by a combination of clinical and dermoscopic
108 assessment, with skin biopsy being the current golden standard diagnostic tool in case of
109 uncertainty.²⁻⁴

110 Dermoscopic BCC diagnostic accuracy depends on several factors, including specific setting, lesion
111 type, and degree of pigmentation. Estimates in previous studies report vary in terms of sensitivity
112 and specificity rates, ranging between 70%-100% and 54-100%, respectively.⁵ Reflectance confocal
113 microscopy (RCM) has proven to be a useful adjunctive tool for skin cancer diagnosis, in both
114 prospective and randomized controlled trials.⁶⁻⁹ Most studies have employed RCM wide-probe,
115 requiring attachment on the skin, which can be difficult on curved surfaces (i.e. face, proximity of
116 eyes, ears, mouth, nose etc.). As a matter of fact, when dealing with BCC diagnosis in clinical
117 practice, the choice of HH-RCM imaging is driven by the need to have a rapid and direct
118 examination of lesions.¹⁰ However, currently literature does not provide any prospective studies
119 focusing on the diagnostic role of dermoscopy and HH-RCM for BCCs.

120 Additionally, considering that recent guidelines have included several non-surgical treatments for
121 BCCs, to improve pre-treatment diagnostic sensitivity and specificity is crucial to increase the rates
122 of appropriate lesion management.

123 The aim of the current multicenter, prospective study is: i) to assess diagnostic accuracy for BCC
124 with dermoscopy with adjunctive HH-RCM assessment versus dermoscopy alone, among a
125 consecutive cohort of patients referred to 3 Skin Cancer Centers in Northern Italy with clinical
126 suspicion of BCC; ii) to develop a BCC multivariate prediction model.

127 **Methods**

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130 This prospective, multicenter study was conducted at 3 Italian centers, located in a Northern Italian
131 Region: Department of Dermatology of the University of Modena and Reggio Emilia, Skin Cancer
132 Center of IRCCS Reggio Emilia, and Skin Cancer Unit of IRST and Ravenna Hospital, Ravenna-
133 Forlì. Patients' access and clinical therapeutic pathways are similar at each collaborating center, as
134 per national and regional healthcare regulations. The study was approved by the local ethics
135 committee (Protocol 3844/C.E.) and a short, translated version of the study protocol is available
136 online (Clinicaltrials.gov; NCT04789421).

137 Criteria specified the inclusion of all consecutive lesions with clinical-dermoscopic suspicion of
138 BCC assessed between August 2017 - June 2019. Lesions without clinician specified possible
139 differential diagnosis of BCC (without any clinical or dermoscopy clues for BCC), or
140 hyperkeratotic, fully ulcerated lesions and/or lesions located on mucosa or palmo-plantar areas or in
141 other body areas not permitting the use of RCM, were excluded. Patients who provided written
142 informed consent were enrolled into the current study.

143 All collaborating centers were equipped with a handheld dermoscopy (Dermlite DL-1, 3Gen, San
144 Juan Capistrano, CA, US) and HH-RCM (Vivascope 3000®, MAVIG GmbH, Munich, Germany).
145 Acquisition procedures for HH-RCM evaluations have been extensively described elsewhere.^{10,11}

146 Study procedure: All lesions with clinical BCC suspicion were enrolled into the study. After
147 obtaining informed consent, each lesion was first documented with clinical and dermoscopic
148 images. For each lesion, a combined clinical-dermoscopic diagnosis of "BCC" or "other" was
149 reported. Clinical-dermoscopy diagnosis was registered, supported by observed clinical-
150 dermoscopic criteria on a pre-defined list of BCC associated features, including palpability (flat,
151 papule, nodule), pigmentation (absent, light or dark), multiple blue-gray dots, large blue grey ovoid
152 nests/globules, leaf-like/concentric/spoke-wheel structure, arbourizing vessels, short fine
153 telangectasia, ulceration, multiple small erosions.

154 All enrolled lesions (independently of demoscopy diagnosis) were then evaluated with HH RCM by
155 a Clinical independent investigator, exploring the entire surface of the lesion and acquiring at least

156 1 Vivastack (with a mean of 2 Vivastack for each case) in the significant region area. After HH-
157 RCM examination was completed, the investigator was asked to report a suspected diagnosis of
158 “BCC” or “other”, and was invited to record the presence/absence of RCM diagnostic criteria
159 associated with BCC diagnoses, on a pre-defined form. RCM criteria included streaming of the
160 epidermis, small tumor islands, big tumor islands, cord-like structures, dark silhouette, clefting,
161 ulceration, erosion, peripheral palisading, increased vascularization, refractile fibrotic collagen, and
162 inflammation.¹²

163 The study diagnostic pathway is summarized in the Flow Chart, **Figure 1**.

164 Lesions with concordant clinical-dermoscopic and RCM diagnosis for BCC were treated with
165 surgery or medical therapies according with diagnostic confidence and medical decision; in the case
166 of a highly certain BCC diagnosis and medical therapeutic approach, histology was not performed
167 according with normal clinical practice, and monitored for at least 2-years follow up. Lesions with
168 dermoscopy or RCM diagnosis of “other”, and lesions with concordant dermoscopic and RCM
169 BCC diagnosis, but not highly confident, were all biopsied.

170

171 ***Data analysis***

172 Lesion images were maintained in dedicated, center-specific databases. Data were collected in a
173 central database and analyzed by an expert statistician (S.K.). All histopathologic diagnoses other
174 than BCC, were grouped as “malignant” (melanoma, malignant non melanocytic tumor), and
175 “benign” (benign melanocytic tumor, benign non-melanocytic tumor, or inflammatory/scar)
176 categories.

177

178 ***Statistical analysis***

179

180 STATA® software version 17 (StataCorp. 2021. Stata Statistical Software: Release 17. College
181 Station, TX: StataCorp LLC.) and MedCalc Statistical Software version 14.8.1 (MedCalc Software
182 bvba, Ostend, Belgium; <http://www.medcalc.org>; 2014) were employed for statistical analysis.

183 BCC diagnosis was defined for all lesions either confirmed by histopathology or with concordant
184 clinical-dermoscopic-RCM diagnosis of BCC undergoing medical treatment. Sensitivity,
185 specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV), and 95%
186 confidence intervals were calculated for BCC diagnosis.

187 Continuous variables (patients [N], mean, standard deviation [SD]) were compared using Unpaired
188 Student's t test (2 groups). Categorical variables (frequency [N, %]) were compared using Pearson's
189 chi-squared test.

190 Logistic regression model (stepwise forward selection) was used for association between
191 parameters and to identify prognostic factors. Intercept-only model was fitted and individual score
192 statistics were evaluated ($p < 0.05$), removing insignificant variables before adding variables. Data
193 were expressed as odds ratio (OR), 95% confidence interval (CI). $p < .05$ was considered
194 statistically significant. A nomogram for predicting BCC probability (including univariate and
195 multivariate logistic regression analyses) was estimated. Based on prognostic factors identified with
196 logistic regression, a score for BCC diagnosis probability was devised. Briefly, each prognostic
197 variable is assigned a score (0-10). Total BCC scores (0 – 52) corresponded to different BCC
198 diagnosis probabilities. We report a worksheet for the application of the BCC multivariate
199 prediction model. In addition, receiver operating characteristic (ROC) curve was constructed and
200 the area under the curve (AUC) obtained. Alpha level was set at 0.05.

201

202

203 **Results**

204 ***Study population***

205 A total of 777 patients (mean age 68.4 ±14.6, range 21-97) with 1005 lesions were included in this
206 study. Of these lesions, 74% (n=740) were BCCs; most cases (n=703 cases; 70%) had concordant
207 clinical-dermoscopy-RCM diagnoses and 531 were not biopsied because of strongly confident BCC
208 diagnosis and medical treatment choice. A total of 474 cases (47%) were biopsied and diagnosis
209 was confirmed with histopathology.

210 A total of 265 cases had a diagnosis of “other” than BCC, corresponding to 97 (37%) benign non-
211 melanocytic lesions (mainly solar lentigos, seborrheic keratoses), 63 (24%) benign inflammatory
212 lesions or scars, 57 (21%) malignant non-melanocytic lesions (21 actinic keratoses but 31 squamous
213 cell carcinomas and 5 other non-melanoma skin cancers), 41 (15%) benign melanocytic lesions and
214 7 (3%) melanomas.

215 The overall study population is described in **Table 1**. Clinically, lesions were mainly amelanotic
216 (n=574, 57%) and hypomelanotic (n=297, 30%), palpable (n=689, 69%, either papules or nodules)
217 and located on the head-neck area (53%). However, BCCs were more frequently flat and located on
218 the trunk than “other” lesions (33% vs 26%, and 37% vs. 27%).

219 ***Diagnostic accuracy***

220 Diagnostic accuracy achieved in the different workflow steps is reported in **Figure 1**.

221 In detail, the clinical-dermoscopic approach alone reached a sensitivity of 93.2% (CI 95% 91.2 -
222 94.9) and a specificity of 51.7% (CI 95% 45.5 - 57.9). Dermoscopy examination alone resulted in
223 50 false negative lesions, represented by 7 superficial, 34 nodular, 8 infiltrative and 1
224 basosquamous lesions. Interestingly, all 50 lesions were all amelanotic (**Supplementary Fig. 1**).
225 Dermoscopy examination alone also identified 128 false positive cases, represented by 40 (32%)
226 benign inflammatory/scar, 45 (35%) benign non-melanocytic, 27 (21%) malignant non-
227 melanocytic, 13 benign melanocytic (10%), and 3 melanomas in situ (2%) lesions. Dermoscopy
228 alone PPV was 84.4 (CI 95% 81.7 – 86.8) and NPV was 73.3 (CI 95% 66.3 - 79.5).

229 The adjunctive use of RCM proved an increased sensitivity and specificity compared to
230 dermoscopy alone (**Figure 1**). Sensitivity and specificity rates were 97.8% (CI 95% 96.5 - 98.8) and
231 86.8% (CI 95% 82.1 - 90.6).

232 With adjunctive RCM examinations, a total of 16 lesions (2% of the total 740 BCCs) were
233 identified as false negatives and 35 false positives. False positives included 15 (43%) benign
234 inflammatory/scar, 9 (25%) benign non- melanocytic tumors, 10 (26%) malignant non-melanocytic
235 tumors, and 1 nevus. Notably, 46 of the 50 (92%) false negative cases identified by dermoscopy
236 alone were correctly diagnosed as BCCs upon RCM examination. Also, RCM correctly classified
237 103 out of 128 dermoscopy false positive.

238 Adjunctive RCM PPV was 95.4 (CI 95% 93.6 – 96.8) and NPV was 93.5% (CI 95% 89.7 - 96.2).

239 ***BCC diagnosis predicting factors***

240 As expected, BCC dermoscopic and RCM diagnostic criteria were significantly associated with
241 BCC diagnosis, (**Supplementary Table 1**).

242 At multivariable analysis, the strongest factor for the likelihood of BCC diagnosis was the presence
243 of concentric/spoke-wheel structures upon dermoscopy (OR=10.8, CI 1.7-66.9, p=0.011), followed
244 by dark silhouettes (OR=5.9, CI 2.8-12.9, p<0.001), small tumor islands (OR=4.4, CI 1.9-9.7,
245 p<0.001) and large tumor islands (OR=4.3, CI 1.6-11.6, p=0.004) upon RCM examination, see
246 **Figure 2**.

247 BCC multivariate prediction model, including predictive variables identified with regression
248 analysis, was developed from the Nomogram analysis, see **Figure 2**. The BCC-I facilitates the
249 calculation of a personalized BCC-I score, corresponding to the probability of a BCC diagnosis.
250 The reported worksheet (**Supplementary Table 2**) enables clinicians to calculate a personalized
251 BCC-I score, corresponding to a probability of a BCC diagnosis. The BCC-I predictive accuracy
252 was high (AUC= 0.95). The BCC-I predictive accuracy was high as calculated by ROC analysis
253 (AUC= 0.95) (**Supplementary Figure 2**).

254

255 **Discussion**

256 In the realm of non-invasive techniques, dermoscopy has been extensively used in clinical practice
257 for skin cancer diagnostic and monitoring purposes. However, evidence in literature of the real-life
258 diagnostic accuracy of dermoscopy for BCC diagnosis is mainly based on retrospectively designed
259 studies. Recently, wide-probe RCM has been applied for skin cancer diagnosis in a prospective,
260 multi-center, randomized study and was proven useful as an adjuvant tool to dermoscopy
261 examination for melanoma diagnosis, remarkably improving diagnostic specificity.⁶

262 The results of our prospective, multicenter study, carried out on 1005 lesions suspicious of BCC at
263 clinical examination, provides real-life diagnostic accuracy of dermoscopy for BCC, as a stand-
264 alone tool and with adjunctive HH-RCM assessment. The sensitivity and specificity of both
265 dermoscopy alone and with adjunctive RCM assessment reported in this study confirm higher rates
266 when adjunctive RCM assessment is included. The rates reported in our study are in line with data
267 currently available in literature.^{5,9} The pooled sensitivity for BCC diagnosis reported in 2 systematic
268 reviews and meta-analyses was 91.2% with dermoscopy alone and 97% with adjunctive RCM,
269 comparing well with the 93.2% and 97.8% reported in our study.^{5,9}

270 Results concerning RCM sensitivity were even higher and overlapping with histopathology when
271 comparing 3-mm punch biopsies (PB) to 4-mm RCM mosaics (RCM 99.0% vs. PB 99.0%; $P =$
272 1.0). and a lower specificity for RCM as compared to PB (59.1% vs. 100%; $P < 0.001$).¹³ Notably,
273 in our study, HH-RCM was able to correctly diagnose 92% of BCCs that were “false negative”
274 upon dermoscopic examination, thus offering the potential of narrowing the need of biopsies to
275 correctly diagnose a given tumor. This implies a faster pre-treatment diagnostic approach in a real
276 clinical scenario.

277 A meta-analysis concerning RCM diagnostic accuracy, reported a summary estimate of sensitivity
278 of 97% and specificity 93%.^{5,9} Although these meta-analyses had a significant heterogeneity among
279 studies with a medium-to-high risk of bias, the RCM diagnostic accuracies reported in these studies

280 are very similar to those reported in our prospective, multicenter study carried out in a real-life
281 setting.

282 The higher specificity of RCM compared to dermoscopy is justified by the correct identification of
283 35 false positive lesions, resulting mainly in benign inflammatory/scar in 15 cases (43%).

284 Navarrete et al.¹⁴ analyzed the potential role of RCM in evaluating clinically negative sites for
285 residual BCC following biopsy in 61 patients. When considering all cases, including high risk
286 subtypes, they found an RCM sensitivity and specificity of 92.8% and 68.4%. Our data corroborate
287 these findings despite different clinical setting and patient inclusion criteria (patients with either
288 clinically or dermoscopically persistent BCC were excluded by Navarrete et al.).

289 Further, results of the multivariate analysis were employed to develop a BCC multivariate
290 prediction model, showing high accuracy (AUC=95%), revealing an increased association between
291 BCC and specific clinical, dermoscopic as well as RCM features. The model highlights that the
292 presence of BCC tumor-specific features (usually detectable in pigmented BCC only), such as leaf-
293 like/concentric/spoke wheel structures, are strongly associated to BCC diagnosis, corroborating the
294 findings of a previous dermoscopic score, underlining that pigmented features were associated with
295 higher BCC diagnostic accuracy and confidence.¹⁵ In this prospective study, the lower specificity
296 observed with dermoscopy in our study can be reasonably related to the study population that
297 included 87% of hypopigmented/amelanotic lesions. In fact, it is well known that dermoscopic
298 accuracy for BCC diagnosis is lower for non-pigmented lesions^{16,17}, compared to those with
299 pigmentation.^{18,19} Comparatively, RCM diagnostic accuracy seems unaffected by the presence or
300 absence of pigmentation, which is supported by data currently available in literature.^{20,21}

301 Regarding RCM criteria, the presence of BCC tumor-specific features, such as tumor islands (small,
302 big or dark silhouette) along with additional tumor characteristics (i.e. clefting) were significantly
303 associated to BCC diagnosis, as previously reported.¹¹

304 On the contrary, the presence of streaming of the epidermis (a non-specific epidermal change) was
305 not relevant for BCC diagnosis in our study. Some previous studies have claimed a diagnostic role

306 for this criterion.^{22,23} In fact, we believe that streaming of the epidermis is a result of epidermal
307 stretching correlated with tumor expansion, regardless the tumor nature.
308 Additionally, our study specifically and systematically explored the use of HH-RCM device for
309 screening of clinically suspicious BCC and demonstrated an excellent accuracy, emphasizing that
310 this handy device could be safely employed in clinical practice for epithelial cancer with the
311 advantage of faster examination of multiple lesions, compared to the wide-probe RCM.^{24,25} This can
312 be explained by the fact that for confirming a BCC diagnosis by means of RCM it is usually
313 sufficient to identify any BCC tumor-specific feature (such as a basaloid nest or a dark-silhouette),
314 that can be easily visualized during tumor live examination.²³ Whereas, for melanoma differential
315 diagnosis a more accurate cytological and architectural evaluation of the entire lesion is required in
316 order to distinguish slight to moderate atypical nevi from an early malignancy, thus requiring the
317 use of wide-probe RCM.

318 ***Limitations***

319 This study is limited by the inherent depth limitation of the RCM device, potentially affecting the
320 diagnostic accuracy for deeply located BCC features. Further, the sensitivity and specificity
321 reported in our study was achieved in 3 tertiary cancer centers with expertise in dermoscopy and
322 RCM imaging, and may not reflect all real-life clinical scenarios. Accuracy of RCM diagnosis may
323 however vary depending on user experience, although literature data²⁶ reports an overall sensitivity
324 for skin cancer diagnosis of 91%, with a sensitivity for basal cell carcinoma of 92% for novice
325 users. Finally, not all lesions were biopsied and verified by histopathology, but this reflects current
326 clinical practice and, for our study, we had diagnostic confirmation with 2 years follow up.
327 The reproducibility of the BCC multivariate predictive model developed in this study-requires
328 validation in other samples prior to being applied to clinical practice.

329 ***Conclusions***

330 Dermoscopy with adjunctive RCM assessment of BCC clinically suspicious lesions permits high
331 BCC diagnostic accuracy with minimal false negative lesions.

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409 **Figure legends**

410 **Figure 1.** Workflow illustrating tumors distribution according to dermoscopic and confocal
411 outcome and main diagnostic classification (STARD diagram flow).

412

413 **Figure 2.** Results of multivariate statistical analysis employed to develop BCC multivariate
414 prediction model, represented in the nomogram

415

416 **Table 1. Demographic, clinical and histopathologic data of a total of 1005 lesions included in**
417 **the study**

	Total (n=1005)	Others (n=265, 26%)	BCC (n=740, 74%)	p-value	OR (CI 95%)
Diagnosis, n (%)					
BCC	740 (74)	0	740 (100)	-	
Other	265 (26)	265 (100)	0		
Diagnosis other than BCC, n (%)					
<i>Benign</i>	201 (20)	201 (76)	0		
Inflammatory/scars	63 (6)	63 (24)	0	-	
Benign non-melanocytic	97 (10)	97 (37)	0		
Benign melanocytic	41 (4)	41 (15)	0		
<i>Malignant</i>	64 (7)	64 (24)	0		
Malignant non-melanocytic	57 (6)	57 (21)	0		
Malignant melanocytic	7 (1)	7 (3)	0		
Age, mean ± SD (range)	68.4 ±14.6 (21-97)	63.8 ±15.9 (21-95)	70.0 ±13.8 (22-97)	<0.001	1.02 (1.01-1.03)^
Sex, n (%)					
Female	417 (41)	135 (51)	287 (39)	0.004	ref.
Male	588 (59)	130 (49)	453 (61)		1.5 (1.1-2.0)^
Location, n (%)					
Head-neck	534 (53)	158 (60)	376 (51)	0.024	ref.
Trunk	346 (34)	71 (27)	275 (37)		1.6 (1.2-2.2)^
Upper limbs	67 (7)	20 (7)	47 (6)		0.9 (0.6-1.7)
Lower limbs	58 (6)	16 (6)	42 (6)		1.1 (0.6-2.0)
Palpability, n (%)					
Flat	316 (31)	69 (26)	247 (33)	0.004	Ref.
Papule	337 (34)	110 (42)	227 (31)		0.6 (0.4-0.8)^
Nodule	352 (35)	86 (32)	266 (36)		0.9 (0.6-1.2)
Clinical pigmentation, n (%)					
Amelanotic	574 (57)	170 (64)	404 (55)	0.001	Ref.
Light	297 (30)	54 (20)	243 (33)		1.9 (1.3-2.7)^
Dark	134 (13)	41 (16)	93 (12)		0.9 (0.6-1.4)

418 ^ Univariate logistic regression significant differences among groups (p-value<0.005)
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