Funded by the European Union NextGenerationEU

Ministero dell'Università e della Ricerca

Italiadomani PIANO NAZIONALE DI RIPRESA E RESILIENZA

UNIVERSITÀ POLITECNICA DELLE MARCHE

Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXIX

FTIR Imaging Spectroscopy: a Novel Tool for Improving the Differential Diagnosis of Human Uterine Lesions

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Introduction & Aim

Background

- ✓ Uterine smooth muscle tumors (USMTs) include a spectrum from benign **leiomyomas** (**LMs**) to malignant **leiomyosarcomas** (**LMSs**) [1,2].
- ✓ LMs arise from smooth muscles in uterine myometrium, while LMs originate from myometrial mesenchymal cells [1,2].
- ✓ Some histological overlap between LMSs and LM variants makes difficult the diagnosis [1].

Objective: Given the need for molecular-level tools to **improve diagnostic accuracy**, this study focuses on identifying **reliable spectral markers** to support the **differential diagnosis** between **LMS** and **LM** subtypes.

Methods

Sample Collection & Preparation

- ~ 5 µm thickness sections from FFPE samples collected from surgical hysterectomy.

N. samples	Uterine lesion
5	LMS
5	LM Cellular
5	LM Usual
3	LM Bizzarre
3	LM Apoplectic

Histological Analysis

- Masson's trichrome staining kit with aniline blue.
- Olympus BM50 optical microscope.

FTIR Imaging Analysis

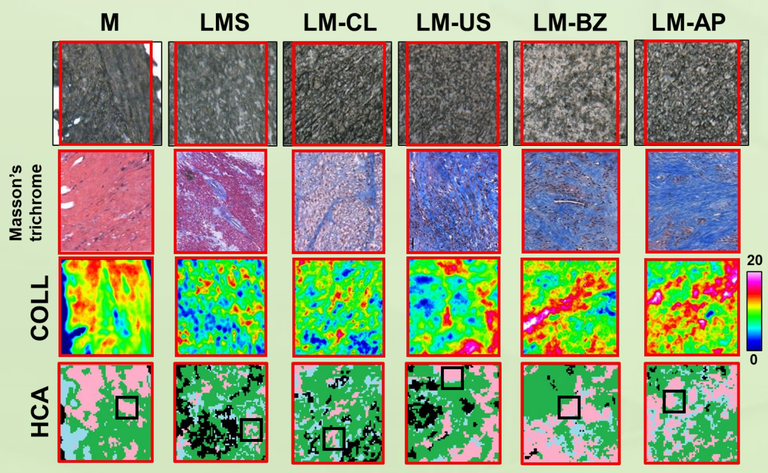
- Bruker INVENIO-R interferometer equipped with a Hyperion 3000 Vis-IR microscope and a Focal Plane Array detector. IR maps (164×164 µm^2 size, 4096 spectra, 2.56×2.56 µm^2 spatial resolution) acquired in transmission mode in the 4000–900 cm^{-1} spectral range (256 scans; 4 cm^{-1} spectral resolution) (OPUS 7.5 software package, Bruker Optics, Ettlingen, Germany).

Data Analysis

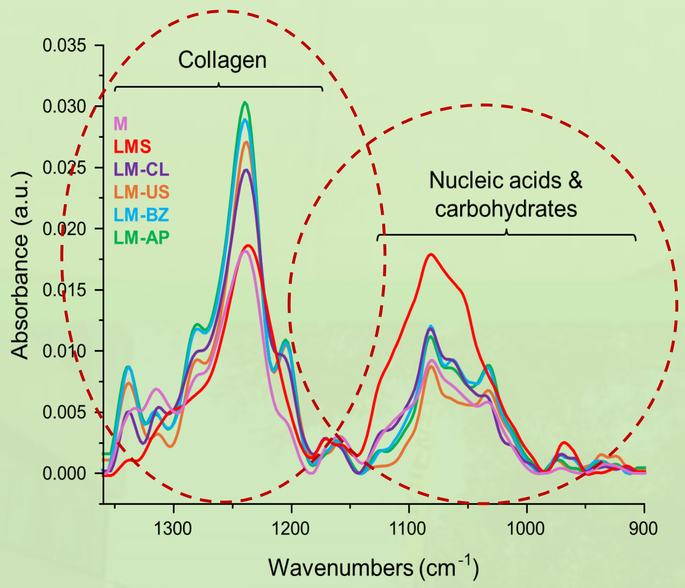
- Hierarchical Cluster Analysis (HCA, Euclidean distance and the Ward's method) (CytoSpec software v. 2.00.01).
- Principal Component Analysis (PCA) (OriginPro 2023 software, OriginLab Corporation, Northampton, MA, USA).
- One-way analysis of variance (ANOVA) multiple comparison test (software Prism6, Graphpad software, Inc., San Diego, CA, USA).

Results & Discussion

High Resolution Imaging Analysis

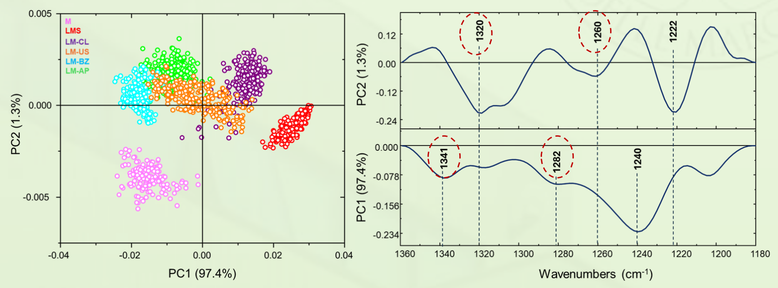


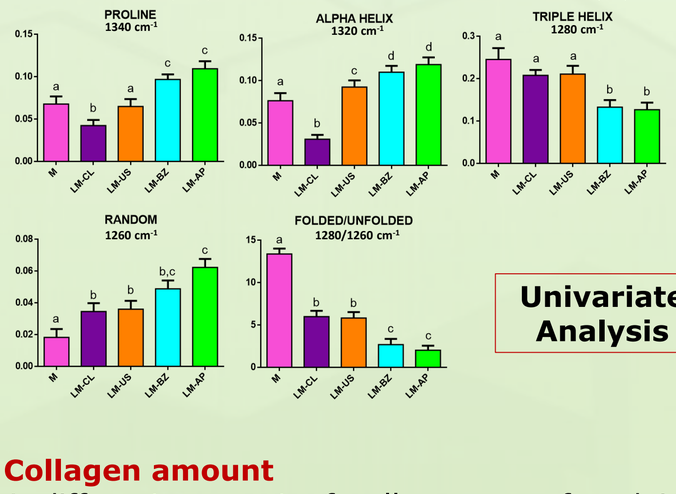
Collagen spectral clusters from which **average spectra** were extracted.



Collagen Features Analysis

Principal Component Analysis & Loading Spectra





Univariate Analysis

Collagen amount

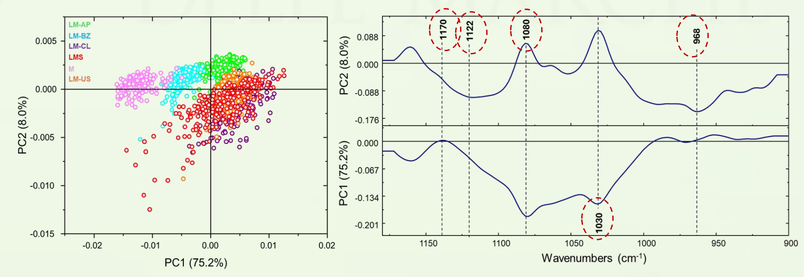
- A different amount of collagen was found in LMs: higher but with an uneven distribution in **LM-BZ** and **LM-AP.** The lowest amount was observed in **LM-CL.**

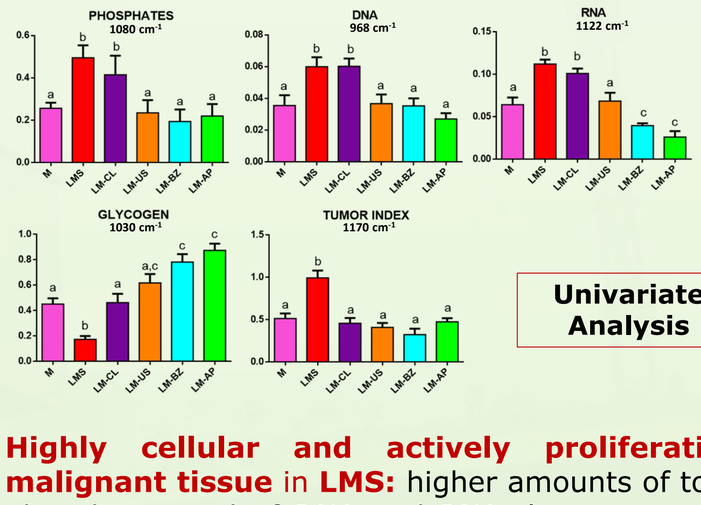
Collagen structural organisation

- A greater degree of collagen organisation (FOLDED/UNFOLDED and TRIPLE HELIX) was observed in **LM-CL** and **LM-US**, whereas **LM-BZ** and **LM-AP** showed a more disorganised protein component.

Cellular component analysis

Principal Component Analysis & Loading Spectra





Univariate Analysis

- **Highly cellular and actively proliferating malignant tissue** in **LMS:** higher amounts of total phosphates and of DNA and RNA; lower amounts of glycogen and carbohydrates. A similar trend is observed in **LM-CL**. **LM-US**, **LM-BZ** and **LM-AP** exhibit minimal cellular components, with lower values.
- The **TUMOR INDEX (1170 cm^{-1})**, associated with phosphorylated proteins and tumorigenicity, reaches its highest value in **LMS,** effectively distinguishing it from all **LM subtypes**.

Conclusions

FTIR imaging, combined with histology and statistical analysis, proved to be a valuable approach for the **differential diagnosis of USMTs**. Spectral markers, including the TUMOR INDEX, enabled reliable **discrimination** between **LMS** and **LM subtypes**. Notably, this method allowed clear **differentiation** between **LMS** and **LM-BZ**, which are histologically similar and difficult to distinguish using conventional methods. In addition, the high spectral similarity observed between **LM-CL** and **LM-US** suggests a possible continuum, with the cellular variant potentially representing an **early stage** of the usual form. These findings highlight the potential of IR-based techniques as **complementary tools** to **improve diagnostic accuracy**.

References

[1] Belloni, A. et al. FTIR Microspectroscopy as a new probe to study human uterine lesions: Characterization of tumor cell lines from uterine smooth muscle cells and evaluation of EPA and DHA in vitro treatments. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, *1870*(1) (2024).

[2] Belloni, A. et al. Uterine leiomyoma as useful model to unveil morphometric and macromolecular collagen state and impairment in fibrotic diseases: An ex-vivo human study. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1868*(12), 166494 (2022).