

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: <http://www.elsevier.com/locate/medici>

Original Research Article

Nevomelanocytic atypia detection by in vivo reflectance confocal microscopy

Ingrida Vaišnorienė^{a,*}, Ričardas Rotomskis^{a,e}, Vytautas Kulvietis^a, Rimantas Eidukevičius^b, Violeta Žalgevičienė^c, Aida Laurinavičienė^d, Jonas Venius^a, Janina Didžiapetrienė^a

^a National Cancer Institute, Vilnius, Lithuania^b Faculty of Mathematics and Informatics, Vilnius University, Vilnius, Lithuania^c Department of Anatomy, Histology and Anthropology of Faculty of Medicine, Vilnius University, Vilnius, Lithuania^d National Center of Pathology, Vilnius University Hospital Santariškių Klinikos, Vilnius, Lithuania^e Biophotonics Group of Laser research Center, Vilnius University, Vilnius, Lithuania

ARTICLE INFO

Article history:

Received 28 April 2014

Accepted 22 September 2014

Available online 1 October 2014

Keywords:

Diagnostic accuracy

Melanocytic atypia

Dysplastic nevus

Melanoma

Reflectance confocal microscopy

ABSTRACT

Background and objective: In vivo reflectance confocal microscopy (RCM) is a promising novel technology for non-invasive early diagnostics of cutaneous melanoma. However, the possibility to detect melanocytic atypia in nevi by means of in vivo RCM remains unknown. The aim of the study was to evaluate the significance of in vivo RCM features of melanocytic atypia for the diagnosis of melanocytic nevi, dysplastic nevi and cutaneous melanoma.

Materials and methods: A total of 138 melanocytic skin lesions comprising 25 melanocytic nevi, 69 dysplastic nevi and 44 melanomas were analyzed by means of dermoscopy, in vivo RCM and routine histopathology. In vivo RCM images were analyzed for the arrangement of keratinocytes in epidermis, pagetoid cells and junctional melanocytic nests and correlated refractivity aspects of nests with histopathology.

Results: Separately and all together taken the in vivo RCM features of melanocytic atypia were significant in differential diagnosis of benign and malignant melanocytic skin lesions, though none of the features was significant in discriminating nevi without cytologic atypia of dysplastic nevi. In vivo RCM feature of dense cell clusters corresponded with melanin containing nevomelanocytes on histopathology though exact correspondence of non-homogeneous and atypical sparse cell clusters remained questionable.

Conclusions: Nevus with histopathologically confirmed nevomelanocytic atypia (dysplastic nevus) could not be distinguished from nevus without atypia using analyzed in vivo RCM features of melanocytic atypia. More accurate diagnostics by means of in vivo RCM needs further investigation on reflectance of single and nested cutaneous melanocytes in benign and malignant skin lesions.

© 2014 Lithuanian University of Health Sciences. Production and hosting by Elsevier Urban & Partner Sp. z o.o. All rights reserved.

* Corresponding author at: National Cancer Institute, Santariškių 1, 08660 Vilnius, Lithuania.

E-mail address: ingrida.vaisnoriene@gmail.com (I. Vaišnorienė).

Peer review under responsibility of Lithuanian University of Health Sciences.



Production and hosting by Elsevier

<http://dx.doi.org/10.1016/j.medici.2014.09.008>

1010-660X/© 2014 Lithuanian University of Health Sciences. Production and hosting by Elsevier Urban & Partner Sp. z o.o. All rights reserved.

1. Introduction

Diagnostics of cutaneous melanocytic lesions is orientated toward the detection of melanoma at the earliest possible stage. Nevi, especially dysplastic ones, are critical not only as simulants, but also as possible precursors to melanoma [1–3]. The possibility to monitor morphological changes of melanocytic atypia/dysplasia in nevi would provide insight into their true nature and importance in melanomagenesis. Detection of dysplasia in nevi by means of non-invasive diagnostics is challenging: nevi with dermoscopic features of atypia do not always show atypia on histopathological examination; conversely, nevi with severe dysplasia are difficult to distinguish from malignant melanoma.

Recent innovations in optical skin imaging technologies provide additional information, and together with dermoscopy, help make more accurate clinical diagnosis of melanocytic lesions. *In vivo* reflectance confocal microscopy (RCM) represents a new imaging technique, which offers the possibility to examine non-invasively superficial (to 250 μm depth) layers of the skin at a cellular resolution [4]. *In vivo* RCM improves diagnostic accuracy of melanocytic skin tumors [5,6].

The main endogenous contrast agents on RCM are melanin and keratin. Melanin containing cells appear bright on RCM imaging. Melanocyte as a melanin-producing cell is expected to be bright on confocal images [7].

Melanosomes/melanin in nested melanocytes provide contrast, making them easily recognizable by *in vivo* RCM. Junctional cell clusters are one of the key features in distinguishing between nevi and melanomas by *in vivo* RCM [8]. Cytologic atypia of nested melanocytes (nevomelanocytes) presented as nuclear enlargement, irregularity, hyperchromasia and prominent nucleoli in histopathology cannot be evaluated by *in vivo* RCM.

According to cytomorphological features of increasing atypia of (nevo)melanocytes, all cutaneous melanocytic lesions histopathologically are sorted in melanocytic nevi, dysplastic nevi and malignant melanomas [9,10].

The aim of the study was to evaluate the significance of *in vivo* reflectance confocal microscopy (RCM) features of melanocytic atypia for the diagnosis of melanocytic nevi, dysplastic nevi and cutaneous melanoma. We were not able to find any data in the literature on differential diagnosis of dysplastic nevi by *in vivo* RCM criteria of melanocytic atypia. So, this may be the first study in the diagnostic of dysplastic nevi by *in vivo* RCM. A comparison of *in vivo* RCM features of melanocytic atypia with changes of (nevo)melanocytes on the histopathological investigation in dysplastic nevi are presented on pictures, discussed below and the significance of *in vivo* RCM features was evaluated by methods of statistical analysis.

2. Materials and methods

2.1. Patients

The participants were patients of the Outpatient Clinic of the National Cancer Institute (Lithuania). The study was approved

by the local ethics committee (protocol number ADT-01) and performed in accordance with the Declaration of Helsinki. Overall 138 patients (90 women, 48 men, standard deviation 16, median age of 42 years, range 18–78 years of age) were recruited over a period of 2 years (November 2010–March 2013). All lesions were excised for histopathological examination. The study was conducted on 138 melanocytic lesions comprising 44 melanomas (median Breslow thickness of 1.35 mm: min – 0 mm (in situ; 9 cases) and max – 9 mm)), 69 dysplastic nevi and 25 melanocytic nevi.

2.2. Instruments and image acquisition procedures

In vivo RCM. Before biopsy, dermoscopic and *in vivo* RCM features of the study lesions were documented using a commercially available, near-infrared reflectance confocal microscope (Vivascope 1500; Lucid Inc., Rochester, NY, USA). The confocal microscope Vivascope 1500 uses a diode laser at 830 nm wavelength and a power of less than 20 mW at tissue level. This provides a lateral resolution of 0.5–1.0 μm , axial resolution of 3.0–5.0 μm (section thickness) and imaging depth from corneal layer to the superficial dermis (up to the 200–250 μm) *in vivo*. Instrument and acquisition procedures are described elsewhere [4]. The skin contact device consists of a metal tissue ring with a coverglass window, which is attached to the skin surrounding the lesion. Dermoscopic images were recorded by a digital camera (Vivacam; Lucid Inc.) connected to the RCM computer by USB cable. After acquiring the dermoscopic image, a 30 \times objective lens of numerical aperture 0.9 was attached to the tissue ring with a diameter of 1 cm. The *in vivo* RCM acquires horizontal tissue images at a 500 \times 500 μm field of view with a resolution of 1024 \times 1024 pixels. An automatic stepper was used to scan up to 8 \times 8 mm field of view in the tissue (depending on the size of the lesion), producing a square mosaic of up to 256 contiguous individual images (VivaBlock). Mosaic images were obtained on three levels: 1st comprises stratum granulosum/stratum spinosum (20–30 μm depth); 2nd level, dermoepidermal junction (60 μm depth), and 3rd level, superficial dermis (90 μm depth). In addition, images were captured with an automatic stepper obtaining sequentially deeper individual images (VivaStack) on the areas of interest.

2.3. Image description

In vivo RCM images were described using terms for the RCM description of melanocytic lesions summarized in a consensus terminology glossary [11]. Pagetoid cells and epidermal disarray were analyzed 30 μm below stratum corneum. Analysis on the cellularity, density and homogeneity of brightness of junctional cell clusters was performed at the level of dermoepidermal junction – 60 μm and 90 μm below stratum corneum – on *in vivo* RCM images. Recognition of the predominant type of junctional clusters (dense or nonhomogeneous) and the presence of irregular discohesive and sparse cell clusters were the focus of *in vivo* RCM examination. The depth limit of 90 μm below stratum corneum was chosen because analysis of cellular aspects of (nevo)melanocytes is possible only in the superficial layers of the skin on *in vivo* RCM imaging.

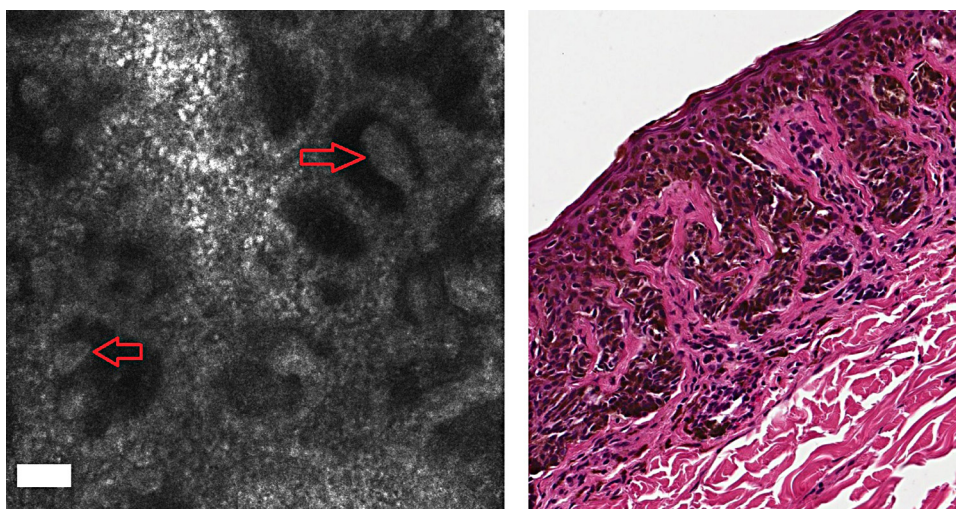


Fig. 1 – In vivo RCM image (0.5 mm × 0.5 mm) obtained at the upper dermoepidermal junction level (30 μ m depth) and corresponding histological image (HE staining, 20 \times). In vivo RCM features: dense junctional thickenings bulge into dermal papillae (arrows) observed together with architectural disorder. Bar = 50 μ m. Histopathological diagnosis: dysplastic nevus with moderate cytologic atypia.

2.4. In vivo RCM features

Five criteria correlating with melanocytic and nevomelanocytic atypia were chosen for statistical analysis, namely: dense clusters, non-homogeneous clusters, irregular discohesive and sparse cell clusters, pagetoid cells and epidermal disarray. Compact aggregates of well-demarcated reflective cells were described as dense cell thickenings or clusters (Fig. 1 left). The term “non-homogeneous junctional thickenings or clusters” was employed for describing aggregates composed of cells with variable brightness/heterogenous reflectivity without clear cellular outlines (Fig. 2 left). Aggregates were defined as “irregular discohesive” when they showed cellular discohesion with irregular margins (Fig. 3 left) and “sparse cell clusters” as

roundish dark (non-reflecting) structures with a well-demarcated border. Pagetoid cells were large reflective round to oval cells observed in suprabasal layers. Loss of epidermal cell demarcation and honeycomb pattern was defined as epidermal disarray.

2.5. Histopathologic examination

Histopathologic diagnosis of dysplastic nevus was based on the presence of architectural disorder, cytologic atypia of (nevo) melanocytes and features indicative of a host response to the nevus cells. The following features of cytologic atypia of (nevo) melanocytes were assessed: nuclear shape, chromatin pattern, size of nucleoli, mitotic figures, nuclear membrane irregularity [1]. The abovementioned features were analyzed in melanocytes

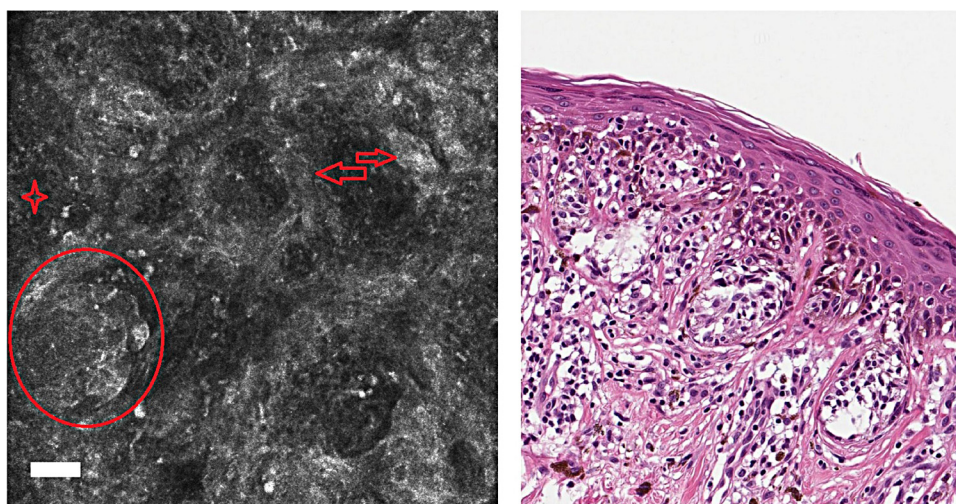


Fig. 2 – In vivo RCM image (0.5 mm × 0.5 mm) obtained at the level of dermoepidermal junction (60 μ m below stratum corneum) and histological image (HE staining, 20 \times). In vivo RCM features: nonhomogeneous junctional thickenings (left arrow) and clusters (circled), bridging of nests between rete (right arrow), fibrosis and inflammatory infiltration (four-point star). Bar = 50 μ m. Histopathological diagnosis: dysplastic nevus with severe cytologic atypia.

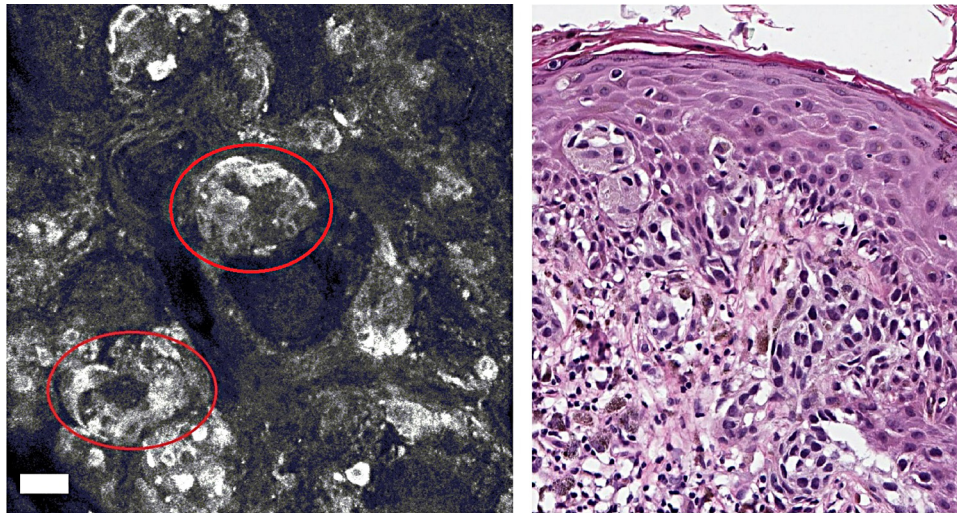


Fig. 3 – In vivo RCM image (0.5 mm × 0.5 mm) obtained at the level of deep dermoepidermal junction 50 μ m below stratum corneum and histological image (HE staining, 20 \times). In vivo RCM features: non-homogeneous junctional thickenings with irregular discohesive nests (circled). Bar = 50 μ m. Histopathological diagnosis: superficially spreading malignant melanoma of 0.9 mm Breslow thickness.

and nevomelanocytes within epidermis and dermo-epidermal junction. Digital images of histological slides were captured using the Aperio ScanScope XT Slide Scanner (Aperio Technologies, Vista, CA, USA) under 20 \times objective magnification (0.5 μ m resolution). On the images of histopathological specimens cytologic features of melanocytic lesions were analyzed and correlated to the in vivo RCM observation.

2.6. Statistical analysis

Statistical analysis was performed using SAS 9.3 statistical software (SAS Institute, Cary NC). Independence was evaluated by means of χ^2 test. Logistic regression was used to estimate prognostic value of various diagnostic features by means of odds ratio (OR), corresponding confidence intervals (CI), ROC curves and area under the curve (AUC). The models in the

tables are sorted according to likelihood score (χ^2) statistics. A P value less than 0.05 was considered statistically significant. In vivo RCM features were statistically analyzed in different groups of melanocytic skin lesions based on histopathological diagnosis (melanocytic nevi vs dysplastic nevi vs melanomas). Diagnostic significance of five in vivo RCM features – predominant type of junctional cell clusters – dense or non-homogeneous, presence of irregular discohesive and sparse cell clusters, pagetoid cells and loss of normal epidermal honeycomb pattern were analyzed.

3. Results

Three in vivo RCM features defining nevomelanocytes – dense, nonhomogeneous and irregular discohesive or sparse cell

Table 1 – Sensitivity and specificity of in vivo reflectance confocal microscope features for melanocytic nevi and dysplastic nevi.

	Nevi ^a n = 25		Dysplastic nevi n = 69	
	Sensitivity, %	Specificity, %	Sensitivity, %	Specificity, %
Dense clusters	72	n = 18 54.87	71.01	n = 49 71.01
Non-homogeneous clusters	16	n = 4 51.33	23.19	n = 16 37.68
Irregular discohesive and sparse cell clusters	4	n = 1 74.34	5.8	n = 4 62.32
Pagetoid cells	24	n = 6 49.56	28.99	n = 20 37.68
Epidermal disarray	0	n = 0 63.72	8.7	n = 6 49.28

^a Melanocytic nevi without cytologic atypia.

Table 2 – Sensitivity, specificity and odds ratios of in vivo reflectance confocal microscope features for nevi and melanomas.

	Nevi ^a n = 94		Melanoma n = 44		OR (95% CI)	P value
	Sensitivity, %	Specificity, %	Sensitivity, %	Specificity, %		
Epidermal disarray	6.38	n = 6 20.45	79.55	n = 35 93.62	57.037 (18.896–172.165)	<0.0001
Nonhomogeneous clusters	21.28	n = 20 11.38	88.64	n = 39 78.72	28.857 (10.059–82.787)	<0.0001
Dense clusters	71.28	n = 67 95.45	4.55	n = 2 28.72	0.019 (0.004–0.085)	<0.0001
Irregular discohesive and sparse cell clusters	5.32	n = 5 43.18	56.82	n = 25 94.68	23.421 (7.95–68.995)	<0.0001
Pagetoid cells	27.66	n = 26 15.91	84.09	n = 37 72.34	13.824 (5.478–34.886)	<0.0001

^a Melanocytic nevi without cytologic atypia and dysplastic nevi. OR, odds ratio; CI, confidence interval.

clusters, together with the in vivo RCM feature of “pagetoid cells” corresponding to presence of single atypical melanocytes in the superficial layers of epidermis and one more feature – “epidermal disarray” characteristic to loss of normal architecture of epidermis were analyzed in nevi, dysplastic nevi and melanomas. The absolute frequencies, sensitivity and specificity together with the odds ratio and 95% confidence interval values for the significant features are reported in Tables 1–3.

The values of dense or non-homogeneous cell clusters as well as other in vivo RCM features were not significant in logistic regression analysis as discriminating factors for melanocytic atypia detection in nevi. Sensitivity and specificity of in vivo RCM features in melanocytic nevi are displayed in Table 1.

All of them together with epidermal disarray, pagetoid cells and irregular discohesive and sparse cell clusters were significant in differentiating nevi and melanoma (Table 2). Epidermal disarray was the most significant feature in malignant melanoma diagnostics by means of in vivo RCM (Tables 2 and 3). The sensitivity of 79.55%, and specificity of 93.62% of RCM epidermal disarray feature showed the best accuracy for cutaneous melanoma diagnostics. Non-homogeneous clusters in pair with epidermal disarray were the most significant for the diagnosis of melanoma according to

likelihood score (χ^2) statistics. The models with three or four independent variables include at least one statistically not significant feature. There is strong dependence among all four of the features (P values ≤ 0.0001).

3.1. In vivo RCM evaluation and correlation with histopathology

Dense junctional clusters predominated in most nevi and in 2 out of 44 melanomas. They corresponded to compact cellular aggregates and well-circumscribed junctional nests composed of melanin containing cells at histopathology (Fig. 1). This feature was characteristic of benign melanocytic skin lesions. Nonhomogeneous junctional thickenings and clusters were characteristic of melanoma, though observed in 4 out of 25 common nevi and in 16 out of 69 dysplastic nevi. They corresponded to irregular junctional nesting and confluence of basilar melanocytes along the rete ridges at histopathology (Fig. 2). Irregular discohesive nests were observed in 25 out of 44 melanomas and were rare in nevi. This feature corresponded to discohesive nests composed of large epithelioid melanocytes histopathologically (Fig. 3).

In vivo RCM feature of dense cell clusters corresponded with melanin containing nevomelanocytes on histopathology

Table 3 – Models of melanoma diagnosis by two in vivo reflectance confocal microscope features.

	AUC	OR (95% CI)	P value
Nonhomogeneous clusters and epidermal disarray	0.9125	8.761 (2.553–30.064) 22.534 (6.824–74.407)	0.0006 <0.0001
Dense clusters and epidermal disarray	0.9206	0.075 (0.015–0.376) 19.254 (5.84–63.48)	0.0017 <0.0001
Irregular discohesive and sparse cell clusters and epidermal disarray	0.892	5.466 (1.351–22.118)	0.0172
Pagetoid cells and epidermal disarray	0.8713	30.349 (9.41–97.876) 0.699 (0.190–2.567) 62.484 (19.364–201.619)	<0.0001 0.5893 <0.0001

AUC, area under the curve; OR, odds ratio; CI, confidence interval.

though exact correspondence of non-homogeneous and atypical sparse cell clusters remained questionable.

4. Discussion

Since the beginning of its use, in vivo RCM researchers have been focused on identification of contrasting cytoplasm and dark (non-reflecting) nucleus containing melanocytes, nevomelanocytes and pagetoid cells [12–16]. According to the standard glossary of terms, the description and interpretation of features of in vivo RCM imaging is that melanin and melanosomes are strong sources of contrast on RCM, pagetoid cells are characterized by dark nucleus and bright cytoplasm refractive cells located in suprabasal layers [5–8,11,13–15], and normal melanocytes may appear as bright (refractive) stellate cells on confocal imaging [17].

In vivo RCM was described as an effective method for the diagnosis of cutaneous melanocytic lesions, which increases specificity above dermoscopic assessment alone [7,18–20]. Atypical and dysplastic nevi have been frequently examined in in vivo RCM studies to define diagnostic criteria for malignant melanoma [13,15,18]. However, until now there are no systematic studies to correlate the confocal aspects of atypical nevi with their histopathological counterparts precisely. Moderate to marked cytologic atypia is characterized in RCM by the presence of large, bright nucleated cells with clearly outlined contours and dark nuclei within the basal layer [11]. Pleomorphic atypical melanocytes may be distinguished although their correlation with histological grading for cytologic atypia has never been proven.

According to our study results, all the analyzed in vivo RCM features statistically and clinically were significant in diagnostics of cutaneous melanocytic lesions. The statistical analysis showed that in vivo RCM helps distinguish between benign and malignant melanocytic lesions according to dense clusters, non-homogeneous clusters, irregular discohensive and sparse cell clusters, pagetoid cells and epidermal disarray. Epidermal disarray was the most significant RCM feature of malignancy according to our results. The correlation of the RCM epidermal disarray and nonhomogeneous clusters with malignancy has been reported in other studies [5,14]. On the other hand, dense cell clusters were characteristic of nevi (sensitivity 71.28%, specificity 95.45%) as reported by others, but the feature was not informative in discriminating melanocytic nevi without atypia and dysplastic nevi.

The results allow us to make an assumption that focusing attention on finding contrasting atypical melanocytes on in vivo RCM imaging is enough for the differential diagnostic of benign-malignant melanocytic skin lesions. More detailed diagnostics of dysplastic nevi needs further investigation on reflectance of melanocytes, nevocytes and atypical melanocytes under RCM.

5. Conclusions

These results show that current criteria of melanocytic atypia by in vivo RCM are limited to differentiation between benign and malignant melanocytic skin lesions. Nevus with

histopathologically confirmed nevomelanocytic atypia (dysplastic nevus) could not be distinguished from nevus without atypia using analyzed in vivo RCM features of melanocytic atypia. More detailed studies on reflectance of single and nested melanocytes in melanocytic skin lesions would improve the diagnostic accuracy of this promising technology.

Conflict of interest

The authors state no conflict of interest.

Acknowledgments

This study was partially supported by two grants from the Research Council of Lithuania (grant numbers LIG-10044, VP1-3.1-SMM-01-V-02-003). No financial support was provided by companies involved with ownership of the in vivo RCM device.

REFERENCES

- [1] Elder DE. Dysplastic naevi: an update. *Histopathology* 2010;56:112–20.
- [2] Elder DE. Precursors to melanoma and their mimics: nevi of special sites. *Mod Pathol* 2006;19:S4–20.
- [3] Arumi-Uria M, McNutt NS, Finnerty B. Grading of atypia in nevi: correlation with melanoma risk. *Mod Pathol* 2003;16:764–71.
- [4] Rajadhyaksha M, Grossman M, Esterowitz D, Webb RH, Anderson RR. In vivo confocal scanning laser microscopy of human skin: melanin provides strong contrast. *J Invest Dermatol* 1995;104:946–52.
- [5] Busam KJ, Charles C, Lohmann CM, Marghoob A, Goldgeier M, Halpern AC. Detection of intraepidermal malignant melanoma in vivo by confocal scanning laser microscopy. *Melanoma Res* 2002;12:349–55.
- [6] Gerger A, Wiltgen M, Langsenlehner U, Richtig E, Horn M, Weger W, et al. Diagnostic image analysis of malignant melanoma in in vivo confocal laser-scanning microscopy: a preliminary study. *Skin Res Technol* 2008;14:359–63.
- [7] Busam KJ, Hester K, Charles C, Sachs DL, Antonescu CR, Gonzalez S, et al. Detection of clinically amelanotic malignant melanoma and assessment of its margins by in vivo confocal scanning laser microscopy. *Arch Dermatol* 2001;137:923–9.
- [8] Pellacani G, Cesinaro AM, Seidenari S. In vivo assessment of melanocytic nests in nevi and melanomas by reflectance confocal microscopy. *Mod Pathol* 2005;18:469–74.
- [9] Hussein MR. Melanocytic dysplastic naevi occupy the middle ground between benign melanocytic naevi and cutaneous malignant melanomas: emerging clues. *J Clin Pathol* 2005;58:453–6.
- [10] Culpepper KS, Granter SR, McKee PH. My approach to atypical melanocytic lesions. *J Clin Pathol* 2004;57:1121–31.
- [11] Scope A, Benvenuto-Andrade C, Agero AL, Malvehy J, Puig S, Rajadhyaksha M, et al. In vivo reflectance confocal microscopy imaging of melanocytic skin lesions: consensus terminology glossary and illustrative images. *J Am Acad Dermatol* 2007;57:644–58.
- [12] Rajadhyaksha M, Gonzalez S, Zavislan JM, Anderson RR, Webb RH. 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] Pellacani G, Cesinaro AM, Seidenari S. Reflectance-mode confocal microscopy for the in vivo characterization of pagetoid melanocytosis in melanomas and nevi. *J Invest Dermatol* 2005;125:532–7.
- [14] Pellacani G, Guitera P, Longo C, Avramidis M, Seidenari S, Menzies S. The impact of in vivo reflectance confocal microscopy for the diagnostic accuracy of melanoma and equivocal melanocytic lesions. *J Invest Dermatol* 2007;127:2759–65.
- [15] Langley RG, Walsh N, Sutherland AE, Propperova I, Delaney L, Morris SF, et al. The diagnostic accuracy of in vivo confocal scanning laser microscopy compared to dermoscopy of benign and malignant melanocytic lesions: a prospective study. *Dermatology* 2007;215:365–72.
- [16] Hofmann-Wellenhof R, Wurm EM, Ahlgrimm-Siess V, Richtig E, Koller S, Smolle J, et al. Reflectance confocal microscopy—state-of-art and research overview. *Semin Cutan Med Surg* 2009;28:172–9.
- [17] Busam KJ, Charles C, Lee G, Halpern AC. Morphologic features of melanocytes, pigmented keratinocytes, and melanophages by in vivo confocal scanning laser microscopy. *Mod Pathol* 2001;14:862–8.
- [18] Guitera P, Pellacani G, Longo C, Seidenari S, Avramidis M, Menzies S. In vivo reflectance confocal microscopy enhances secondary evaluation of melanocytic lesions. *J Invest Dermatol* 2009;129(0):131–8.
- [19] Longo C, Farnetani F, Ciardo S, Cesinaro AM, Moscarella E, Ponti G, et al. Is confocal microscopy a valuable tool in diagnosing nodular lesions? A study on 140 cases. *Br J Dermatol* 2013;169:58–67.
- [20] Longo C, Rito C, Beretti F, Cesinaro AM, Pineiro-Maceira J, et al. De novo melanoma and melanoma arising from pre-existing nevus: in vivo morphologic differences as evaluated by confocal microscopy. *J Am Acad Dermatol* 2011;65:604–14.