16th Meeting of the International Confocal Working Group (ICG), Copenhagen, Denmark

Location: Fiolstræde 44, 1171 København K, Denmark, +45 33 42 66 08
http://www.kosmopol.nu

October 8th, 2015
18:00-21:00

AGENDA:
18.10 Introduction Salvador González

18.10-18.30 COMPUTATION MODELING OF THE DERMAL-EPIDERMAL JUNCTION IN REFLECTANCE CONFOCAL MICROSCOPIC IMAGES OF SKIN

Kivanc Kose, Sila Kurugol, Sindhu Gantha, Christi Fox, Jennifer Dy, Dana Brooks, Milind Rajadhyaksha

Memorial Sloan-Kettering Cancer Center, Caliber ID & Northeastern University

18.30-18.45 REFLECTANCE CONFOCAL MICROSCOPY OF PLAQUE, GUTTATA, PALMOPLANTAR AND ERYTHRODERMIC PSORIASIS


Dermatology Unit. Pathology Unit. Hospital Universitario Fundación Alcorcón. Madrid. Spain.
18.45-19.00 THE STATE OF THE ART OF REFLECTANCE
CONFOCAL MICROSCOPY IN VESICOBULLOUS DISORDERS

Francesco Lacarrubba, Anna Elisa Verzì, Giuseppe Micali
Dermatology Clinic, University of Catania, A.O.U. Policlinico-Vittorio Emanuele, Catania, Italy

19.00-19.15 CONFOCAL MICROSCOPY VERSUS DERMOSCOPY FOR THE DIAGNOSIS OF SCABIES

E. Cinotti, M., B. Labeille, A. Biron, C. Chol, F. Cambazard, A. Leclerc, C. Jaffelin, J. L. Perrot
Dermatologie, CHU Saint Etienne, France

19.15-19.30 MELANOMA AND NAEVI WITH GLOBULAR PATTERN: CONFOCAL MICROSCOPY AS AN AID FOR DIAGNOSTIC DIFFERENTIATION.

Elisa Benati¹, MD, Giuseppe Argenziano², MD, Athanassios Kyrgidis¹, MD, PhD, Elvira Moscarella¹, MD, Silvana Ciardo³, BS, Sara Bassoli³, MD, Francesca Farnetani³, MD, Simonetta Piana⁴, MD, Anna Maria Cesinaro⁵, MD, Aimilios Lallas¹, MD, Stefania Borsari¹, MD, Giovanni Pellacani³, MD, Caterina Longo¹*, MD, PhD
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BREAK


TC Grazziotin, I Alarcon, RR Bonamigo, C Carrera, M Potrony, P Aguilera, JA Puig-Butillé, J Brito, C Badenas, L Alós, J Malvehy, S Puig
Dermatology Department, Melanoma Unit, Hospital Clinic I Provincial de Barcelona, IDIBAPS, Universitat de Barcelona, Barcelona, Spain

19.55-20.10 REFLECTANCE CONFOCAL MICROSCOPY GUIDED MICROBIOPSY

Marco Ardigo¹, Lynlee L Lin², Lisa Tom², Ross Flewell-Smith², Van Hoang², H Peter Soyer², Tarl W Prow²

¹San Gallicano Dermatological Institute-IRCCS, Rome, Italy; ²Dermatology Research Centre, School of Medicine,, University of Queensland, Woolloongabba, QLD, Australia

ICG ACTIVITIES

20.10-20.25 FINAL UPDATE OF THE ICG FOR INFLAMMATORY DISORDERS STUDY

M Ardigo and ICG members
San Gallicano Dermatological Institute-IRCCS, Rome, Italy

20.25- STUDIES, COURSES AND MEETINGS UNDER ICG
J Malvehy, E Cinotti, G Pellacani
BOOK OF ABSTRACTS
COMPUTATION MODELING OF THE DERMAL-EPIDERMAL JUNCTION IN REFLECTANCE Confocal Microscopic Images of Skin

Kivanc Kose, Sila Kurugol, Sindhu Gantha, Christi Fox, Jennifer Dy, Dana Brooks, Milind Rajadhyaksha

Memorial Sloan-Kettering Cancer Center, Caliber ID & Northeastern University

We will present preliminary development of computational models and algorithms for localizing the dermal-epidermal junction (DEJ) and characterizing its morphology in reflectance confocal microscopic (RCM) images of skin. Our motivation is to create quantitative approaches to automate and standardize acquisition of RCM images and mosaics, to guide reading and analysis of images, to assist expert and novice clinicians (RCM readers) for performing noninvasive diagnosis, and also tools for education, training and research. Our models for localizing the DEJ are based on three approaches: texture with depth, intensity and resolution with depth (i.e., imaging physics), and shape (i.e., morphology). Testing on 30 RCM stacks of normal skin, 15 each of dark and fair, with validation against labeling by experts, suggests that the DEJ may be localized to within 2-20 μm. Most of the errors are within the thickness (~15 μm) of a basal layer. Modeling of the cellular morphologic patterns at the DEJ is based on texture, shift invariant feature transform (SIFT) and bag-of-words approaches. Testing on 30 RCM mosaics of benign and malignant melanocytic lesions, again, with validation against labeling by experts, shows variable sensitivity of 65-80% but promisingly high specificity of 80-99% for detecting the main patterns (ring, clod, mesh, non-specific). Of course, this data is all very preliminary and we have a long way to go toward clinical utility.

REFLECTANCE Confocal Microscopy of Plaque, Guttata, Palmoplantar and Erythrodermic Psoriasis


Dermatology Unit. Pathology Unit. Hospital Universitario Fundación Alcorcón. Madrid. Spain.

Reflectance confocal microscopy (RCM) for plaque psoriasis has shown to have a good correlation with histopathology. We present a prospective study of 44 patients with different types of psoriasis (25 plaque, 15 guttate, 2 palmoplantar and 2 erythrodermic) studied by
reflectance confocal microscopy. Clinical and dermoscopic pictures were obtained. Reflectance confocal microscopy was performed to analyse the stratum corneum, spinosum and dermoeipidermal junction. The parameters analysed were parakeratosis, thickening of str. Corneum, neutrophils, epidermal inflammation, epidermal pattern, acanthosis, dilated blood vessels and dermal inflammation.
Punch-biopsies of 27 patients were made and processed horizontally instead of vertically to have a better correlation with confocal microscopy images.
Hematoxylin-eosin staining was made, and the parameters analysed were parakeratosis, presence of neutrophils in stratum corneum, suprapapillary thinning, decrease of the granular layer, dilated blood vessels, epidermal and dermal inflammation.
Our results were comparable with the previous reports. Acanthosis was present in all of the cases. We observed that the presence of “en face honeycomb” was more frequent in plaque psoriasis in comparison of guttate type. Moreover dermal inflammation was present in all of the plaque psoriasis cases and in lower percentage in guttate psoriasis patients.
Reflectance confocal microscopy was proven to have a good correlation with histopathology in different morphologic types of psoriasis, as it was demonstrated in previous studies with plaque psoriasis. It can be also a useful tool to evaluate the response to different treatments in different types of psoriasis.

THE STATE OF THE ART OF REFLECTANCE CONFOCAL MICROSCOPY IN VESICOBULLOUS DISORDERS

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Vesicobullous disorders are characterized by intraepidermal or subepidermal blistering resulting from different mechanisms. The diagnosis is generally based on clinical examination and semi-invasive/invasive procedures such as cytology and histopathology. In vivo reflectance confocal microscopy (RCM), a non-invasive technique for real-time, high-resolution, en face imaging of the epidermis and upper dermis with resolution close to conventional histopathology, may play an important role in the differential diagnosis of these disorders, allowing a “virtual biopsy” of the skin. It may support the clinical diagnosis and direct dermatologists to the appropriate patient management. The handheld device, which allows a rapid examination of several skin lesions in real time and a better access to difficult anatomic locations such as folds, scalp, nose, eyelids, ears and mucosae, appears to be particularly suitable for
such evaluations. RCM features of different vesicobullous diseases are reported along with literature review.

**CONFOCAL MICROSCOPY VERSUS DERMOSCOPY FOR THE DIAGNOSIS OF SCABIES**

E. Cinotti, M., B. Labeille, A. Biron, C. Chol, F. Cambazard, A. Leclerc, C. Jaffelin, J. L. Perrot

Dermatologie, CHU Saint Etienne, France

**Introduction:** The gold standard for the diagnosis of scabies was so far the optical microscopy examination of scales obtained by skin scraping for the identification of the parasite. Recently, dermoscopy (DS) and reflectance confocal microscopy (RCM) have been proposed for a rapid and noninvasive diagnosis of scabies. These techniques have the advantage over optical microscopy that they can examine the entire body surface without being invasive. We compare the sensitivity and specificity as well as the time required for the diagnosis with these two techniques.

**Material and Methods:** A prospective study was performed on 148 consecutive patients, including 100 women and 48 men, aged from 1 month to 95 years (mean age 40 years) referred for suspected scabies. Patients were examined by two different investigators: one starting first with DS (FotoFinder) and the other one starting with RCM (VivaScope 3000).

**Results:** DS had a sensitivity of 94.8% and 90.9% and a specificity of 97.2% and 100% respectively when used before or after RCM. RCM had a sensitivity of 93.5% and 92.2% respectively when used after and before DS, and a specificity of 100% with both procedures. The median time for the diagnosis in positive cases was 10 seconds for DS and 20 and 30 seconds for RCM when used after or before DS respectively.

**Discussion:** DS is more sensitive than RCM for the diagnosis of scabies, whereas RCM is more specific. DS should be used to perform an initial screening, in order to target suspicious areas for a subsequent RCM examination.

**Conclusions:** RCM is a second-level examination to confirm the diagnosis of scabies made by DS.

**MELANOMA AND NAEVI WITH GLOBULAR PATTERN: CONFOCAL MICROSCOPY AS AN AID FOR DIAGNOSTIC DIFFERENTIATION.**

Elisa Benati¹, MD, Giuseppe Argenziano², MD, Athanassios Kyrgidis¹, MD, PhD, Elvira Moscarella¹, MD, Silvana Ciardo³, BS, Sara Bassoli³, MD, Francesca Farnetani³, MD, Simonetta Piana³, MD, Anna Maria Cesinaro³, MD, Aimilios Lallas¹, MD, Stefania Borsari¹, MD, Giovanni Pellacani³, MD, Caterina Longo¹*, MD, PhD
Background: Dermoscopically, one of the most common finding in melanocytic lesions is globular pattern. A regular globular pattern is a common finding in congenital, compound and dermal naevi. In contrast to the uniform appearance of globular naevi, Spitz naevi reveals globules that are much more irregular in size and color and larger at the periphery. Of note, also melanoma can show a globular pattern, with globules typically irregular in size, colour and distribution. Globular structures in dermoscopy and their substrate in RCM have been previously described, reporting that melanoma and Spitz naevi show atypical nesting and cytologic atypia while common naevi tend to display compact aggregates of cells (dense nests).

Objectives: The aim of our study was to investigate the likelihood of diagnosing melanoma according to a distinct dermoscopic aspect and the presence of nest-type pattern as seen on confocal microscopy.

Methods: We analyzed dermoscopic and confocal aspects of 83 excised melanocytic lesions dermoscopically showing globules. Previously described dermoscopic and confocal criteria have been evaluated. Univariate and multivariate analysis were performed.

Results: Our study population included 39 acquired melanocytic naevi, 16 Spitz naevi and 28 melanomas. Univariate analysis showed that regular distribution of globules on dermoscopy is associated with a 9-fold lower risk for melanoma whereas an irregular distribution is associated with an almost 10-fold increased risk for melanoma. Concerning confocal features, dense nests are associated with a 5-fold lower risk for melanoma, whereas loosely arranged nests are associated with an almost 6-fold risk for melanoma; moreover, focusing on the single cells that compose the nests, the presence of round cells is associated with a 17-fold lower risk for melanoma whereas pleomorphic cells are associated with an almost 16-fold risk for melanoma. The multivariate model yielded the presence of regular nests to be associated with at-least 5 times smaller adjusted risk for melanoma. Conversely, the presence of pleomorphic cells is associated with a 16-fold higher adjusted risk for melanoma.

Conclusions: A combined approach using dermoscopy and RCM is useful for the in vivo characterization of melanocytic lesions displaying a globular pattern. In order not to miss melanoma, clinicians should carefully analyze globular lesions in adults, focusing in particular on the distribution of globules and on the presence of confocal cytologic atypia.
The purpose of this study, in the context of high risk patients (familial melanoma and multiple primary melanoma), was to correlate morphological patterns by RCM with clinical data, genetic variants, dermoscopic features and histologic criteria.

A cross-sectional, retrospective, hospital-based study was performed from March 2010 to August 2013 including patients treated and followed-up at the Melanoma Unit of the Hospital Clinic, Barcelona, Spain. Fifty-seven melanomas from 50 patients were included in the study according to the following inclusion criteria: 1) history of at least two melanomas (multiple melanoma) or familial melanoma (two or more patients affected by melanoma in first or second degree relatives) with diagnosis of melanoma proven by histopathological examination (Breslow Index≤3mm) and documented by photographic dermoscopic and confocal examination and with confirmation of CDKN2 status (wild type or mutation). Patients with genetic conditions such as Li-Fraumeni Syndrome, Xeroderma Pigmentosum and Albinism were excluded, as well as patients undergoing systemic treatment for advanced melanoma. Lesions documented as melanoma metastasis or tumor recurrence were also excluded from the study.

The classification of melanomas according to morphological subtypes on confocal resulted in 23 dendritic-cell type (40.4%), 21 round-cell type (36.8%), 2 dermal nests type (3.5%), 2 combined type (3.5%) and 9 non-classifiable type (15.8%).

Individuals with dendritic-cell melanoma were in average 10.5 years older than round-cell melanoma patients (60.4 years, SD = 17.5 years x 49.9 years, SD = 15.6 years; p = 0.043). Comparing to round-cell type, dendritic-cell melanoma patients referred a more intense solar exposition after 18 years (43.5% versus 10%, p = 0.019) and the presence of moderate to severe solar lentigines was more frequently found (38.9% versus 6.7%, p = 0.046). The majority of patients of dendritic-cell type had less then 100 nevus (63.6%) and had no family history of melanoma or pancreatic cancer (60.9%). The frequency in this group of CDKN2A mutations was 5/23 (21.7%).

Round-cell melanomas presented a trend to occur more often in a familial context (66.7%) than dendritic-cell melanomas (39.1%, p = 0.068). Fototype I was more prevalent than in dendritic cell type (23.8% versus 0.0%, p = 0.019).
and 50% of the patients in this group had more than 100 nevus. Only three of the round-cell melanomas occurred in a CDKN2A mutation carrier (14.3%). Non-classifiable type melanomas on confocal were more often located on the trunk (77.8%). They were in average thinner than the other melanoma confocal types (mean thickness = 0.3 mm, SD = 0.2 mm, p = 0.027). Five were in situ melanomas. Two patients (22.2%) were carriers of a CDKN2A mutation (G101W and -34G>T). Non-classifiable type was associated with absence of pagetoid cells on confocal (p = 0.000) and lower frequency of marked atypia on basal cells compared to other types (11.1% versus 50.0%, p = 0.032). In non-classifiable type RCM scores were lower when compared to other types (Barcelona Score = -0.2 SD = 0.9 versus 1.2 SD = 0.9, p = 0.002; Pellacani Score = 3.2 SD = 1.7 versus 5.3 SD = 1.5, p = 0.007). Dermal nest type and combined-type were associated with thicker tumors, while non-classifiable type was in average thinner than other groups. Combined-type and non-classifiable type melanomas presented the higher and lower RCM scores, respectively, the same way as occurred with tumor thickness. It is reasonable thinking that they represent opposite sides of a morphological spectrum. Non-classifiable type was also associated with lower TDS scores. In fact, milder morphological expression in dermoscopy and confocal examination of non-classifiable type is characteristic of early-stage tumors that are frequently difficult to diagnose by in vivo techniques. Presence of CDKN2A mutations did not correlate with any of the melanoma types on RCM.

REFLECTANCE CONFOCAL MICROSCOPY GUIDED MICROBIOPSY

Marco Ardigo¹, Lynlee L Lin², Lisa Tom², Ross Flewell-Smith², Van Hoang², H Peter Soyer², Tarl W Prow²

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Refined clinico-pathologic correlation of melanocytic lesions using dermoscopy has lead to support pathologists to come to more reliable diagnostic conclusions. Despite the promising premise, this integration between dermoscopy and histology was discovered to be effective only in a limited number of scientific works. More recently, according to the necessity of a more detailed correlation, in-vivo reflectance confocal microscopy demonstrated to increase sensitivity of dermoscopy alone, but the really problematic melanocytic lesions still remain an unsolved issue.

In the era of molecular biology, ex-vivo dermoscopy guided sampling of melanocytic lesions has opened a new prospective of integration between non-invasive method of diagnosis, histology and molecular biology with the creation
of a biobank. Following, sub-millimetre skin punch biopsy devices, named microbiopsies, for minimally invasive and suture-free skin sampling for molecular diagnosis have been proposed for in-vivo dermoscopy guided biobanking.

Based on a preliminary experience with dermoscopy guided microbiopsies in-vivo sampling, we recently developed a targeted microbiopsy device for RCM pre-selected targets based on the virtual grid provided by RCM mosaics. In order to test the efficacy of the new device, melanocytic lesions (reticular and globular nevi) from volunteers have been considered and RCM selected microscopic structures have been targeted.

Our preliminary test showed good approximation in targeting the RCM pre-selected areas with an error that was estimated to be less than 0.5 mm. RCM performed after the sampling procedure demonstrated good integrity of tissue with no major damage of the skin surrounding the site of microbiopsies. The micro-samples obtained underwent to RNA extraction; later, obtained RNA was examined with real time qRT-PCR for tyrosinase that was positive in all the samples examined.

Our new device for RCM targeted microbiopsy allows a direct and more precise correlation between in vivo detectable microscopic features and molecular biology. Moreover, it seems to be more feasible to avoid area of hyperkeratosis, acanthosis, regression area or area with limited number of melanocytes in order to provide more adequate sampling for molecular biology and limit sampling errors. Moreover, the device for RCM guided sampling seems to better “stabilize” the microbiopsy, providing a more successful sampling.

In conclusion, our RCM guided microbiopsy device combines a non-invasive, real time “quasi histology” microscopic technique for melanocytic lesions with the rapid molecular biobanking.

FINAL UPDATE OF THE ICG FOR INFLAMMATORY SKIN DISORDERS STUDY
Marco Ardigo & ICG
San Gallicano Dermatological Institute-IRCCS, Rome, Italy

After several years from the recruitment of the centres involved in the multicentre study on reflectance confocal microscopy and inflammatory skin diseases conclusive results have been collected and a final conclusions have been made.

In this preliminary evaluation of the datasets collected by a consortium of the ICG member centers, we have demonstrated the potential of RCM for the identification of confocal patterns consistent with the major features present in the 3 major superficial inflammatory skin disease diagnostic groups: psoriasiform dermatitis, interface dermatitis and spongiotic dermatitis. Moreover, an efficient multivariate method for clinical diagnosis that includes the
development of a tree diagram for simple clinical application of RCM analysis has been defined. Follow-up studies are needed to confirm the significance of our results. It would also be useful to evaluate the reproducibility of the criteria between “data collecting centers” and blind evaluation by the “coordinator center”, determine if the image acquisition methodology should be modified and test of the diagnostic decision tree on real life cases.