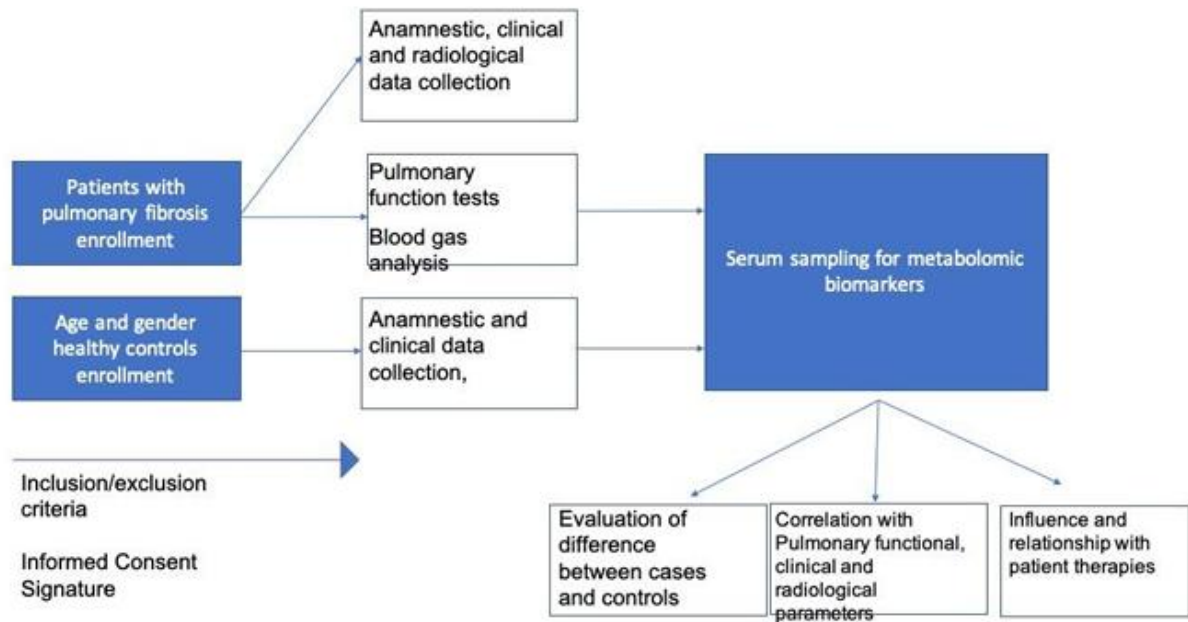**Evaluation of Metabolomic Biomarkers in Pulmonary Fibrosis**

Study Protocol and Statistical Plan

We designed a single-center, prospective, cross-sectional investigation involving patients diagnosed with Pulmonary Fibrosis. Participants are recruited from the Interstitial Lung and Rare Diseases Outpatient Clinic at the Vanvitelli Respiratory Diseases Clinic, Monaldi Hospital, Naples, Italy. Patients were included if they met the following criteria: 1) Confirmed IPF diagnosis based on the 2018 ATS/ERS/JRS/ALAT guidelines [PMID: 30168753] within the past five years 2) diagnosis of other fibrotic interstitial lung disease different from IPF 3) age ≥ 40 years, 4) ability to provide informed consent. Exclusion criteria included: 1) a current diagnosis of asthma or chronic obstructive pulmonary disease (COPD), 2) an acute Pulmonary Fibrosis exacerbation within the past three months. Demographic and anthropometric data—including sex, smoking history, and comorbidities—are systematically recorded. All participants underogo pulmonary function testing, including spirometry, body plethysmography, and single-breath diffusing capacity of the lungs for carbon monoxide (DLCO), following the 2022 ATS/ERS guidelines [PMID: 34949706]. Tests are performed using the Vyntus BODY system (Vyaire Medical), and the following parameters were measured: FEV_1, $FEV_1\%$, FVC, FVC%, FEV_1/FVC, TLC, TLC%, RV, RV%, DLCO, and DLCO% [PMID: 22743675, PMID: 28893868]. Additionally, arterial blood gas (ABG) analysis was conducted to assess pH, partial pressure of oxygen (pO_2), partial pressure of carbon dioxide (pCO_2), bicarbonate (HCO_3^-), and lactate levels. Functional capacity is evaluated using the 6-minute walk test (6MWT), performed according to ATS guidelines [PMID: 12091180]. The Gender-Age-Physiology (GAP) index was calculated to stratify IPF patients into three risk stages based on clinical (sex, age) and physiological (FVC, DLCO) parameters, providing an estimate of 1-, 2-, and 3-year mortality [PMID: 22586007]. To enhance prognostic accuracy, the Distance-Oxygen-Gender-Age-Physiology (DO-GAP) index was also determined by incorporating 6MWT distance and exertional hypoxemia [PMID: 37228268].

Study Protocol Design

Statistical plan

Baseline population characteristics, comparing the control group and Pulmonary Fibrosis patients. In particular, the X² test or Spearman's rank correlation (Rho) was applied to categorical variables according to their distribution, while the Wilcoxon-Mann-Whitney test was used for continuous variables to identify significant differences between the two groups (controls and PF). For univariate correlations, Pearson's or Spearman coefficient are used as appropriate. These statistical methods provide a comprehensive understanding of the clinical and biochemical differences between the two groups and the relationships among the fatty acids studied. Finally, we evaluated the diagnostic potential of significant FAs for IPF using Receiver Operating Characteristic (ROC) curves. A p-value < 0.05 was considered statistically significant.