Analysis of selected cytokines and miRNA profile in patients with rheumatoid arthritis and ankylosing spondylitis

Rheumatic diseases are autoimmune chronic inflammatory disorders including rheumatoid arthritis (RA) and ankylosing spondylitis (AS). Symmetric inflammation of joints leading to the destruction of cartilage and bone tissue is a characteristic manifestation of RA. AS affects sacroiliac joints and the spine, and may also involve peripheral joints. RA is described with the presence of rheumatoid factor, while AS is classified as a seronegative disease. The prevalence of both disorders differs worldwide. In Poland, RA affects approximately 0.9%, whereas AS affects 0.01% of the population. RA incidence is disproportionately more common in women than men, and the opposite is true in AS. All patients suffer from pain, stiffness, reduction of mobility and poor quality of life. Even though susceptibility for both RA and AS is relatively well-known to be associated with environmental and genetic risk factors, the pathogenesis has not been fully elucidated. However, it has been indicated that T helper (Th) cell differentiation plays a crucial role in these diseases, especially the imbalance between Th17 and regulatory T lymphocytes. First-line treatment strategies comprise non-steroidal anti-inflammatory drugs, glucocorticoids, and disease modifying antirheumatic drugs. The introduction of biological therapy remarkably improved patients' quality of life. Unfortunately, at least 30% of patients do not respond to the treatment with TNF-α, inhibitors, which constitutes a major, unsolved healthcare problem. Other dominant proinflammatory cytokines involved in RA and AS are interleukin (IL)-6 and IL-17 respectively. These cytokines are also mediators of pain, thus blocking their signalling not only impairs disease progression but also alleviates the chronic pain.

This doctoral thesis is presented as a series of publications. The aim was to identify biomarkers associated with susceptibility to selected rheumatic disorders, disease progression, and anti-TNF treatment outcomes. Clinical samples were collected from Polish RA and AS patients at baseline, as well as after three and six months of treatment with TNF-α inhibitors. Additionally, experimental data were compared to healthy controls.

In the first publication, a group of 130 patients with RA and 112 healthy controls were genotyped for the *IL6* rs1800795 single nucleotide polymorphism (SNP) located in the promoter region. Serum IL-6 levels were also analysed. It was observed that IL-6 concentrations were significantly higher in RA patients before initiation of anti-TNF therapy compared to healthy controls. In addition, RA patients with the homozygous *CC* genotype were characterized with the highest level of this cytokine before biological treatment and high disease

activity compared to *G* allele carriers. These results suggest that the rs1800795 *CC* genotype, associated with increased IL-6 production, plays an adverse role in RA patients.

In the second paper, genetic variants of IL-17A, IL-17F, and their receptors were analysed. Potential associations between the *IL17A* rs2275913, *IL17F* rs763780, *IL17RA* rs4819554 and *IL17RC* rs708567 alleles with clinical parameters and anti-TNF treatment responses were investigated. The study cohort consisted of 138 AS patients and 190 healthy controls. The *IL17F* rs763780 *AG* genotype was found to be associated with higher disease activity, as well as a lack of response to therapy after six months. Moreover, the *IL17RA* rs48419554 *G* allele was identified as a potential marker of AS severity.

In the third paper, repertoires of miRNAs in exosomes from serum of RA and AS patients before and after three months of anti-TNF therapy were determined. The analysis was performed using NanoString technology, which enabled the analysis of the expression profile of 800 miRNA molecules. Twelve miRNAs differentially expressed between RA and AS patients at baseline were detected. A comparison of the expression profiles in patients before and after three months of TNF-α inhibitor intake showed differences in four and fourteen miRNAs in RA and AS patients respectively. Additionally, we carried out an *in silico* analysis of potential miRNA targets and associated pathways.

The publication series closes with a review paper describing the usage of miRNAs as potential markers of treatment response in rheumatic diseases.

The studies described herein established an important role of polymorphisms within genes encoding proinflammatory cytokines and miRNAs profiles in predicting rheumatic disease outcome. The investigated SNPs were shown to modulate therapeutic responses to TNF-α inhibitors and influence clinical parameters such as inflammatory markers or disease activity. These results demonstrate that serum miRNA profiling may have a diagnostic and prognostic potential in rheumatic disease.