Oral Pathology Follow-up by means of Micro-Raman Spectroscopy on Tissue and Blood Serum Samples: an Application of Wavelet and Multivariate Data Analysis

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We report on the results of a micro-Raman spectroscopy study on tissue and blood serum samples from ill, recovered and under therapy Pemphigus Vulgaris patients. To obtain reliable information on the patient illness stage wavelet techniques and advanced multivariate analysis methods have been developed and applied to the spectra analysis. Promising results have been obtained.

1 Introduction

Pemphigus vulgaris (PV) is a potentially fatal autoimmune disease that causes blistering of the skin and oral cavity. It is characterized by disruption of cell-cell adhesion within the suprabasal layers of epithelium, a phenomenon termed acantholysis. In these years, the application of optical techniques and, in particular, of Raman spectroscopy to a wide variety of biophysical, technological biomedical and industrial questions has given very promising results in all cases. We have recently proposed a simple linear regression analysis on micro-Raman spectra from blood serum samples for PV follow-up monitoring [1]. Moreover, multivariate analysis methods have been recently applied to oral tissue Raman spectra. In particular, Malini *et al.* [2] using Principal Component Analysis (PCA) methods combined with multiparameter limit test have been able to classify oral tissue in different categories depending on their disease stage (normal, inflammatory, premalignant and malignant). In this frame, we have investigated the possibility of using Raman spectra and supervised pattern recognition methods (Partial Least Square, PLS) to monitor PV follow up. This approach has been employed to study both tissues and blood serum samples from patients with histological and immunofluorescence confirmed PV, from patients on therapy and on recovered patients. The supervised pattern recognition procedure has been applied to untreated and deconvolved Raman spectra. A wavelet deconvolution procedure which has shown to be very useful to subtract noise from biological and liquid samples Raman spectra [3] has been also employed. The results have been discussed in the view of routine application of Raman spectroscopy in monitoring PV follow-up.

2. Materials and Methods

Raman spectra were collected from biopsies and serum samples obtained by informed patients. In particular, 2 subjects had an active PV, 2 patients were under treatment and 2 patients were in a remission stage of illness. From each patient, blood samples and two oral biopsies were obtained. One was used for direct immunofluorescence techniques, and the other for Raman investigation. For details on micro-Raman spectrometer see ref. 1. An automatic numerical data treatment based on wavelet algorithm was used in order to suppress the noncorrelated signal, to subtract the background signal and to increase the quantitative readibility of the Raman signal [3]. For multivariate analysis the software employed in this work was properly written for Raman tissue analysis purposes and is based on the PLS toolbox 3.5 for MATLAB, from Eigenvector Research, for PCA, cluster analysis and PLS-DA, and the iPLS toolbox for MATLAB, implemented by Lars Noorgard (*http://www.models.kvl.dk/source/ipls/*). The iPLS method was applied to Raman spectra of tissues and serum samples before and after a wavelet deconvolution method had been used.

3 Results and discussion

In Figs. 1, 2, 3 and 4 the principal results of our investigation are reported. As is evident from Figs.1 and 3 iPLS approach [4] is able to discriminate between serum and tissue Raman spectra belonging to patients in different stages of illness. However, the extracted illness stage parameter can lead to some false negative or false positive cases because of the dispersion of values

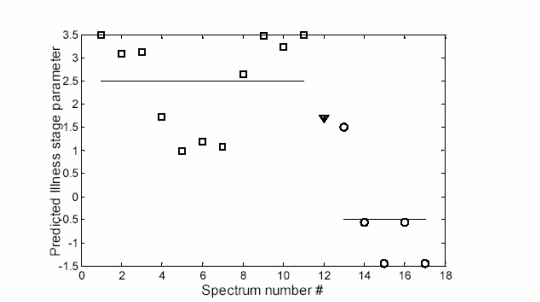

Fig. 1 *Predicted illness stage parameter for serum raw Raman spectra vs spectrum number. Symbols. Squares-recovered, circles – under therapy, triangles-ill.*

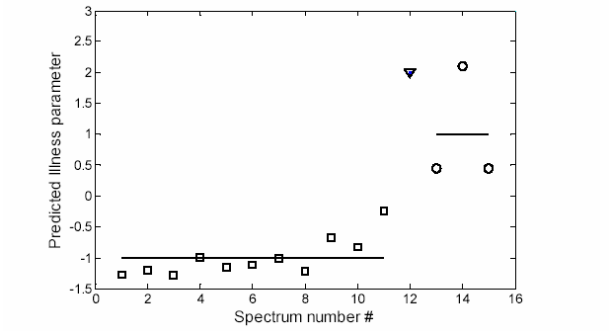

Fig. 2 *Predicted illness stage parameter for serum wavelet treated Raman spectra vs spectrum number. Symbols. Squares-recovered, circles – under therapy, triangles-ill*

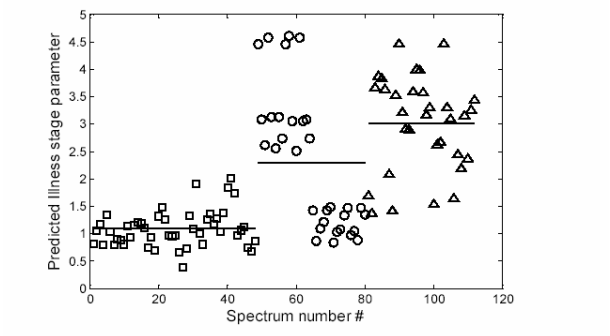

Fig. 3 *Predicted illness stage parameter for tissue raw Raman spectra vs spectrum number. Symbols. Squares-recovered, circles – under therapy, triangles-ill.*

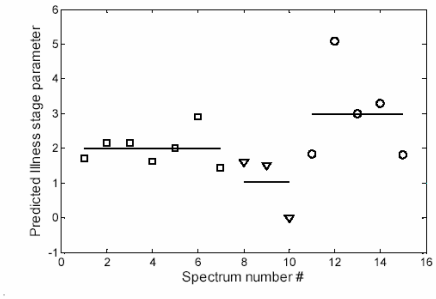

Fig. 4 *Predicted illness stage parameter for serum wavelet treated Raman spectra vs spectrum number. Symbols. Squares-recovered, circles – under therapy, triangles-ill.*

obtained for spectra characterized by the same illness stage. To reduce this variability in the predicted illness stage parameter, the wavelet data analysis procedure has been applied to blood serum and tissue Raman spectra before of iPLS analysis. From Figs. 2 and 4 it is clear that wavelet deconvolution analysis enabled the iPLS model to be more precise.

4 Conclusions

Raman spectra of tissues and blood serum samples from patients in different PV illness stages were analysed by means of iPLS multivariate procedure before and after the application of a wavelet deconvolution method. The results demonstrated that iPLS method is able to discriminate the illness stage of the patients. For a more reliable prediction the application of the wavelet deconvolution procedure is required.

5 References

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