STSM REPORT

STSM: Confocal microscopy evaluation of occupational contact

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Occupational dermatitis is one of the most frequent occupational diseases, having an important economical impact by direct (hospitalization, treatments) and indirect costs (lack of productivity, vacation). Appling the right treatment and preventive measures plays an important role in decreasing the burden of this condition.

My field of interests includes the study of occupational contact dermatitis. By using confocal microscopy we intend to analyze skin structure alteration and inflammation. With the help of this imagistic method our goal is to have a better understanding of the inflammatory process. Also, we would like to evaluate the efficiency of treatments and the penetration of topical products into the skin by monitoring the evolution of these inflammatory diseases from onset to remission. We want to analyze the efficacy of protective methods (eg. protective gloves) by using both clinical and confocal microscopy, to evaluate the opportunity of work reintegration or professional reorientation among the patients that are affected by professional dermatosis.

Confocal laser scanning microscopy is an optical imaging technique used in immunofluorescence studies of the skin. Compared to conventional fluorescence microscopy, this technique offers several advantages as high resolution images, reconstruction on 3D, absence of artifacts, shallow depth of field and ability to collect serial optical sections from thick specimens. In dermatology, confocal microscopy can be used in vivo or in vitro, each application having its benefits. For *in vitro* studies, fluorescence confocal laser scanning microscopy is more sensitive than conventional fluorescence microscopy and with the help of specific fluorophores, subcellular structures can be identified, analyzed and reconstructed 3D point by point. In vivo reflectance confocal microscopy is a non-invasive technique that allows monitoring of a skin lesion over time without having to perform a biopsy, which makes it a valuable method for treatment monitoring.

In order to acquire basic principles of confocal microscopy technique, I have applied for the Grant from the COST Action TD1206 STANDERM, which gave me the opportunity to visit the team of Prof Dr Erwin Tschachler, at the Department of Dermatology, University of Vienna Medical School, in Vienna, Austria. The Short Term Scientific Mission (STSM) took place between 6th to 18th of April 2014, in Vienna, at the Research Division of Biology and Pathobiology of the Skin which is part of the Department of Dermatology from the University of Vienna Medical School. The purpose of my visit was to learn basic principles of confocal

microscopy and to be able to analyze confocal microscopy aspects of cutaneous inflammatory lesions.

To get familiar with this technique, the objectives of the stay were to learn to retrieve antigens from paraffin embedded tissue, to perform immunofluorescence and double immunofluorescence staining on tissue sections and to analyze immunofluorescence staining on conventional immunofluorescence and laser scanning microscopy. I have analyzed biopsy samples of inflammatory lesions that were brought from patients treated in the 2nd Department of Dermatology at Colentina Clinical Hospital in Bucharest, Romania.

Throughout my stay in the laboratory, I benefit of hands-on training in order to obtain quality samples for conventional microscopy and confocal laser analysis. From preparation to analysis, I went through the following steps:

1. Sectioning paraffin blocks in thin sections (5 µm) for conventional immunofluorescence and thick sections (30-40 µm) for laser scanning microscopy analysis.
2. Immunofluorescence staining of paraffin section that included the following steps: deparaffinization and rehydration of samples, demasking of antigens, preparation for the first step antibody and the second step antibody as double immunofluorescence staining was also performed.
3. Analysis of the thin section samples at the conventional 3 channel immunofluorescence microscope in order to validate the staining and to observe the stained structures.
4. Analysis of the thick section samples at the confocal laser microscope and building 3D images of the tissue samples, with visualisation of cells structure and blood vessels. For each step, photo documentation was performed.

The visit at the Research Division of Biology and Pathobiology of the Skin in Vienna helped me to get familiar with the technique of laser scanning confocal microscopy. The hands-on training in preparation of tissue samples along with the technical steps as calibration of the laser, choosing the appropriate parameters for analysis, performing various analysis patterns, building 3D images of the samples will be useful for my future projects in investigating professional contact dermatitis.

In the future, I am hoping to continue our research collaboration in the field of occupational contact dermatitis. I want to establish a occupational contact dermatitis database that will contain photo documented clinical aspects of lesions, in vivo reflectance confocal microscopy images and biopsy samples that will be analyzed with the help of in vitro fluorescence confocal laser scanning microscopy. The purpose of using these imaging methods is to set up a set of characteristics for in vivo confocal microscopy based on the information obtained from the in vitro samples, in order to asses a non-invasive, precise evaluation method and to have a better understanding on the pathophysiology of the inflammatory process in this skin condition. This will help to evaluate the efficiency of treatments and protective methods and hopefully will decrease the burden of this condition.