Chapter 3

Objectives
3.1 Identification of differentially expressed transcripts/mRNAs between RA and OA by DNA microarrays

**Rationale**

RA and OA are complex musculoskeletal disorders that are heterogeneous in nature. Their mechanism of pathogenesis is not completely understood. DNA microarray technology is a powerful tool for gaining insights into the molecular complexity of these two diseases. Identification of the differentially expressed transcripts between RA and OA would provide a list of genes that might help in their differential diagnosis and open new avenues for stratification of patients based on the molecular criteria. A whole genome oligonucleotide microarray platform consisting of ~41,000 transcripts was performed using synovial fluid mononuclear cells (SFMCs) obtained from RA and OA patients. SFMCs are a reasonable target than PBMCs or synovial biopsies as they are exudative cells, filtered from the circulatory system into the areas of inflammation.

3.2 Identification and validation of proteins present in the synovial fluid of osteoarthritis patients by high resolution mass spectrometry

**Rationale**

Osteoarthritis is a chronic musculoskeletal disorder characterized mainly by progressive degradation of the hyaline cartilage. Patients with osteoarthritis often postpone seeking medical help, which results in the diagnosis being made at an advanced stage of cartilage destruction. Sustained efforts are needed to identify specific markers that might help in early diagnosis, monitoring disease progression and in improving therapeutic outcomes. An in-depth analysis of the synovial fluid proteome from patients with osteoarthritis was carried out in this study using multiple fractionation strategies followed by high resolution mass spectrometry using Fourier Transform LTQ-Orbitrap Velos mass spectrometer. A
few proteins were also validated by multiple reactions monitoring (MRM) which is a mass spectrometry-based complementary approach in order to demonstrate the reproducibility of mass spectrometry data. The catalog of proteins generated in this study will further enhance our knowledge regarding the pathophysiology of osteoarthritis and should assist in identifying better biomarkers for early diagnosis.

3.3 Identification and validation of synovial fluid proteins differentially expressed between RA and OA patients by high resolution mass spectrometry

Rationale

Despite high prevalence rates, etiological factors involved in RA and OA disorders remain largely unknown. Dissecting the molecular aspects of these disorders will significantly contribute to improving their diagnosis and clinical management. In order to identify the differentially expressed proteins between these two diseases, a quantitative proteomic experiment was carried out using iTRAQ labeling of synovial fluid proteins from RA and OA followed by high resolution mass spectrometry analysis using Fourier Transform LTQ-Orbitrap Velos mass spectrometer. Validation of differentially expressed protein was carried out by multiple reaction monitoring (MRM) and Western blot. The differentially expressed proteins obtained from this study might aid in early diagnosis, prognosis as well as in the evaluation of disease progression of RA and OA.