

FOURIER-TRANSFORM INFRARED (FT-IR) SPECTROSCOPY OF PLASMA AS A LIQUID BIOPSY FOR MONITORING ACUTE MYELOID LEUKEMIA AND EXTRAMEDULLARY RELAPSE FOLLOWING ALLO-HSCT

A. Leoni^{1,2}, B. Xhahysa^{1,2}, L. Garuffo^{1,2}, A. Cavalleri^{1,2}, F. Trenta^{1,2}, F. Re^{1,2}, S. Mutti^{1,2}, L. Paolini^{3,4}, M. Farina², E. Morello², D. Avenoso², D. Russo², M. Malagola², S. Bernardi^{1,2}

¹Centro di Ricerca Emato-oncologico ALL, ASST Spedali Civili di Brescia; ²Unit of Blood Diseases and Bone Marrow Transplantation, Cell Therapies and Hematology Research Program, Department of Clinical and Experimental Science, University of Brescia, ASST Spedali Civili di Brescia; ³Department of Medical and Surgical Specialties, Radiological Sciences and Public Health - DSMC, University of Brescia; ⁴Center for Colloid and Surface Science -CS-GI- Sesto Fiorentino.

Relapse of Acute Myeloid Leukemia (AML) remain a major cause of treatment failure. Among relapse patterns, extramedullary disease, referred to as myeloid sarcoma, representing a rare but clinically significant scenario, characterized by infiltration of leukemic blasts in tissues outside the bone marrow. Diagnosis is challenging and requires a multimodal approach integrating imaging, histopathology and immunophenotyping to confirm the myeloid origin of blasts. Prognosis is influenced by localization, disease burden and characteristic and response to therapy.

In this single-Centre study, we employed a liquid biopsy approach based on Fourier-Transform Infrared (FT-IR) spectroscopy to analyze plasma samples from AML patients collected at diagnosis, pre- and post-allogenic hematopoietic stem cell transplantation (allo-HSCT), bone marrow (BM) relapse, and myeloid sarcoma, as well as from healthy controls. Spectral data were normalized using standard normal variate and analyzed with multivariate and machine-learning approaches (Random Forest) with leave-one-patient-out cross-validation. The overall comparison between healthy and pathological subjects demonstrated high discriminatory power (AUC=0.866; Accuracy=0.826), with optimized sensitivity of 100% and specificity of 80%, highlighting the presence of a characteristic FT-IR spectral signature associated with the AML and sarcomatous pathology (Figure 1A). The comparison between the diagnostic phase and BM relapse or myeloid sarcoma showed excellent discrimination (Figures 1B and

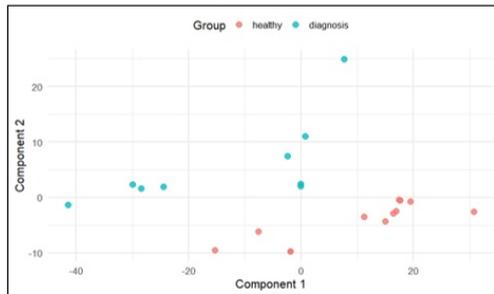
1C), as well as the difference between medullary and sarcomatous relapse (Figure 1D). This suggests that, although differences exist, the metabolic, molecular and structural profile detected by FT-IR differs in the two types of relapse, indicating peculiar biochemical features related with the clinical presentations. Furthermore, analysis of post-HSCT spectra revealed a gradual return to a physiological condition, with normalization observed in 88.8 and 94.4% of samples at +30 and +90 days, respectively, consistent with partial metabolic recovery.

In conclusion, our findings suggest for the first time that FT-IR spectroscopy applied to plasma represents a promising liquid biopsy tool for monitoring AML and its post-HSCT relapse. The method's ability to distinguish the diagnostic phase from relapse, the relapse types, and to capture a gradual normalization of the spectral profile in post-transplant samples (up to 94.4% of patients at 90 days) indicates that plasma biochemical changes accurately reflect the patients' clinical and metabolic status, supporting its potential use in clinical practice.

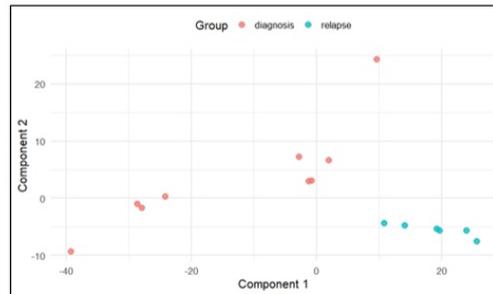
FT-IR offers a sensitive, non-invasive approach to detect biochemical alterations, potentially providing complementary biomarkers for disease monitoring and early relapse prediction. Further studies are warranted to explore these FT-IR-derived signatures and to integrate this approach into precision monitoring strategies for AML patients.

STEM CELL TRANSPLANTATION, IMMUNOTHERAPY AND CELL THERAPY

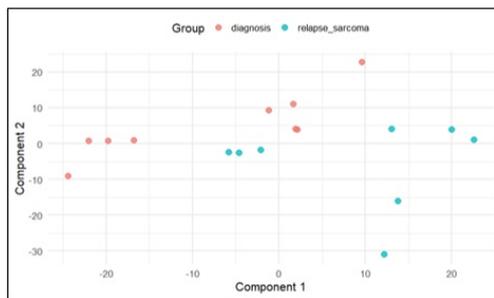
1A – PLS-DA - Healthy vs AML diagnosis



1B – PLS-DA - AML diagnosis vs BM relapse



1C – PLS-DA - AML diagnosis vs Myeloid Sarcoma



1D – PLS-DA – BM relapse vs Myeloid Sarcoma

