WP4 Reliability Assessment with ELISA

Technical Flowchart

WP4.1. Quantification of inflammation markers in synovial joint fluid

IL-1β, IL-6, IL-8, IL-10, IL-12p70, IFN-α, IFN-β, IFN-λ1, IL-29, IFN- in joint fluid isolated from patients It will be performed using the LEGENDplexTM (Biolegend, USA) kit with the multiplex ELISA method with λ2/3, IL-28, IFN-γ, TNF-α, IP-10 and GM-CSF factors. For this, antibody-immobilized bead, washing solution and standard solutions in various ratios provided by the kit manufacturer will be prepared in accordance with the manufacturer's instructions and the samples will be diluted. Analysis of the prepared kit solutions and samples will be carried out using the V-bottom culture plate (Biolegend, USA). 25 µl of bead will be added to the samples and solutions placed on the culture plate, the plate will be closed with a sealer and incubated on a shaker for 2 hours at 800 rpm at room temperature in the dark. Then, it will be centrifuged in the bucket rotor with the microplate adapter at 1050 rpm for 5 minutes, and then the supernatant will be removed from the microplate. 25 µl of detection antibodies are added to the microplates and the plate will be incubated on a shaker at 800 rpm for 1 hour at room temperature in the dark. After incubation, washing will be done with washing solution and the samples will be read on the flow cytometry device (NovoCyte, Agilent, USA). After reading, the data will be analyzed in LEGENDplexTM Data Analysis (Biolegend, USA) software.

WP4.2. Determining the amount of cartilage formation and destruction markers in synovial joint fluid

COL2, HA, COMP and MMP3 markers (Nepenthe, Turkey) will be evaluated in joint fluid isolated from patients by ELISA method and the protocol specified by the kit manufacturer will be followed. Accordingly, the joint fluid will be centrifuged at 2500 rpm for 20 minutes, and the biotinylated conjugate, streptavidin HRP, washing and standard solutions included in the kit will be prepared in various dilutions. Standard solutions and samples will be added to the wells and incubated for 80 minutes at 37oC. After incubation, the wells will be washed with washing buffer and the biotinylated conjugate will be added to each well and incubated at 37oC for 50 minutes. Washing will be done and streptavidin-HRP will be added to each well and incubated for 50 minutes at 37oC. After washing, TMB substrate solution will be added and incubated for 20 minutes at 37oC. Then, the interaction with the stopping solution will be stopped and it will be read with a microplate reader (Molecular Devices, USA) at a wavelength of 450 nm.

WP4.3. Comparative evaluation of Omics and ELISA data

The data obtained within the scope of IP3 in joint fluid isolated from patients will be evaluated together with the data on COL2, HA, COMP and MMP3 markers determined by the ELISA method. At this point, any correlations that may arise will be analyzed with the IBM SPSS Statistics (IBM, USA) program and the reliability of the results will be evaluated with ELISA.