Raman spectroscopy as a novel clinical tool for improved diagnosis of thyroid pathologies

Julietta V. Rau[1], Marco Fosca[1], Valerio Graziani[1], Chiara Taffon[2], Marco Caricato[2], Anna Crescenzi[2]

[1]Istituto di Struttura della Materia (ISM-CNR), via del Fosso del Cavaliere 100, 00133 Roma, Italy.
email: giulietta.rau@ism.cnr.it
[2] Policlinico Universitario Campus Bio-medico, via Álvaro del Portillo 200, 00128 Roma, Italy

Inter-observer variability and cancer over-diagnosis are emerging clinical problems, especially for follicular patterned thyroid lesions. A very recent paradigm change versus the reduced thyroid cancer overtreatment necessitates for a new clinical tool to reliably identify neoplastic lesions and to improve the efficiency in differentiation between benign and malignant neoplasms, especially what regards small carcinomas and growing number of thyroid nodules. Raman spectroscopy (RS) is a promising label-free non-invasive technique capable to increase diagnostic reliability, providing specific information on biochemical composition. The combined histological and Raman microscopy analyses approach could allow clear-cut integration of morphological and biochemical observations, with dramatic improvement of efficiency and reliability in the differential diagnosis of neoplastic thyroid nodules.

In this work, we applied RS microscope to investigate frozen thyroid tissues, in order to support histologic analysis. Our aim was to investigate the ability of RS to discriminate between healthy and Papillary Thyroid Carcinoma (PTC) tissues, between the two PTC variants (classical and follicular); between normal parenchyma and pathologic follicular patterned thyroid nodules, and between benign and malignant follicular lesions (adenoma *versus* carcinoma). Our Raman database of various thyroid pathologies includes 30 thyroid patient cases, containing reach biochemical information.

To generate tissue classification models, a supervised statistical analysis of the obtained Raman spectra was performed. It consisted in the sequential data treatment by Principal Component Analysis and Linear Discriminant Analysis, followed by internal (leave-one-out) and external (independent) dataset validation. The obtained results allow to affirm that RS is able to discriminate between healthy and PTC tissues of thyroid with 100% of sensitivity, specificity and accuracy and between classical and follicular variants of PTC with 93% of sensitivity, 100% of specificity and 95% of accuracy [1]. The achieved diagnostic sensitivity, specificity and accuracy are compatible with the clinical use, both for the PTC diagnosis and for the differential diagnosis between classical and follicular variants of PTC, the latter being a significant challenging point for thyroid nodules evaluation.
Furthermore, the achieved results demonstrate 92% of accuracy for RS based diagnosis in distinguishing between healthy and neoplastic follicular patterned thyroid tissues, while for the very challenging follicular lesions (carcinoma *versus* adenoma) approximately 90% of accuracy can be assured [2]. In comparison, for this latter result, histopathology provides about 70% of reliability. The 20% increase of diagnosis reliability for follicular lesions demonstrates the great potential of RS biochemical fingerprints to contribute to clinical decision-making.

Moreover, our study demonstrates that RS translation into intra-operative diagnosis on frozen sections and in pre-operative analysis of biopsy samples can be helpful to reduce unnecessary surgery in patients with indeterminate cytological reports. Furthermore, RS biochemical information allows the identification of molecular species involved in thyroid tumorigenesis and progression.

The combination of histological and Raman microscopy analyses, yielding improved diagnostic accuracy and reduced inter-observer variability, may open a new way to integrative findings with wide implications for basic pathology, tumour classification schemes and therapeutic strategies.

References

[1] J.V. Rau, V. Graziani, M. Fosca, C. Taffon, M. Rocchia, P. Crucitti, P. Pozzilli, A. Onetti Muda, M. Caricato, A. Crescenzi, *Sci. Rep.* **6,** 35117 (2016).
[2] J.V. Rau, M. Fosca, V. Graziani, C. Taffon, M. Rocchia, M. Caricato, P. Pozzilli, A. Onetti Muda, A. Crescenzi. *Sci. Rep. submitted* (2017).