Master Thesis Proposal

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TITLE: Discovery of anti-TNF α agent´s efficacy in ankylosing spondylitis patients using a metabolomic approach

BACKGROUND

Ankylosing Spondylitis (AS) is amongst the most common forms of inflammatory arthritis. It is characterized histopathologically by the presence of inflammation at the site of insertion of tendons or ligaments into bone, leading to new bone formation in affected joints.

Inflammatory back pain is a characteristic symptom and syndesmophyte formation with vertebrae fusion an x-ray hallmark of the disease. AS affect people around 20-30 years of age promoting physical function deterioration and work disability, with strong impact in quality of life.

The introduction of biologic therapies, such as anti TNF-α agents contributed for several benefits regarding clinical management and prognosis. Although most AS patients respond well to these therapies, partial response is still frequent. Current markers of response include younger age, HLA-B27 carriage and elevation of acute phase reactants. Whilst these are statistically significant biomarkers, it is clear that they do not have a relevant impact in terms of medical therapeutic decision. Identification of new and more discriminant biomarkers is an unmet need.

Under the framework of a multicenter national clinical study, AS patient´s sera samples were collected from responders and non-responders to anti TNF-α blockers at several time-points during treatment. The levels of variation of serum proteins from the collected samples were evaluated by a proteomic approach. Obtained results provide evidence that a panel of proteins might be able to predict the response to adalimumab therapy in AS patients, even before the beginning of treatment.

OBJECTIVES

Establishment of a selective and sensitive signature of biomarkers for the prediction of therapy efficacy in daily clinical practice. Promising proteomics results will be complement with the metabolites levels determination by NMR metabolomics, aiming the identification of compounds for which the level of variation is tightly associated to the clinical response to anti TNF-α blockers.

PROJECT DESCRIPTION

To identify specific biomarkers for prediction of therapy efficacy to anti TNF-α treatment, differential metabolomics will be performed with blood collected from responders and non-responders AS patients before and 3 time-points after starting treatment.

- Task 1: The serum preparation protocol was established previously after optimization of serum thawing time, sample stability and ultrafiltration conditions with the main goal of improving reproducibility and minimizing sample degradation. Thawed serum is passed through a centrifugal filter to remove macromolecules shown to interfere with the NMR metabolite signals.
- Task 2: Proton (^{1}H) spectroscopy will be performed on an 800 MHz Bruker AvanceII+ spectrometer equipped with a Avance III 800 CRYO, at 298 K, at the Magnetic Resonance Centre António Xavier at ITQB NOVA.
- Task 3: Metabolite identification and quantification will be performed using Chenomx NMR Suite 8.1 software, using its internal reference library (Version 10), the Human Metabolome Data Base (HMDB 3.6) and the Biological Magnetic Resonance Bank (BMRB). 2D ^{1}H-^{13}C HSQC spectra will be used to aid metabolite identification.
- Task 4: Univariate and multivariate analysis are used to search for metabolic biomarkers that separate anti TNF-α treatment responders and non-responders AS patients. Metabolites with VIP >1 and p-value<0.05 are applied for metabolic pathway analysis alone and integrated with the protein biomarkers already validated, together will the known molecular mechanisms associated with AS.
- Task 5: Writing of master thesis

TIMELINE

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10
Task 1										
Task 2										
Task 3										
Task 4										
Task 5										