1. INTRODUCTION

1.1 RHEUMATOID ARTHRITIS

History

Rheumatic diseases were first recognized by Hippocrates in the fourth century B.C. The term “rheuma” indicates continuous flow of pain through the different joints of the body. Goemaere et al\textsuperscript{1} reported the appearance and distribution of lesions in ancient skeletons suggesting that rheumatoid arthritis may have existed in North America at least 3000 years ago. The first clinical description of rheumatoid arthritis is credited to Augustin-Jacob Landre Beauvais (1800) in his thesis.\textsuperscript{2}

Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory systemic disease of unknown etiopathogenesis. It is one of the commonest autoimmune diseases.\textsuperscript{3} The major characteristic feature is the chronic, erosive synovitis of peripheral joints due to the uncontrolled proliferation of synovial tissue which leads to severe joint destruction.\textsuperscript{2} In addition to early disability and premature mortality, symmetric swelling of peripheral joints is the hallmark of the disease.\textsuperscript{4}

Symptoms of Rheumatoid arthritis

The symptoms of rheumatoid arthritis mainly involving the joints; typically affected are those of the hands, wrists, knees and feet. The synovial joint inflammation which leads to cartilage destruction, bone erosions and joint deformities is the important aspect of rheumatoid arthritis. The characteristic feature of the disease is experiencing pain,
warmth, swelling, tenderness, stiffness and limitation of motion.\textsuperscript{5,6} Extra-articular involvement is another feature of RA and this can range from rheumatoid nodules occurring most commonly over bony prominences to life-threatening heart nodules and vasculitis.\textsuperscript{7}

The course of rheumatoid arthritis can be quite variable, and can differ in different patients. Some RA patients may experience only a mild illness for brief duration involving one or two joints with minimal joint damage, whereas others may have a relentless progressive polyarthritis with noticeable functional impairment and disability.\textsuperscript{2} Rheumatoid arthritis is also associated with symptoms of fatigue, pain, and sleep disturbances that can overlap with or mimic symptoms of depression. Depressive symptoms are highly co morbid with RA and may occur with at least mild severity in about 40-42\% of RA patients. Rheumatoid arthritis and depression contribute to mortality, decreased quality of life, increased health care costs, and disability. Inflammatory pathways may hold the key to a link between depression and RA, and cytokines have been a major target of research in this area.\textsuperscript{8}

\textbf{Prevalence}

Rheumatoid arthritis is prevalent worldwide among all races. Several studies of rheumatoid arthritis on incidence and prevalence indicated occurrence of the disease varies significantly among different populations. In one of the recent Indian study by Sudha \textit{et al} has mentioned RA has been reported in approximately 1\% of the general population with an estimated prevalence of 1 to 2\% worldwide. Another recent African studies
also reported with the prevalence of RA is 0.6 and 0.9% in adults.\textsuperscript{10} Previously Spector \textit{et al}\textsuperscript{11} has reported it is the most common inflammatory arthritis, affecting 0.8-1% percent of the adult population worldwide. There is geographic and ethnic variations with high prevalence found among North American Indians (3.5 to 5.3%) and a low prevalence has been reported in rural South African blacks and in Japanese (0.1%).\textsuperscript{12}

The disease equally afflicts people of all races and it can begin at any age. In some families, multiple members can be affected, suggesting a genetic basis for the disease. Sung \textit{et al}\textsuperscript{13} has reported the incidence of RA in 2008 was estimated at 42:100,000 in the general population of South Korea which is comparable to values for other countries in Asia. Rheumatoid arthritis affects approximately 1.3 million people in the United States according to a 2008 census data with the average annual incidence is about 70 per 100,000 annually.\textsuperscript{14} An age associated increase in the prevalence of RA has also been observed in both males and females.\textsuperscript{15}

\textbf{Role of age and gender on Rheumatoid arthritis}

Prevalence increases with age, approaching 5% in women over age 55. Both incidence and prevalence of rheumatoid arthritis are two to three times greater in women than in men. Although rheumatoid arthritis may present at any age, patients most commonly are first affected in the third to sixth decades with women twice as likely to develop the disease as men. According to Mitchell \textit{et al}\textsuperscript{16} the prevalence of RA is clearly higher in females, the estimated ratio being around 3:1. But later studies Lee and
Weinblatt\textsuperscript{5} reports RA affects about 1\% of the Caucasian population in a female to male ratio of 2.5:1.

According to studies by Jonsson \textit{et al}\textsuperscript{17} young women below the age of 50-years who have rheumatoid arthritis are at a greater risk of developing fractures than women without rheumatoid arthritis. The prevalence increases with age, approaching 5\% in women over age 55 years and sex differences diminish in the older age group\textsuperscript{6}. Sex hormones are believed to contribute to RA not only because of the disease's higher prevalence in female especially during the child-bearing years, also because of the dramatic improvements seen during pregnancy.\textsuperscript{18}

**Prevalence in India**

The prevalence of rheumatoid arthritis in India is quite similar to that reported from the developed countries. It is higher than that reported from China, Indonesia, Philippines and rural Africa. A study reported in India says that the prevalence of rheumatoid arthritis is 0.75\%, similar to the developed countries. These findings are keeping with the fact that the North Indian population is genetically closer to the Caucasians than to other ethnic groups.\textsuperscript{19} The overall impression is that the prevalence of RA is slightly less compared with the west and follows a milder course. There may be differences in the articular expression of the disease, with the wrist and forefoot less commonly affected than in Caucasian studies.\textsuperscript{20} One of the recent Indian study predicted that about 70\% of patients with RA are women. The 2- to 3-fold higher prevalence of the disease in women,
primarily due to an increased female incidence before menopause, has been interpreted as indicating a role for hormonal or reproductive factors.\textsuperscript{21}

**Risk factors for Rheumatoid arthritis**

Studies have also indicated that in addition to genetic, age and sex as predisposing factors, a number of other factors including socio-economic status, education and stress have also play an important role in predisposition in rheumatoid arthritis.\textsuperscript{2} Some more factors which are associated with an increased risk for developing rheumatoid arthritis includes positive family history, silicate exposure, and smoking\textsuperscript{22} where as high vitamin D intake,\textsuperscript{23} tea consumption\textsuperscript{24} and oral contraceptive use\textsuperscript{1} are associated with decreased risk of rheumatoid arthritis.

Tobacco smoking has long been known to play a role in the pathogenesis of rheumatoid arthritis. Studies suggest that smoking accounts for more than one third of cases of the most common form of RA and for more than 50% of RA diagnoses among smokers who are genetically susceptible to the development of this disease.\textsuperscript{25} Another study found that there is an association between smoking and presence of anti-citrullinated anticyclic antibodies in the serum of RA patients.\textsuperscript{26} Citrullinated peptides have been detected while doing broncho-alveolar lavage in RA patients who smoke regularly.\textsuperscript{27}

**Criteria for Diagnosis of Rheumatoid arthritis**

Rheumatoid arthritis primarily is a clinical diagnosis and no single laboratory test is diagnostic. It is characterized by persistent synovial joint tissue inflammation eventually, leading to bone erosion, destruction of
cartilage, and complete loss of joint integrity.\textsuperscript{28} There are no specific clinical or laboratory features that can be used to define the disease clearly.\textsuperscript{29} The diagnosis of RA is based on the presence or absence of combinations of clinical, laboratory and radiological abnormalities in individual patients. Earlier, the most widely used criteria to estimate the prevalence of RA have been those of the 1958 American rheumatoid association.\textsuperscript{30} A modified definition of RA referred to as the American college of Rheumatology (ACR) 1987 revised criteria for the classification of RA was published in 1988.\textsuperscript{31} These criteria distinguish RA from other rheumatic conditions with a specificity of 89% and sensitivity between 91-94% among affected patients.

1987 Revised American Rheumatism Association criteria for classification of Rheumatoid Arthritis

1. Morning stiffness - Morning stiffness in and around the joints lasting at least one hour before maximal improvement.

2. Arthritis of three or more joint areas - At least three joint areas simultaneously having soft tissue swelling or fluid observed by a physician (the 14 possible joint areas are (right to left) proximal interphalangeal joint (PIP), metacarpophalangeal joint (MCP), wrist, elbow, knee, ankle and metatarsophalangeal joint (MTP).

3. Arthritis of hand joints - At least one joint area swollen as above in wrist, MCP or PIP joint.

4. Symmetric arthritis - Simultaneous involvement of the same joint on both sides of the body (bilateral involvement of PIP, MCP or MTP - joints is acceptable without absolute symmetry).
5. Rheumatoid nodules - subcutaneous nodules over bony prominences or extensor surfaces or in juxta-articular regions observed by a rheumatologist.

6. Serum rheumatoid factor - Demonstration of abnormal amounts of serum rheumatoid factor by any method that has been positive in less than 5% of normal control subjects.

7. Radiographic changes - Changes typical of RA on hand wrist radiographs which must include erosions or unequivocal bony decalcification localized to most marked adjacent to the involved joints.

For classification purposes a patient is said to have RA if he/she has satisfied at least four of the seven criteria. Criteria, one through four must be present for at least 6 weeks.

Diagnosis of rheumatoid arthritis is difficult most of the time, as revised American College of Rheumatology (ACR) criteria (1987) for diagnosis of RA are based mostly on clinical manifestations which may not be present, typically in early disease. Rheumatoid factor may not be detected in many cases particularly in early disease and it is no longer the unchallenged gold standard autoantibody but has competition from the anti-citrulline antibodies. Many researchers have agreed that only 52-80% of early RA patients fulfill ACR criteria for diagnosis of rheumatoid arthritis. Hence, early intervention is crucial for preventing progression to irreversible joint damage.
New criteria-The 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for rheumatoid arthritis classification (2010 ACR/EULAR RA classification) was released in 2010 in an attempt to improve the previous approach in an eligible group of patients with early disease. This new classification system redefines the current paradigm of RA by focusing on features at earlier stages of disease that are associated with persistent and/or erosive disease, rather than defining the disease by its late-stage features. This new approach is with a specific emphasis on identifying patients with a relatively short duration of symptoms who may benefit from early institution of drug therapy or entry into clinical trials of promising new agents that may halt the development of disease that currently fulfills the 1987 ACR criteria.37

Management of Rheumatoid arthritis

Rheumatic diseases are a huge burden on the health care systems of countries worldwide and account for significant disability, lost productivity and reduction in quality of life.2 Rheumatoid arthritis is associated with pain, deformity, decreased quality of life, and disability, which in turn affect patient’s ability to lead a normal and productive life as shown in Figure 1.1. Recent studies have shown that 5 years after the onset of the disease, approximately one third of patient’s with RA are no longer able to work, and within 10 years, half of the patients have substantial functional disability.38 Consequently, RA imposes an important economic burden on society and
considerable data also suggest that RA is associated with lowered life expectancy.

If it is untreated, about 20-30% of patients with RA become permanently work-disabled within two to three years of diagnosis of rheumatoid arthritis. Although laboratory testing and imaging studies can help to confirm the diagnosis and prognosis of the disease progress since it is a chronic inflammatory disease which has an enormous socioeconomic impact wherein, there is limitation of activity which decreases the quality of life.\textsuperscript{39}

To achieve adequate rehabilitation for RA patients some of the common consequences like depression, anxiety and poor-self esteem have to be addressed. Patients are best managed within the context of a multidisciplinary team including medical specialist, nursing, occupational therapy, physiotherapy and surgical specialists.\textsuperscript{29} Patients with RA not only have a progressive and debilitating disease and severe functional impairment, but can also experience a reduced life expectancy due to frequent involvement of the major organ systems. Environmental factors influencing both susceptibility and progression have been sought, and smoking has emerged as a powerful factor. Steady progress in genetics is finally being made and one day in future it may be possible to predict RA before onset of symptomatic disease.

Current paradigms for management of patients with rheumatoid arthritis dictate early aggressive therapy in treatment-to-target strategies, aiming for remission of symptoms which may in turn prevents joint
destruction and associated co-morbidities, including cardiovascular complications. A particular concern regarding these principles, however, is that some patients with RA may remit with less aggressive treatment regimes, exposing a proportion of patients to unnecessary medications and their associated risks. Ideally it should be possible to study baseline clinical characteristics and laboratory biomarkers of RA patients and prescribe according to a predictive algorithm, so-called personalised medicine. Current algorithms, however, while predictive at a population level, have insufficient power to guide treatment of the individual patient.

Management of rheumatoid arthritis has been improved over the last 10 years. These improvements have been achieved not only by new drugs, but also by the overall approach toward treating patients. One need to keep in mind that positive result for rheumatoid factor or anti-CCP alone never proves the diagnosis of RA. After diagnosis therapy should be started immediately, recruiting physiotherapy, pain medication, corticosteroids and disease-modifying anti-rheumatic drugs (DMARDs), primarily methotrexate. At the latest after failure of two DMARDs biologics like TNF-α-blockers, an Interleukin-6-Receptor-antibody, a B-cell-specific antibody or a rather T-cell-specific biologic will be initiated.

Pharmacotherapy for rheumatoid arthritis generally involves a non-steroidal anti-inflammatory drug (NSAID) for control of pain, with selective use of low-dose oral or intra-articular glucocorticoid, and initiation of disease-modifying anti-rheumatic drugs. Most commonly, non-steroidal anti-inflammatory drug, salicylates, or cyclooxygenase-2 inhibitors are used
for initial treatment of rheumatoid arthritis to reduce joint pain and swelling. Steroids at low dosages as recommended by rheumatologist daily are highly effective for relieving symptoms of rheumatoid arthritis and can slow joint damage. Biologics in RA have been successful; however, safety concerns and pharmaco-economical issues are still debated. Protein kinase inhibitors have been developed, and can be called "molecular-targeting antirheumatic drugs" (MTARDs), as opposed to "disease-modifying anti-rheumatic drugs."

Therapeutics options for RA have increased tremendously in the past decade with the introduction of biological agents in 1999. Several different cellular and cytokine targets have been identified, with specific inhibitors, including the tumor necrosis factor (TNF) antagonists, an interleukin 1 (IL1) antagonist, an inhibitor of T cell co-stimulation and a selective depleter of B cells. Significant advances have been made in our understanding of the events involved in the onset and persistence of RA, and these have provided the rationale to modulate them. New therapeutic approaches aim at suppressing inflammation and establishing tolerance without compromising the immune system. Experimental immunotherapeutic strategies in RA treatment include the use of regulatory T cells, mesenchymal stem cells (MSCs), and tolerogenic dendritic cells.

Several new drugs with novel mechanisms of action have emerged in recent years like drugs act as a competitive inhibitor of an intracellular enzyme needed for de novo pyrimidine synthesis by activated lymphocytes in RA patients (Leflunomide), tumor necrosis factor (TNF) antagonists,
recombinant interleukin-1 receptor antagonist (Anakinra), Anti–interleukin-6 receptor antibodies also are being evaluated. A number of additional, non-pharmacologic treatments for rheumatoid arthritis have been tried like therapeutic fasting, dietary supplementation of essential fatty acids, along with have spa therapies and exercise.

1.2 PATHOGENESIS OF RHEUMATOID ARTHRITIS

The etiology of Rheumatoid arthritis is not yet fully understood. Studies of RA heritability in two northern European regions have demonstrated that an average of 60% of the disease variance can be attributed to genetic factors.46

STRUCTURE OF A SYNOVIAL JOINT

Synovial joints (also known as diarthroses) are the most common type of joint in the body. As with most other joints, synovial joints achieve movement at the point of contact of the articulating bones. They are characterized by having capsules surrounding the articulating surfaces and the presence of lubricating synovial fluid within that capsule (synovial cavity). The cavity (also called joint cavity) is the space between two articulating bones.

Synovial joints consist of five different tissue types including bone, cartilage, synovium, synovial fluid and tensile tissues such as ligament and tendon. Articular cartilage covers and protects the bone ends and the articular capsule encloses the joint structure. It consists of an outer layer, the fibrous membrane, and an inner lining, the synovial membrane. Ligaments are fibrous thickenings of the articular capsule that help provide
stability. Beneath the surface of the cells lining the synovium is a network of capillaries important for gas and nutrient exchange and the development of synovial inflammation. The synovium is therefore permeable to water, gases, nutrients, small molecules and proteins, but not to large proteins, proteoglycans, glycosaminoglycans and oligosaccharides. Hyaluronic acid (HA) is an important molecule that makes the synovial fluid viscous. This property allows the synovium to trap synovial fluid and osmotically active load resistant molecules within the cavity. During normal and pathological turnover, degradation products of matrix proteins and glycoproteins are released to the synovial fluid and to the circulation.\textsuperscript{47}

**ARTICULAR CARTILAGE**

Articular cartilage is an avascular and alymphatic connective tissue with unique biological and mechanical properties. Its load-bearing function depends on the structural design of the tissue and the interactions between its unique resident cells, the chondrocytes, and the extracellular matrix (ECM) that makes up the bulk of the tissue. Chondrocytes are the only cells present in cartilage. They are architects and designers of the ECM, building the macromolecular framework of the ECM from three distinct classes of macromolecules: fibrillar and non-fibrillar collagens, proteoglycans, and non-collagenous proteins. Of the collagens present in articular cartilage collagens type II, IX, and XI form a febrile meshwork that gives cartilage tensile stiffness and strength\textsuperscript{48}

Genetic and environmental factors play a role in pathogenesis. Even though infectious agents such as viruses, bacteria and fungi have been
suspected none has been proven as the cause. According to Figure 1.2, exogenous (infectious) agents like human T-cell lymphotropic virus type 1, herpes virus, mycoplasm, Epistein-Barr virus have been proposed as etiological agents of rheumatoid arthritis. Endogenous proteins such as collagen and IgG are most often implicated in the progression of rheumatoid arthritis and RF is considered amplifiers of RA inflammation. 49

The disease process leading to rheumatoid arthritis begins in the synovial membrane, a protective sac that surrounds a joint filled with lubricating liquid called the synovial fluid. In addition to cushioning joints, this fluid supplies nutrients and oxygen to cartilage. Joint cartilage is composed primarily of the structural protein collagen, which forms a mesh to give support and flexibility to joints. 50

In Rheumatoid arthritis, an abnormal immune system produces destructive molecules that cause continuous inflammation of the synovium. Fluid and immune system cells accumulate in the synovium to produce a pannus, a growth composed of thickened synovial tissue. The pannus can attack articular cartilage and then it can destroy the soft subchondral bone. 51 The pannus produces more enzymes that destroy nearby cartilage, aggravating the area and attracting more inflammatory white cells, thereby perpetuating the process.

In early stages of Rheumatoid arthritis edema is seen in cells of the synovium. 4 During inflammatory process, these cells grow and divide abnormally making the synovium thick and resulting in swollen joints affecting cartilage, bone and organs in other parts of the body. White blood
cells that are part of the normal immune system travel to the synovium and cause inflammation, which is called synovitis, and it results in the warmth, redness, swelling, and pain that are typical symptoms of rheumatoid arthritis.\textsuperscript{52}

The pathogenesis of Rheumatoid arthritis is a complex phenomenon and includes synovial cell proliferation, fibrosis, pannus formation and bone and cartilage erosion. The pathophysiology of rheumatoid arthritis is mediated by an inter-related network of cytokines, proteolytic enzymes and prostanoids. Interleukin I (IL-1) is a key mediator of pannus formation and synovial inflammation also believed to be a contributor to the hampering of tissue repair processes and the damage to bone and cartilage in rheumatoid arthritis.\textsuperscript{53} It is also mediates inflammation by recruiting neutrophils into the joint, activating macrophages, and stimulating T- and B-cell proliferation and differentiation. Synoviocytes, on their exposure to IL-1, proliferate and produce IL-6, matrix metalloproteases (MMPs) which further causes proteoglycan degradation, which ultimately results into cartilage destruction.\textsuperscript{54}

Lymphocytes, both B-cells and T-cells play an important role in the pathogenesis of rheumatoid arthritis. B-cells that carry surface immunoglobulin produce auto antibodies known as rheumatoid factors, which form immune complexes, subsequently complement fixation, neutrophil activation and inflammation. T-cells help in autoantibody production and thus responsible for inflammation. Heat shock proteins produced by all the cells in response to stress facilitate cross reactivity of
lymphocytes with host cells and trigger immunological reaction in rheumatoid arthritis. Reactive oxygen species (ROS) produced during cellular respiration resulting in oxidative stress can serve as a intracellular signaling molecule for amplification of synovial inflammatory response in rheumatoid arthritis.

Cytokines also play an important role in rheumatoid arthritis by regulating the broad range of inflammatory processes that are implicated in the pathogenesis of rheumatoid arthritis. There is chronic inflammation with lymphocytes and plasma cells leading to excessive cytokines, autoantibodies and rheumatoid factor production in the pannus which are probably responsible for the cartilage and bone destruction via further local macrophage activation, complement activation and polymorph nuclear cell infiltration.

Abnormal production of some other inflammatory mediators like tumor necrosis factor alpha (TNF-alpha), interleukins, transforming growth factor beta (TGF-beta), fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF) is also seen in RA patients. Inflammation and abundant proliferation of synovium gives rise to destruction of several tissues, which include cartilage, tendons, bones, blood vessels and ligaments as shown in Figure 1.3. Although the major sites of inflammation and damage are the articular structures, other tissues are severely affected. The joint damage in rheumatoid arthritis begins with the proliferation of synovial macrophages and fibroblasts after a triggering incident, possibly autoimmune or infectious.
Several studies have established that mast cell have a critical role in the pathogenesis of synovitis in rheumatoid arthritis.\textsuperscript{5} Mast like cells express high levels of surface HLA-DR and production of cytokines thus effectively mediating the inflammatory reaction in rheumatoid arthritis. Studies have shown that the genetic basis is dependent on at least two genes, one of which is HLA-DR4, which is found in majority of Caucasian patients. Analysis of genetic markers has demonstrated an association between the development of RA and HLA-DRBI including HLA-DR4 alleles.\textsuperscript{60} Neutrophils in the rheumatoid synovium ingest immune complexes and release lysosomal enzymes, destructive oxygen free radicals thus damaging cartilage and joint structure. Fibroblast like cells is responsible for synovial lining hyperplasia and local invasion by pannus in rheumatoid arthritis.\textsuperscript{61}

**Early Rheumatoid arthritis**

Early indications of rheumatoid arthritis are swelling and pain of the PIP and MCP joints. Later, the larger joints become affected, especially those of the knee, elbow and ankle. Large numbers of activated leukocytes infiltrate the synovial membrane, causing hyperplasia and inflammation, which in most cases lead to progressive destruction of cartilage and bone. Since RA is a systemic autoimmune disease, other parts or organs of the body may become affected at a later stage. An example of this is the formation of rheumatoid nodule. Peak onset typically occurs in the fourth and fifth decades of life.\textsuperscript{62}
Early RA tends to affect the smaller joints first, such as the joints in the wrists, hands, ankles and feet. As the disease progresses, joints of the shoulders, elbows, knees, hips, jaw and neck can also become involved. Unlike other arthritic conditions that only affect areas in or around joints, RA is a systemic disease which can cause inflammation in extra-articular tissues throughout the body including the skin, blood vessels, heart, lungs and muscles.8

More sophisticated and effective as well as aggressive therapies are available nowadays, which can control the disease at an early stage thus preventing an irreversible damage. Hence, there is a need for a sensitive as well as specific serological marker to diagnose RA at an early stage.

1.3 BIOMARKERS ASSOCIATED WITH RHEUMATOID ARTHRITIS

A biomarker is classically defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological pathogenic or pharmacological responses to a therapeutic intervention.63 Diagnostic markers of disease ideally fulfill three requirements: (i) good sensitivity, to detect a high percentage of patients; (iii) good specificity, to limit false-positive results as much as possible; and (iii) early presence, to facilitate early diagnosis.

The term biomarker includes not only proteins and protein fragments, but also metabolites, carbohydrate biomarkers, genomic biomarkers, cellular biomarkers and imaging biomarkers. Consequently, there is an overwhelming interest in biomarker research within both the basic and clinical sciences.47
Significance of study of markers in Rheumatoid arthritis

Biomarker discovery and validation for Rheumatoid arthritis have accelerated over the past several years, coincident with an evolving understanding of joint tissue molecules and their complex interactions, and the compelling need for improved RA outcome measures in clinical trials. One of the main potential uses of biochemical markers would be to identify patients at high risk of rapidly progressive joint destruction. Indeed, clinical indices such as pain and physical function score are poorly related to the destruction of joint structure.\(^{64}\)

Change in joint space width assessed by radiography remains the gold standard, but it allows neither either early detection of joint tissue damage nor efficient monitoring of the effectiveness of treatment aimed at preventing joint destruction, because of its poor sensitivity and relatively large precision error. Evidently, for identifying patients at high risk for destructive osteoarthritis (OA) and for monitoring the efficacy of new structure-modifying therapies, there is a need for better techniques than plain radiography. Magnetic resonance imaging (MRI) is currently being optimized for this purpose.\(^{65}\) Alternatively, specific and sensitive biochemical markers reflecting abnormalities of cartilage, synovium, and bone tissues may be useful for the investigation and monitoring of rheumatoid arthritis.

Molecular or biochemical markers of tissue turnover have been used for decades in clinical trials of osteoporosis, but only recently in rheumatoid arthritis. Biomarkers are directly reflective of structural damage to joints in
contrast to serum C-reactive protein which is only a nonspecific indicator of systemic inflammation. Since RA involves joint inflammation, bone remodeling and destruction of the extracellular matrix (ECM) of articular cartilage with degenerative changes generate collagen and proteoglycan fragments, which are potential biomarkers that can be detected in blood, synovial fluid and urine.\textsuperscript{66}

Biochemical markers are particularly useful for patient selection and treatment, but can be used in a variety of ways to accelerate clinical trial and reduce the uncertainty and cost of drug development. An increasing body of evidence suggests that a combination of these markers could provide useful adjuncts to radiography and disease activity indices for identifying early RA patients at risk of rapid joint destruction, potentially before any joint damage is detected by radiography. The rapid responsiveness of markers should prove valuable in the clinical development of drugs for preventing joint destruction, and in monitoring their treatment effects in patients with early rheumatoid arthritis.\textsuperscript{67} Good evidence exists for the role of several of these markers in perpetuating disease in RA patients.

More recently developed molecular markers of bone, cartilage and synovial tissue turnover may provide better indication of destructive activity of the disease. The extracellular matrix of these tissues is composed primarily of collagens including type I (bone and synovium), type II (cartilage) and type III (synovium) associated with proteoglycans- (e.g. aggrecan in cartilage) and other glycoproteins. Potential biological markers
for RA include matrix components (and/or their breakdown products), cytokines, and proteases like matrix metalloproteases.\textsuperscript{68}

The joint is a complex organ and rheumatoid arthritis alters the metabolism of different tissues including bone, cartilage and synovium. The disease can irreversibly change the structure and function of a joint to the degree that other degenerative changes may occur, especially in the weight bearing joints of the body. Thus joint destruction can progress to the degree that joint motion is significantly limited and joints can become markedly unstable. To evaluate the mechanism involved in the joint destruction, the simultaneous assessment of markers for these tissues will prove to be useful.

**Synovial inflammation in Rheumatoid arthritis**

Synovitis plays a predominant role in the pathophysiology of rheumatoid arthritis. It is a condition that develops when a joint lining (synovial lining) becomes irritated and inflamed. Each joint is enclosed in a capsule lined with membrane tissue known as the synovium or synovial membrane. The synovium secretes a lubricating synovial fluid and is able to adapt to different motions of a joint by expanding and contracting. The inflamed synovium increases fluid production, resulting in warmth, tenderness, and swelling in and around the joint.\textsuperscript{69}

Synovitis is more commonly found in RA (Figure 1.4) than in other forms of arthritis, and can thus serve as a distinguishing factor, although it can present to a lesser degree in osteoarthritis. Long term occurrence of synovitis can result in degeneration of the joint. Synovitis causes joint
tenderness or pain, swelling and hard lumps, called nodules. When associated with rheumatoid arthritis, swelling is a better indicator than tenderness. Because synovial activity is also likely to have a pivotal role in the pathogenesis of RA, a marker that specifically reflects its turnover would be useful.

Several markers have been proposed to assess synovitis. Most important among them are serum hyaluronan, a high molecular weight heteroglycan. It is not only present in synovium but also in articular cartilage, skin and eye. The degradation of polymeric structure of hyaluronan is characteristic of inflammation reaction as seen in rheumatoid arthritis. The concentration of hyaluronan in serum has been correlated with, disease activity and morning stiffness in rheumatoid arthritis. Some other new but non specific markers also been proposed to assess inflammatory synovial activity like Matrix metallo proteinases (MMP) and YKL-40.

Rheumatoid arthritis is characterized by an invasive and tissue destructive infiltrate of lymphocytes, macrophages and synoviocytes. Matrix metallo proteinases and tissue inhibitors of metallo proteinases (TIMPs) are produced by these cells are important in remodeling of articular tissue. Normally, a tight balance exists between Matrix metallo proteinases and tissue inhibitors of metallo proteinases, however in pathological situations such as RA an MMP/TIMP imbalance is present, which leads to an excess of activated MMPs, which have an important role in the chain of events leading to excessive cartilage degradation. Increased levels of MMPs are
found in tissue, in the synovial fluid and in the systemic circulation of patients with rheumatoid arthritis.\textsuperscript{76} YKL-40, a glycoprotein produced in large amount by activated macrophages, but its expression is detected only at a late stage of macrophage maturation in RA patients.\textsuperscript{77} It has been suggested that it is a surrogate marker of synovial inflammation and joint destruction in rheumatoid arthritis and osteoarthritis.\textsuperscript{78}

**Cartilage degradation in Rheumatoid arthritis**

Rheumatoid arthritis is characterized by progressive destruction of articular cartilage and by subchondral bone and synovial tissue reaction. The extensive extra cellular matrix of hyaline cartilage contains a dense network of composite collagen fibrils and large proteoglycans of articular cartilage, which consist of core proteins with different oligosaccharide and glycosaminoglycan chains linked to them.

Cartilage is predominantly an acellular tissue, consisting of chondrocytes embedded in a highly structured matrix. In chronic arthritic diseases such as rheumatoid arthritis and osteoarthritis, the normal balance between matrix synthesis and degradation in cartilage is disturbed and shifts toward reduced synthesis and enhanced breakdown. This shift leads to the subsequent progressive destruction of the matrix, which in turn leads to irreversible joint damage and finally destruction of the joint. Such joint damage is detectable by radiography, but unfortunately at a stage too late for intervention.\textsuperscript{79}
Cartilage breakdown can be evaluated by an important non-collagenous protein, cartilage oligomeric matrix protein (COMP). \(^8^0\) During the destructive process, however, increased amounts of matrix macromolecules like COMP are released into the synovial fluid from where they then reach the blood stream as a consequence of the increased tissue degradation. This increased presence of matrix macromolecules in the bloodstream offers the possibility to use them as ‘biomarkers’ to identify the severity of reactions in the cartilage of the joint. \(^8^1\)

**Bone turnover in Rheumatoid arthritis**

Bone is a specialized form of metabolically active, mineralized connective tissue. It is a “dynamic tissue” that is remodeled constantly throughout life. \(^8^2\) Bone is composed of mineral and protein matrix of which Type I collagen is the main organic constituent, making up about 80-90% and the remaining 10-15% is of non-collagenous proteins including osteocalcin, glycoproteins and proteoglycans. The collagen matrix is made sturdy and durable by mineralization with hydroxyapatite crystals. *In vitro* studies demonstrate that osteocalcin binds strongly to hydroxyapatite, the mineral phase of bone with the help of the gamma-carboxyglutamic acid side chains. \(^8^3\)

Osteoblasts are mononuclear cells that attach to bone surfaces and form new bone, most commonly at sites that recently underwent resorption. They produce type I collagen and other matrix components of osteoid, and they also mineralize the osteoid with hydroxyapatite. In elderly women, osteoblasts may increase in number in response to the increase in bone
resorption brought on by estrogen deficiency. In elderly men, osteoblast activity may decrease\textsuperscript{84} possibly because of decreasing levels of serum insulin-like growth factor-1 and testosterone.\textsuperscript{85} A variety of factors can contribute to bone loss, including glucocorticoid therapy, hypogonadism, vitamin D deficiency, immobility, and elevated levels of bone-active cytokines.\textsuperscript{86}

In Rheumatoid arthritis an interaction between antibodies and antigens occurs which causes alterations in the composition of the synovial fluid affecting the normal performance of the functions. The changes in the synovium and synovial fluid are responsible for a large amount of joint and soft tissue destruction. The destruction of bone eventually leads to laxity in tendons and ligaments resulting in the deformities frequently seen in patients with rheumatoid arthritis. Bone destruction occurs at areas where the hyaline cartilage and the synovial lining do not adequately cover the bone.\textsuperscript{87}

The levels of bone formation markers are generally low during glucocorticoid therapy and those of bone resorption markers are either normal or low.\textsuperscript{88} The incidence of osteoporosis and fractures is also increased in patients with rheumatoid arthritis.\textsuperscript{89} Bone mass peaks at 30 years in both men and women and approximately 0.4% of bone is lost per year after this peak bone mass is achieved. It is important to note that because bone resorption and formation are coupled, an increase in either process will increase bone marker levels. In chronic steady-state conditions, most markers reflect both formation and resorption because
only a small imbalance exists. In addition, biochemical markers of bone are not disease specific, but reflect alterations in skeletal metabolism regardless of the underlying cause. These markers could be useful in making therapeutic decisions because, in general, the higher the bone remodeling and bone loss rates the greater the concentration of bone biochemical markers in blood or urine as reported by an Indian study by Apurva et al.\textsuperscript{90}

In Rheumatoid arthritis the normal joint homeostasis breaks down resulting in destruction of cartilage and loss of joint integrity. Markers of bone formation are products released by the osteoblasts or peptides derived from the amino-terminal or carboxy-terminal of Type I procollagen which by itself is a precursor of Type I collagen.\textsuperscript{91}

Biochemical monitoring of bone metabolism depends upon measurement of enzymes and proteins released during bone formation and of degradation products produced during bone resorption. There are various biochemical markers are now available that allow a specific and sensitive assessment of the rate of bone formation and bone resorption. They are osteocalcin, skeletal alkaline phosphatase and amino terminal propeptide of Type I collagen. Among them the most important biomarker is osteocalcin.\textsuperscript{92}

**Traditional markers of systemic inflammation in Rheumatoid arthritis**

Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are the acute-phase reactants, belongs to the class of serum glycoproteins,
whose concentration in the blood increases after various stimuli such as trauma or inflammation tissue injury, infection and cancer.\textsuperscript{93}

C-reactive protein belongs to the pentraxin family of proteins. It is exclusively made in the liver and is secreted in increased amounts within 6 hours of an acute inflammatory stimulus.\textsuperscript{94} It is a serum marker for inflammation or infection and acts by binding to phosphocholine found on the surface of dead or dying bacterial cells in order to activate the immune system via the complement system. The level of CRP is rapidly increasing when inflammation is present and rapidly decreasing as inflammation improves. This makes CRP a good monitoring tool for disease activity and its levels are usually elevated in untreated rheumatoid arthritis; the degree of elevation correlating roughly with the severity of inflammation to assess the effectiveness of a specific arthritis treatment and monitor periods of disease outburst.\textsuperscript{95}

Fat cells release factors that stimulate the liver to produce CRP, and serum levels greater than 10 mg/dl are generally considered indicative of an infectious or inflammatory process. After an inflammatory stimulus, serum CRP levels may exceed 500 times the baseline, so CRP is used in all medical specialties to help diagnose inflammation and infection. The magnitude of the acute-phase protein response is roughly proportional to the severity of the stimulus. In the body, CRP plays the important role of interacting with the complement system, an immunologic defense mechanism.\textsuperscript{96}
Another blood test often ordered along with C-reactive protein is known as erythrocyte sedimentation rate. Both CRP and ESR give similar information about non-specific inflammation yet CRP appears and disappears more quickly than changes in ESR. Therefore, persons CRP level may drop to normal following successful treatment, whereas ESR may remain elevated for a longer period.97

In general CRP and ESR are used steadily in chronic inflammation where as ESR has become less widely used because of its slow response following an inflammatory stimulus, so making use of the historical tendency to record the ESR and CRP as an alternative and independent marker of inflammation in patients with rheumatoid arthritis.98 There is also a high sensitivity CRP test (hs-CRP) in addition to the regular CRP test. The hs-CRP measures very low amounts of CRP in the blood and is typically used to assess risk for heart problems.40 Erythrocyte sedimentation rate and C-reactive protein are the most widely used assays to measure the laboratory aspect of the acute-phase response, being of great value in monitoring disease activity in rheumatoid arthritis.64

C-reactive protein as a general indicator, not specific for RA alone and in most infections and inflammations it is found to be very high. It appears to be a better test regarding measurement of the acute phase response because ESR is sensitive to immunoglobulins and RF, it may measure general severity better than CRP, even though it is a poorer measure of inflammation. The combination of ESR and CRP yields useful information that is often not apparent when only a single test is used.
Although patients with chronic inflammatory diseases, such as RA have raised levels of CRP, levels are still highly variable.\textsuperscript{99}

Both erythrocyte sedimentation rate and C reactive protein have been used as indicators of inflammation and disease progression and outcome in rheumatoid arthritis. The persistent elevation of C reactive protein in the acute phase of RA is associated with functional deterioration and elevation of erythrocyte sedimentation rate is strongly correlated with radiographic progression of the disease.\textsuperscript{100}

**Autoantibodies**

Auto antibodies can be detected in a spectrum of rheumatic diseases where they may be highly associated with distinct clinical syndromes. These are often helpful for diagnosis, and to some extent, prognosis. Since rheumatoid arthritis is characterized by the presence of various auto antibodies in serum and synovial fluid the auto antigens recognized by these auto antibodies include cartilage components, chaperones, enzymes, nuclear proteins and citrullinated proteins. This demonstrates that RA is not characterized by single autoreactivity or a single autoantigen but by accumulated autoreactivities in both B and T cells.\textsuperscript{101} The presence of these antibodies early in disease development opens a window of opportunity for early custom-tailored treatment of rheumatoid arthritis

Autoantibodies are proved to be useful as diagnostic tools for different kinds of rheumatic and non-rheumatic autoimmune disorders. They are a common and characteristic feature of rheumatic autoimmune
diseases. Even though the majority of autoantibodies do not seem to playing a major role in the pathogenesis of these disorders they are extremely useful as diagnostic tools and indicators of disease activity.\textsuperscript{7}

Rheumatoid arthritis is accompanied by the occurrence of many auto antibodies in the serum of the patient. Most of these antibodies are not specific for RA because they also occur in other inflammatory conditions. Other antibodies appear to be more specific and are, in some cases, almost exclusively present in rheumatoid arthritis. We should distinguish between RA-associated antibodies, which are present in RA but also in other diseases, and RA-specific auto antibodies, which appear to be exclusively present in RA. It is, of course, the category of RA-specific antibodies that is most relevant for the clinician. The possible pathogenic nature of auto antibodies in RA is still controversial.\textsuperscript{7}

**Rheumatoid Factor (RF)**

Rheumatoid factor (RF) found to be associated with rheumatoid arthritis was the first autoantibody which was described by Waaler and Rose\textsuperscript{102}. They are a family of circulating autoantibodies which recognizes the fraction crystallizable (Fc) part of Immunoglobulin $\gamma$ (IgG) molecules which itself is an antibody. Rheumatoid factor exists as IgG, Immunoglobulin $\alpha$ (IgA) and Immunoglobulin $\mu$ (IgM) isotypes and both RF and IgG join to form immune complexes that contribute to the disease process in rheumatoid arthritis.
Physiological role of Rheumatoid factor

Rheumatoid factors are produced by B cells present in lymphoid follicles and germinal like structures that developed locally in the inflamed synovium.\textsuperscript{103} Under normal conditions, the physiological roles of RF is to enhance immune complex clearance and helping B cells to uptake immune complex thereby, efficiently present antigens to T cells. Rheumatoid factor also facilitates complement fixation by binding to IgG containing immune complexes as shown in Figure 1.5. Normally immune complexes and polyclonal B-cell activators like lipopolysaccharides and Epstein-Barr virus induces transient production of low-affinity IgM Rheumatoid factor.\textsuperscript{104}

Rheumatoid factor and Rheumatoid arthritis

Rheumatoid factor is an immunologic marker in the body, found in low titer in a number of infectious diseases, viral diseases, chronic bacterial infections and other acute/chronic conditions. It is also found in approximately 5% of healthy elderly persons. Rheumatoid factor is an antibody that is not usually present in the normal individuals but it may sometimes be present in normal individuals without diseases particularly in people with family members who have rheumatoid arthritis.

Rheumatoid factor can be present years before the onset of rheumatoid arthritis and rheumatoid factor test is among the diagnostic tests commonly ordered to help diagnose rheumatoid arthritis. The highest levels of rheumatoid factor are usually found in rheumatoid arthritis. Clinically, higher titers tend to correlate with more severe and sustained
disease, joint deformities, rheumatoid nodules and other extra-articular features of the disease.\textsuperscript{105}

The pathogenic implication of Rheumatoid factor in rheumatoid arthritis may involve formation of immune complexes able to mediate tissue damage by complement activation or Fc receptor ligation.\textsuperscript{106} Fc receptors (FcγR I, FcγR II and FcγR III) are cell surface receptors expressed on various leukocytes specifically binding IgG and IgG immune complexes (ICs) crosslink these FcγRs and activate leukocytes effector functions, such as respiratory cellular burst, cytokine secretion, antibody-dependent cellular cytotoxicity and phagocytosis.\textsuperscript{107}

Song and Kang\textsuperscript{101} demonstrated that accumulated somatic mutations and the presence of isotope switching indicate RF production is T-cell mediated. Although T cells infiltrate RA synovium and contain auto reactive clones they have been shown to be polyclonal and lack specificity for any particular auto antigen. The distinguished feature of Rheumatoid factor in RA patients is that they exhibit affinity maturation where as RF in healthy individuals are polyreactive, low affinity for antigen and does not exhibit affinity maturation. Pike \textit{et al}\textsuperscript{108} proposed that RF could be utilized as diagnostic criteria in RA and among different types of RF isomers IgM RFs are the major species in RA along with other isomers like IgG (RF) to be detected in 60–80\% of RA patients.

**Serological marker of Autoimmunity**

Rheumatoid factor has been the only serologic marker in the American College of Rheumatology (ACR) classification criteria for several
decades. It is a valid prognostic indicator, but the antibody’s usefulness for early detection of RA is limited by its moderate sensitivity and relatively low specificity.\textsuperscript{109} Rheumatoid factor can be a very good prognostic indicator because they are closely associated with increased severity of RA as the presence of erosive disease indicates more rapid disease progression. Although RF may be involved in RA pathogenesis, their role is still not entirely clear and also interestingly, a study on animal models report that RF do not occur in experimental models of rheumatoid arthritis.\textsuperscript{110}

Rheumatoid factor (IgG) has four subclasses and each have distinct biological properties.\textsuperscript{107} IgG1 and IgG3 are able to activate all types of Fc receptors. It might be expected that IgG1 and IgG3 would be mainly involved in the immunopathology associated with IgG mediated autoimmune inflammatory conditions. Chapuy-Regaud \textit{et al}\textsuperscript{111} reported subclass distribution of the IgG response to divergent antigens in RA patients is variable. Therefore, examination of the patterns of distribution of serum IgG subclass concentrations may provide insight into the immunological process involved in rheumatoid arthritis.

It is not clear why IgA RF should be more closely associated with fluctuations in disease activity than the other RF isotypes. Perhaps an outbreak of RA is normally accompanied by polyisotypic RF production of which the IgG RF and IgM RF contribute to the inflammatory reaction by activation of complement and phagocytes. This in turn may lead to rapid clearance and turnover of IgG RF and IgM RF in the joints and only a limited spillover into the peripheral circulation.\textsuperscript{112}
Though IgM RF is measured in most studies its specificity for diagnosing RA is limited concluding that not only a very low level IgM-RF is present in sera of normal people and a high concentration of IgM-RF is detected in individuals with viral and bacterial infections or chronic inflammations other than RA which can induce polyclonal stimuli to B cells.

Rheumatoid factor can also be a cryoglobulin (antibody that precipitates on cooling of a blood sample). The presence of rheumatoid factor in serum can also indicate the occurrence of suspected autoimmune activity unrelated to rheumatoid arthritis. In such instances, RF may serve as one of several serological markers for autoimmunity.

**Anti-cyclic citrullinated peptide (Anti-CCP)**

Anti-citrullinated protein/peptide antibodies (Anti-CCP) are a group of autoantibodies frequently detected in RA patients. According to Nienhuis and Nienhuis and Mandema these antibodies were originally described as ‘Anti-perinuclear factor (APF) and subsequently as Anti-keratin (AKA) and Anti-filaggrin antibodies.

**Antigens directed against citrulline**

Analysis of Anti-keratin antibodies (AKA) and Anti-perinuclear factor (APF) auto-antibodies showed that most of the reactivity present against these antigens was directed against citrulline residue as shown in Figure 1.6. This discovery led to the development of assays employing cyclic citrullinated peptides (CCP) to measure antibodies recognizing citrullinated antigens as a diagnostic test for rheumatoid arthritis. The citrulline moiety of the autoantigen is the essential determination proteins.
recognized by APF and AKA and it has become evident since 1998 that APF, AKA target citrullinated proteins.  

Citrulline is a non-standard amino acid generated by the post-translational modification of arginine by peptidylarginine deiminase (PAD) during inflammation and other variety of biologic processes. During inflammation citrulline is incorporated enzymatically into protein by a process called citrullination. Conversion of arginine into citrulline involves the replacement of an amine group by an oxygen atom in the side chain of this amino acid, and is associated with the loss of a positive charge (at neutral pH). Although this conversion results in a relatively small chemical alteration of the protein involved, the reactivity of autoantibodies with citrulline-containing epitopes seems to be critically dependent on the presence of a citrulline residue. Peptidyl arginine deiminase mediates citrullination of arginine in the presence of sufficient concentrations of Calcium ions. Apoptotic granulocytes create an environment for PAD activation, in which cytosolic Calcium ion level rises due to caspase-mediated plasma membrane Ca^{2+} pump cleavage. When the apoptotic cells are not cleared efficiently as in an inflammatory environment, intracellular citrullinated proteins and/or PAD are released into the extracellular space, where the former are taken up by antigen-presenting cells and the latter induces the citrullination of synovial proteins. While these antibodies are referred to as different names depending on the antigens used for their detection, citrulline is a common critical constituent
of the antigenic determinant of these antibodies, as its absence leads to a lack of recognition by antibodies.\textsuperscript{101}

Schellekens \textit{et al}\textsuperscript{120} proved that anti-CCP antibodies bind to antigenic determinant containing citrulline which is formed from arginine, also pointing that such antigenic determinants also have been found in various proteins such as fillagrin, vimentin and fibrinogen.

\textbf{Anti-cyclic citrullinated peptide antibodies versus Rheumatoid factor as Diagnostic markers in Rheumatoid arthritis}

The most important requirements for a good diagnostic marker are that it must be specific for the disease and present in a relatively high percentage of patients with the disease. Next to being sensitive and specific, good markers of disease should be detectable as early in the disease process as possible. Though the exact cause of the RA remains still elusive several studies reported the presence of characteristic autoantibodies like IgM, IgG and IgA fraction RF and anti-CCP prior to the appearance of disease manifestations strongly providing evidence of a preclinical, asymptomatic, phase of the disease.\textsuperscript{121}

Several studies using the anti-CCP test showed this test indeed provides a good sensitivity/specificity balance in RA patients.\textsuperscript{118} One of the German studies by Zendman \textit{et al}\textsuperscript{122} clearly states that in general anti-CCP has better diagnostic value than RF in terms of sensitivity and specificity and importantly is seen in early rheumatoid arthritis.
Combined role of Anti-CCP and RF in Rheumatoid arthritis

Several studies support the view that both RF and anti-CCP is highly specific and moderately sensitive for RA thereby making this combination of auto antibodies a powerful serologic tool in the assessment of rheumatoid arthritis. Schellekens *et al.*[^120^] reported the specificity for RA can be further increased by combining the presence of anti-CCP antibody with the presence of Rheumatoid factor, and anti-CCP and RFs of all isotypes predated the onset of RA by several years. The presence of anti-CCP and IgG-RF predicted the development of RA, with anti-CCP antibody having the highest predictive value. This indicates citrullination and the production of anti-CCP and RF autoantibodies are early processes in rheumatoid arthritis.[^123^]

Anti-CCP in early Rheumatoid arthritis

It is mandatory to differentiate between RA and other forms of arthritis early after symptom development. Rheumatoid arthritis patients are often difficult to diagnose in the early stages of disease because they do not always show typical signs and symptoms and they may not fulfill the ACR (American college of Rheumatology) classification criteria. Anti-CCP antibodies and RF are superior to several genetic markers in predicting the diagnosis of RA from undifferentiated arthritis in early arthritis patients.

Early recognition and treatment of rheumatoid arthritis is important to prevent irreversible joint damage. Hence, anti-CCP has been suggested for early diagnosis. Assays that detect anti-CCP antibodies have become popular in recent years for diagnosing early RA because they are believed
to be more specific than RF tests with similar sensitivity. Studies by Penny et al.\textsuperscript{124} concludes that the sensitivity is higher in studies of established RA than in those of early RA patients for whom testing have greatest potential benefit recommending anti-CCP tests should be used routinely to diagnose early rheumatoid arthritis.

Anti-cyclic citrullinated peptide antibodies correlate with the development of erosive disease in the early stages of rheumatoid arthritis.\textsuperscript{125} Most importantly, anti-CCP antibodies are present early in the disease process and may even pre-date the onset of RA by many years. The presence of anti-CCP antibodies in RA is predictive of the development of erosive disease. This effect seems to be greatest in those patients who are seronegative for Rheumatoid factor.\textsuperscript{126}

**Hyaluronic Acid**

Hyaluronic acid (HA) was known as a “goo” molecule until the late ‘70’s and is part of synovial fluid found in between the joints. This fluid lubricates the joints and reduces friction. Hyaluronic acid was identified about 50 years ago as a major component of the skin and was thought to originate solely in the dermis until a few years ago. This belief was held due to the fact that the dermis is the major part of the skin and the role that hyaluronic acid played in forming matrices in connective tissue was known.\textsuperscript{127} The ground substance in the connective tissues and supporting structures in the body is made up of primarily two compounds- chondroitin sulphate and hyaluronic acid.
The word Hyaluronic acid is derived from Greek, in which “Hyalos” means “for vitreous” and uronic acid because it was first isolated from the vitreous humour which possesses a high uronic acid content. The term hyaluronate refers to the conjugate base of hyaluronic acid. Because the molecule typically exists in vivo in its polyanionic form, it is most commonly referred to as hyaluronan.\textsuperscript{128}

**Structure of Hyaluronic acid**

Hyaluronic acid is one of the most physiologically important anionic non-sulfated glycosaminoglycans (GAGs) along with sulfated chondroitin sulfates, keratin sulfate (KS), and heparin. All these GAGs are the major components of joint cartilage, synovial fluid, and other soft connective tissues like connective, epithelial and neural tissues.\textsuperscript{129} It is a high molecular weight acidic mucopolysaccharide composed of repeating disaccharide units, i.e., 1,4-glucuronic acid (GlcUA) and 1,3-N-acetylglucosamine (GlcNAC).

**Source of Hyaluronic acid**

Hyaluronic acid is synthesized by bioactivity of enzyme hyaluronan synthase (HAS), which has been reported to have three isoforms (HAS1, HAS2, and HAS3) in humans.\textsuperscript{130} It is produced by type B (fibroblast-like) synoviocytes and chondrocytes appears to reach the blood via the lymphatic systems and is rapidly eliminated by the liver.\textsuperscript{131}

It is synthesized in the plasma membrane of fibroblasts and other cells primarily in connective tissue and synovial membranes by addition of sugars to the reducing end of the polymer, whereas the non-reducing end
protrudes into the pericellular space. The polysaccharide is catabolized locally or carried by lymph to lymph nodes or the general circulation, from where over 90% is cleared rapidly by the liver via receptor facilitated uptake and catabolism in liver endothelial cells.\textsuperscript{73} As connective tissue matrix component the overall turnover rate is surprisingly rapid for hyaluronic acid.\textsuperscript{132} The half life of HA in the circulation of healthy subjects is 2–5 minutes.\textsuperscript{133} Normal serum HA concentrations are age dependent with a decline from infancy to adulthood, and rise again in the elderly.\textsuperscript{134}

Hyaluronic acid is degraded by a family of enzymes called hyaluronidases (HAase). In humans, there are at least seven types of hyaluronidases-like enzymes, several of which are tumor suppressors. The degradation products of hyaluronan are the oligosaccharides and very low-molecular-weight hyaluronan, exhibit pro-angiogenic properties. Study reported that the optimum pH of hyaluronidases is 4.0 and no HAase activity could be detected at pH 5.0–7.0. However, systemic arterial pH is maintained between 7.35 and 7.45, so serum HAase may not hydrolyze serum HA in vivo.\textsuperscript{135}

In addition, one of the studies showed that hyaluronan fragments and not the native high-molecular mass of hyaluronan, can induce inflammatory responses in macrophages and dendritic cells in tissue injury and in skin transplant rejection.\textsuperscript{136} The hyaluronic acid level is reported to change in response to physical activity, and along with other biomarkers related to matrix turnover it shows significant diurnal variation.\textsuperscript{137}
Physiological role of Hyaluronic acid

Hyaluronic acid is also referred as sodium hyaluronate; a viscoelasticity substance that occurs naturally in synovial fluid found to increase the viscosity of the fluid and is thought to play an important role in lubricating, protecting, and maintaining the health of articular cartilage as shown in Figure 1.7. In the synovial fluid each molecule of HA consists of a long linear polymer of disaccharide (N-acetyl glucosamine and glucuronic acid) repeats and in this forms it is important for joint lubrication. It is also an important articular cartilage glycosaminoglycans component, binding and retaining multiple aggrecan monomers and it is present as a coat around each chondrocytes.

Hyaluronan production increases in proliferating cells and the polymer may play a role in mitosis. It seems to play an important role during development and differentiation and has other cell regulatory activities.\textsuperscript{138} Being one of the chief components of the extracellular matrix HA not only contribute significantly to cell proliferation and migration but also thought to be involved in tissue formation and cell retention as a matrix material.\textsuperscript{139} Extensive Hyaluronidases sensitive coats have been identified around mesenchymal cells. They are either anchored firmly in the plasma membrane or bound via hyaluronan-specific binding proteins (receptors). Hyaluronan has been assigned various physiological functions in the intercellular matrix like in water and plasma protein homeostasis. Because of its free-radical scavenging property, it can protect against free-radical damage to cells.\textsuperscript{140}
Recent investigations have continued to reveal the variety of actions of HA on cartilage like working as a mechanical/physical barrier, anti-inflammatory and analgesic functions.\textsuperscript{141} Hyaluronic acid provides a cushion effect between the joints so we are able to move easier and feel less pain. It also acts as a "reservoir of water" within the skin thereby improving the elasticity and texture of the skin. It reduces bacterial infections and has been found to raise the white cell count in the bloodstream.\textsuperscript{142}

Studies have been shown that Hyaluronic acid attenuates prostaglandin- or bradykinin-induced pain in experimental osteoarthritis animals and suppresses pain.\textsuperscript{143} Several studies also demonstrated that HA can enhance synthesis of chondroitin sulfate and proteoglycans and reduce the production and activity of matrix metallo proteinases.\textsuperscript{144}

Hyaluronan is also used to treat osteoarthritis of the knee called visco-supplementation, are administered as a course of injections into the knee joint, and are believed to supplement the viscosity of the joint fluid, thereby lubricating and cushioning the joint, and producing an analgesic effect. It has also been suggested that hyaluronan has positive biochemical effects on cartilage cells.\textsuperscript{145} Another study reported, along with the destruction of RA joint tissue, a remarkable quantity of various MMPs, GAG molecules, especially hyaluronic acid are released from the extracellular matrix of the synovium\textsuperscript{146} which is a key feature of RA progression. Because GAGs are the basic structural components of joint cartilage,
synovial fluid, and soft tissues the RA synovium produces an abundance of free GAGs during tissue destruction.

The molecular weight of hyaluronic acid from the pathological fluids was somewhat lower than from the normal fluids and HA increases the viscosity of synovial fluid than other proteins. Studies indicate that in rheumatoid arthritis the total HA and protein content of the joint and connected cavities is increased.\textsuperscript{128}

**Hyaluronic acid and Rheumatoid arthritis**

Emlen et al\textsuperscript{147} reported the high levels of serum hyaluronic acid seen in rheumatoid arthritis are produced by proliferating synovial B cells, discharged into the synovial fluid and then released into the blood via lymph ducts. Pitsillides et al\textsuperscript{148} found the ratio of free hyaluronic acid to bound form was significantly increased in the RA as compared with the healthy synovium, although the total concentration of hyaluronan was not increased in the rheumatoid synovium. The study also proved that hyaluronan was concentrated in the lining layer of non inflamed synovial membrane of most of the RA patients.

On the other hand, the HA level is very low among various other tissues. This finding helps to explain why RA occurs and develops in joint tissue because the inflamed RA synovium is uniquely rich in free HA and other GAGs, along with extracellular matrix degeneration.\textsuperscript{149} Hyaluronic acid in synovial tissue may leak into the circulatory system during synovial inflammation because of this, serum HA levels are expected to be elevated.
in rheumatic diseases with synovial involvement such as rheumatoid arthritis.

**YKL-40**

YKL-40 is a mammalian member of family of a glycosyl hydrolases, also called human cartilage glycoprotein 39 or Chondrex. It is secreted by chondrocytes, synovial cells, macrophages and neutrophils and also secreted by the human osteosarcoma cell line by articular human cartilage chondrocytes and by human synovial fibroblasts. It was first discovered in mouse breast cancer cells and named BRP-39. It is originally identified in the whey secretions of nonlactating cows. The protein was named YKL-40 based on its three N-terminal amino acids Tyrosine (Y), Lysine (K) and Leucine (L) and its molecular mass of 40 kDa. Human YKL-40 contains a single polypeptide chain of 383 amino acids and has a calculated molecular mass of 40,476 Da with an isoelectric point of about 7.6. It shares significant amino acid sequence identity to bacterial chitinases and to seven other “mammalian chitinase-like proteins.

**Physiological role of YKL-40**

The exact physiological function of YKL-40 is still not known. It is a chitin binding lectin but has no chitinases activity. Even though it binds with chitin most of the studies on this chitinase-like protein focus on its endogenous “chitin independent” effector functions. It appears to be important in the homeostasis of the body. In another study Hakala et al proved the rapid induction of YKL-40 production in normal human cartilage
after introduction to cell culture conditions indicating YKL-40 is involved in the cartilage-remodeling process.

YKL-40 is a growth factor for connective tissue cells; it also stimulates the migration of endothelial cells.\(^\text{156}\) Moreover, it is a trans-membrane protein in which cleaved components bind to an unidentified receptor and the expression of YKL-40 is regulated by various inflammatory cytokines and hormones.\(^\text{157}\) It has recently been shown that it binds specifically to collagen types I, II, and III and modulates the rate of type I collagen fibril formation.\(^\text{158}\) Studies reported chondrocytes from normal cartilage are found to be YKL-40 negative\(^\text{159}\) and another finding demonstrated that no YKL-40 mRNA expression by normal human chondrocytes.\(^\text{160}\)

**YKL-40 and Rheumatoid arthritis**

Verheijden et al\(^\text{161}\) has shown that immunization with YKL-40 could induce RA-like arthritic lesions in a mouse model. These findings suggested YKL-40 may be an auto antigen derived from cartilage in rheumatoid arthritis. In another study Jacques et al\(^\text{162}\) observed increased levels of YKL-40 in serum and synovial fluid are found in patients with active RA compared to normal subjects concluding serum YKL-40 is a more direct measure of joint inflammation though in general CRP and ESR, the gold standards of biochemical assessment of the disease activity in RA and indirect measure of joint inflammation thus proving the role of YKL-40 in RA pathogenesis.
In the synovium, YKL-40 positive cells were found in synovial lining and macrophages and the number of YKL-40 positive cells was related to degree of synovitis in rheumatoid arthritis.\textsuperscript{163} In arthritic cartilage YKL-40 positive cells is located in chondrocytes and its level in synovial fluid are higher in RA patients with moderate/severe or none/slight synovitis of the knee joint. Thereby, knee joint derived YKL-40 influences the level of serum YKL-40.\textsuperscript{161} In one of the studies Volck et al\textsuperscript{164} reported that it may be involved in the pathophysiology of the arthritic processes and reflect local disease activity, it is considered as an important marker of synovial inflammation in rheumatoid arthritis.

Studies by Vos et al\textsuperscript{165} proved that the glycoprotein YKL-40 whether produced locally at the site of inflammation or systemically, is widely expressed and thus may be a prominent target for specific T cells which makes YKL-40 is a promising candidate antigen for tolerance induction in rheumatoid arthritis. Several present and previous studies support this hypothesis by finding the highest level of YKL-40 expression in patients with rheumatoid arthritis.

**YKL-40 and Hyaluronic acid**

The expression and function of YKL-40 is closely related to hyaluronan, like it has been connected with embryogenesis, morphogenetic processes, cell proliferation and tissue remodeling and is involved in acute and chronic inflammatory processes. It is not known if YKL-40 binds hyaluronan, but YKL-40 has two potential hyaluronan binding motifs (location 147-155 and 369-377).\textsuperscript{77} It may recognize hyaluronan, or its
precursor, as a substrate in the ECM and interfere with its synthesis, which could affect local hyaluronan levels and consequently influence the extent of cell adhesion and migration during the tissue remodeling processes that take place during inflammation, fibrosis, atherogenesis and metastasis.\textsuperscript{166}

**Matrix Metalloproteinases**

Matrix Metalloproteinases (MMP) are a family of more than 25 enzymes that are intimately involved in the breakdown of extracellular matrix of many normal physiological processes such as embryonic development and growth, tissue remodeling and in diseases like RA and cancer metastasis.\textsuperscript{167}

These zinc-dependent and calcium-dependent endopeptidases consist of several subsets of enzymes, including collagenases, stromelysins and gelatinases and are involved in the degradation of the extra cellular matrix (ECM) that forms the connective material between cells and around tissues.\textsuperscript{168} They can cleave adhesion molecules, cytokines, chemokines, growth factors as well as their receptors, and binding proteins.\textsuperscript{169} One of the earliest studies stated that the MMP family contains the only vertebrate proteinases that can specifically degrade triple-helical collagens type I, type II and type III.\textsuperscript{170}

*In vitro* studies by Murphy *et al*\textsuperscript{171} have demonstrated the ability of active MMPs to denature virtually all cartilage components. In the absence of disease they take part in normal tissue remodeling, but in pathologic states they contribute to tissue destruction. Matrix metalloproteinases cleave characteristically and specifically at a single locus in all three
collagen chains, at a point approximately three-quarters from the N-terminus of the molecule. At physiological temperature, the cleaved triple helix then unwinds, becoming susceptible to attack from other, less specific, proteinases. Matrix metalloproteinases are now acknowledged as key players in the regulation of both cell–cell and cell–extracellular matrix interactions as shown in Figure 1.8. They are involved in modifying matrix structure, growth factor availability and the function of cell surface signaling systems, with consequent effects on cellular differentiation, proliferation and apoptosis.\textsuperscript{172}

The interstitial collagens (types I, II and III), are the principle targets of destruction and the secreted collagenases (MMP-1 and MMP-13) have the major role in this process.\textsuperscript{173} In general, MMPs are induced in response to the cytokines and growth factors usually found in arthritic joints.\textsuperscript{174} Thus, the collagenases (MMP-1, MMP-8 and MMP-13) have the unique ability to cleave the triple helix of collagen, thereby allowing the chains to unwind; the chains then become susceptible to further degradation by other matrix metalloproteinases.

**Tissue inhibitors of metalloproteinases (TIMP)**

Tissue inhibitors of metalloproteinases (TIMPs) are naturally occurring, specific MMP inhibitors that form noncovalent, tight-binding complexes with active matrix metalloproteinases.\textsuperscript{175} It is a small glycoprotein with a molecular weight of 28KD and it is produced locally by the same cells that secrete matrix metalloproteinases. Disease processes associated with the MMPs are generally related to imbalance between the
inhibition and activation of MMP leads to chronic stimulation of MMP, resulting in excessive degradation of the extracellular matrix in disease like osteoarthritis, rheumatoid arthritis etc. However, MMPs and TIMPs are not subject to the same regulatory influences, so in RA and excess of MMPs over TIMPs results in an imbalance favoring proteolysis leading to joint destruction.\textsuperscript{176}

Another study with the similar outcome reported that MMPs and TIMP are regulated differently, despite the fact that they are produced by the same cells within the joint and a disproportionately low level of TIMP secretion may be part of the acquired functional abnormalities of RA synovial cells.\textsuperscript{177}

**Source of origin of Matrix metalloproteinases**

Matrix metalloproteinases are secreted by the macrophages and lymphocytes present in joint tissues as well as by invading cells. They are active around neutral pH at the same time they have the combined ability to degrade all the components of the extracellular matrix. The matrix metalloproteinases in fact may have a dual effect on chemokines, as proteolytic cleavage can inactivate some chemokines simultaneously activating others.\textsuperscript{178}

**Subclass of MMP**

Based on the substrate specificity, the family of MMP enzymes is subdivided into subgroups such as stromelysins (MMP-3, -10, and -11), collagenases (MMP-1, -8, -13), gelatinases (MMP-2, -9), and membrane-type MMPs (MMP-14, -17, -22, -24, -25).\textsuperscript{179} According to studies by Pendas
there are two types of collagenases which are products of two
distinct genes called MMP-1 (fibroblast-type collagenase) and MMP-8
(neutrophil collagenase).

**Matrix Metalloproteinase -1**

Interstitial collagenase (E.C.3.4.24.7) also known as MMP-1 is a
member of the MMP gene family that cleaves the collagen triple helix to
yield characteristic products. The primary structure of MMP-1 was first
published by Goldberg et al. Matrix metalloproteinase -1, as the other
members of matrix metalloproteinases family, is structurally formed by
different protein building blocks.

**Matrix Metalloproteinase-1 and Rheumatoid arthritis**

The destruction of joint tissue is a primary feature of RA and in the
inflamed RA synovium produce proliferating macrophages and colonizing
lymphocytes together with persistent angiogenesis produce large amounts
of matrix metalloproteinases that destroy the surrounding cartilage and
extracellular matrix of connective tissue. Matrix metalloproteinase-3 and
MMP-1 are abundantly expressed in active rheumatoid synovium, and
serum level of MMP-3 along with MMP-1 is a useful marker not only for the
diagnosis of rheumatoid arthritis but also for the evaluation of prognosis in
the joint destruction. In one of the studies So et al. reported the MMP-3
(stromelysin-1) and MMP-1(collagenase-1), have been studied for their
ability to predict progression of RA in terms of joint damage and MMP
inhibitors are expected to be useful for the treatment of joint disorders like
rheumatoid arthritis.
Matrix Metalloproteinases have been shown to have an important role in the invasion of the synovial tissue in cartilage, cartilage destruction, and bone erosion formation which contributes to joint articular tissue destruction in rheumatoid arthritis.\textsuperscript{53} In another study Kaneko et al\textsuperscript{76} has shown that progressive degradation of the ECM that comprises joint tissues, including articular cartilage, bone and even intra-articular ligaments and tendons, is a major feature of the arthritic diseases like rheumatoid arthritis suggesting, family of MMPs playing a prominent role along with other proteinases in the breakdown of ECM leading to permanent loss of function in rheumatoid arthritis.

Three apparently distinct processes are involved in cartilage degradation: the hypertrophic synovial cells produce MMPs and release them into synovial fluid, where the enzymes degrade the articular cartilage matrix; fibroblasts at the cartilage-pannus junction secrete MMPs and directly degrade the cartilage matrix; and chondrocytes produce and secrete MMPs and then digest their own matrix.\textsuperscript{185}

\textbf{Cartilage Oligomeric Matrix Protein (COMP)}

Cartilage Oligomeric Matrix Protein was first described in 1992 by the research group of Professor Dick Heinegard\textsuperscript{186} University of Lund, Sweden. It is also called thrombospondin-5. It is a noncollagenous, extracellular glycoprotein and is a member of the thrombospondin family of calcium-binding proteins. It is a large acidic protein with a total molecular weight of 435KD according to Figure 1.9 it is composed of five identical subunits, disulphide bonded near their N-terminal end whereas the C-
terminal end adopts a globular conformation, altogether forming a bouquet-like structure.\textsuperscript{187}

**Physiological role of COMP**

The biological significance of COMP is not very clear but being the structural component of cartilage it catalyzes collagen fibrillogenesis by direct interactions with collagen. The function of which is to bind to type II collagen fibres and stabilize the collagen fibre network in the articular cartilage. It has been proposed that COMP molecules are important for maintaining the properties and integrity of the collagen network and contribute to the material properties of biological tissue.\textsuperscript{188}

**Source of origin**

It was originally isolated and characterized from cartilage.\textsuperscript{189} It is known to be produced by chondrocytes as well as synovial cells, tendon fibroblasts, osteoblasts but localized extracellularly. Cartilage Oligomeric Matrix Protein is secreted and assembled normally into the matrix of tendon, demonstrating that the accumulation of COMP in chondrocytes is a cell-specific phenomenon. It also has been detected in nasal, tracheal, and meniscal cartilage, and most prominently in articular cartilage.\textsuperscript{187} The COMP concentrations are 10 times higher in synovial fluid than in serum which suggests that it is locally produced in the joints.\textsuperscript{190}

Several findings support the theory that COMP is released in response to damage to the cartilage matrix. This can be either due to destabilization of the extracellular matrix or increased protease activity against COMP at the disease site. It may also occur due to increased
synthesis of new COMP as an attempt to repair by the chondrocytes or synovium.\textsuperscript{191}

**COMP and Inflammation**

Cartilage Oligomeric Matrix Protein is the first extracellular matrix protein for which an active role in inflammation has been demonstrated \textit{in vivo}. Simultaneously it can activate one complement pathway and inhibit another. The net outcome of these functions is most likely determined by the type of released COMP fragments, which may be disease specific.\textsuperscript{192} Studies on inflammatory joint diseases such as rheumatoid arthritis and osteoarthritis show that changes in serum concentrations of COMP correlate to changes in cartilage turnover.\textsuperscript{193}

**COMP and Rheumatoid arthritis**

In the serum of the normal population the COMP level is very low and elevated amounts of COMP are found in serum during increased turnover of cartilage associated with active joint disease, such as RA and osteoarthritis.\textsuperscript{194} One of the studies reported an increased level of COMP in the synovial fluid in the early stage of RA whereas in advanced stages of RA, the level of COMP was found to be decreased.\textsuperscript{195}

An interesting feature of COMP is that it is associated with cartilage breakdown perhaps released from cartilage during the erosion of the tissue as seen in severe arthritis. This makes COMP a potential diagnostic and prognostic indicator as well as a marker of disease severity in diseases such as rheumatoid arthritis.\textsuperscript{189}
Osteocalcin

Osteocalcin or Bone Gla Protein (BGP) is a vitamin K-dependent bone specific protein. It is the major and most thoroughly characterized noncollagenous protein in mature human bone, where it constitutes 1-2% of the total protein and about 10–20% of the non-collagen protein. It was discovered in chicken bone and in bovine bone. The complete covalent structure of the 49-residue bovine and human proteins has maximum three residues of the vitamin K-dependent amino acid, gamma-carboxyglutamic acid residues and hence the proteins are designated bone gamma-carboxyglutamic acid-containing protein.

Physiological role of osteocalcin

Osteocalcin is also found in dentin other than bone. The exact physiological function of osteocalcin is still unclear, but it has a high affinity for calcium and exhibits a compact, calcium dependent α-helical conformation, in which the γ-carboxyglutamic acid (Gla) residues binds and promote absorption to hydroxyapatite in the bone matrix, indicating that it might be associated with the process of mineralization of bone. One of the recent studies raise the surprising possibility that osteocalcin is a hormone that influences energy metabolism by modulating the production and action of insulin.

A large number of studies show that the circulating levels of osteocalcin are associated with changes in the rate of bone turnover in metabolic bone diseases such as osteoporosis.
It is a small protein (49 amino acids) with a molecular weight of 5800 Da.\textsuperscript{202} It is synthesized by mature osteoblasts, odontoblasts, and hypertrophic chondrocytes as shown in Figure 1.10. Around 10% to 30% of the osteocalcin synthesized by osteoblasts is released into the circulation.\textsuperscript{203} As seen commonly in other secreted proteins, osteocalcin has a signal sequence that is removed in the rough endoplasmic reticulum to give pro-osteocalcin. Before secretion from the osteoblasts, specific glutamic acid residues are carboxylated by a post-translational, vitamin K dependent enzymatic carboxylation to form gamma-carboxyglutamic acid (Gla).\textsuperscript{204}

Serum osteocalcin levels follow a circadian rhythm characterized by a decline during the morning, a low around noon, and a gradual increase to a peak after midnight. It reportedly varies significantly during the menstrual cycle, with the highest levels observed during the luteal phase.\textsuperscript{205} It has been observed that higher serum-osteocalcin levels are relatively well correlated with increases in bone mineral density. Circulating levels of osteocalcin and its fragments reflect both bone formation and resorption.\textsuperscript{206}

After cleavage of the propeptide and secretion, a large proportion of the native osteocalcin is incorporated into the mineralizing matrix (bone matrix), assisted by the calcium binding properties of the Gla residues\textsuperscript{207} and after biosynthesis of osteocalcin, native calcium-binding molecule and the propeptide appear in the serum. Osteocalcin fragments are released in the plasma after degradation of the matrix-bound material. In addition, lack
of adequate carboxylation can produce non-carboxylated molecules because it is rapidly cleared by the kidney as the half life of circulating osteocalcin is approximately 5 minutes.\textsuperscript{208}

**Osteocalcin as bone formation marker**

Serum alkaline phosphatase (ALP) and serum osteocalcin (OC) have been most extensively studied as markers of bone formation and both are products of osteoblasts.\textsuperscript{209} Serum osteocalcin is considered as a specific marker of osteoblasts function and its levels correlate with the rate of bone formation rate.

The major advantages of using osteocalcin as a clinical index of bone turnover are its tissue specificity, its wide availability, and its relatively low within-person variation. In general, serum levels are elevated in patients with diseases characterized by a high bone turnover rate, and the serum levels reflect the expected changes in bone formation following surgical and therapeutic intervention.\textsuperscript{210}

**Osteocalcin and Rheumatoid arthritis**

Rheumatoid arthritis is associated with severe progressive joint destruction and erosion of periarticular bone. There may be generalized as well as localized bone loss in these patients. Studies of bone status in patients with RA have shown varying results for osteocalcin measurements. Because bone demineralization is a common finding in patients with RA, osteocalcin produced by osteoblasts is often used as a marker for the bone formation process.\textsuperscript{211}
1.4 REVIEW OF LITERATURE

In a recent Indian study Shankar et al\textsuperscript{212} suggested that anti-CCP antibodies are highly specific for rheumatoid arthritis and might aid in predicting development of RA in Indian population.

Heidi et al\textsuperscript{213} reported that the anti-CCP antibodies exist as different isotypes, among them IgM and IgG anti-CCP antibodies is most commonly present in both early and established RA patients.

Lakos et al\textsuperscript{214} also reported the presence of IgA anti-CCP antibodies in RA patients.

Syversen et al\textsuperscript{215} observed among the different isotypes, IgG anti-CCP antibodies are associated with radiographic progression in RA patients.

Studies by Visser et al\textsuperscript{33} and Staikova et al\textsuperscript{216} have shown that both antiCCP and RF are serological markers which are associated with more severe joint damage in RA.

Though study by Vittecoq et al\textsuperscript{217} explained that RF was the main factor predicting radiological progression compared to anti-CCP, but anti-CCP are highly specific for RA and precede the onset of disease symptoms, indicating its role in the pathogenesis of rheumatoid arthritis.

Male et al\textsuperscript{218} reported that RF is an immunological hallmark of RA and may be directly involved in the pathogenesis.
Dorner et al$^{219}$ revealed that RF exists as different isotypes and the prevalence of RA is markedly increased in individuals with RF antibodies of more than one isotype, mostly a combination of IgM and IgG.

Victoria R and Clifton$^{220}$ reported that the prevalence of RA is markedly increased in individuals with RF antibodies of more than one isotype, like IgG, IgM and IgA. So determination of IgG subclass concentrations in RA seems to provide a useful insight into the pathological process operating in the disease.

Dahlqvist et al$^{23}$ and Nielen et al$^{21}$ concludes that RF, an autoantibody directed against the constant region of IgG is elevated in 75% of patients with RA and widely used in clinical practice. In addition to RF, anti-CCP antibodies are frequently observed in patients with RA, especially in early disease.

Munthe and Natvig$^{222}$ published that intracellular IgG (RF) complexes have been found to be particularly prominent in synovial tissues from seronegative RA patients.

Showman et al$^{223}$ concluded that anti-CCP auto antibodies which are found in 70–90% of RA patients and have high disease specificity of about 90–95% suggesting they are rarely found in other diseases or in healthy individuals.

Winchester et al$^{224}$ stated RF is detected in 70–80% of RA patients with established disease and there is no association of RF with the level of disease activity but it is an integral part of the definition of this disorder.
Two different studies also showed that IgG RF frequency ranges from 43-68% in RA patients.\textsuperscript{125,225}

Study by Caspi \textit{et al}\textsuperscript{226} reported that higher level of anti-CCP in the synovial fluid of RA patients than patients with other arthritic diseases such as osteoarthritis or psoriatic arthritis.

Interestingly a study by Bizzaro \textit{et al}\textsuperscript{227} however reported a lower sensitivity of anti-CCP when compared to Rheumatoid factor.

The anti-CCP antibodies are actively produced or enriched at the inflammatory synovial joints and may play an active role in the pathogenesis of RA by enhancing oxidative stress in the joint tissue.\textsuperscript{228}

In another study Levent \textit{et al}\textsuperscript{229} also observed these antibodies are closely related to increased oxidant activity in the synovial fluid of RA patients concluding there were no relationships between anti-CCP antibody existence with oxidant status and antioxidant defense system in blood or serum.

Vencovsky \textit{et al}\textsuperscript{230} reported patients with positive anti-CCP are more associated with higher probability of erosive disease in long-term follow-up studies.

Another study by Sockalingam \textit{et al}\textsuperscript{231} reported a positive value for anti-CCP predicts the future development of erosive RA with more severe disease outcome.

Several studies concluded that anti-CCP antibody response may occur years before any clinical symptoms, as shown by the presence of anti-CCP antibodies several years before the clinical onset of arthritis.\textsuperscript{221,232}
So inclusion of anti-CCP in the diagnostic criteria would promote earlier detection of inflammatory arthritis and allow the initiation of more aggressive treatment to prevent joint damage.\textsuperscript{233}

\textbf{Schelleken et al\textsuperscript{20}} was the first to report on the diagnostic properties of the rheumatoid arthritis specific anti-CCP auto antibodies using the first generation CCP test concluding that determination of anti-CCP and IgG-RF contribute to the prediction of clinical disease activity and joint damage.

Another study by \textbf{Raza et al\textsuperscript{234}} demonstrated the importance of anti-CCP antibodies for diagnosis in RA citing they are more specific than RF in early and late RA patients concluding these antibodies may predict the eventual development into RA when found in undifferentiated arthritis and they may be detected in healthy individuals long before onset of clinical symptoms of disease.

One of the recent studies by \textbf{Guigao and Jinming\textsuperscript{235}} made clear that determination of IgG (RF) subclass concentrations in RA seems to be useful in the better understanding the pathological process in the RA patients.

Though several studies were performed on the IgG (RF) concentrations profiles in the RA patients, the results from these studies were discrepant.\textsuperscript{236,237}

In a most recent report by \textbf{Couderc et al\textsuperscript{238}} concluded RF is 45% positive in first 6 months of disease onset and 85% positive with
established disease. Though not specific for RA, high titer of RF in early stages of RA is a bad sign of diseases progression.

Skoumal et al\textsuperscript{239} proved that the serum COMP is most extensively studied cartilage marker and studies have found an association between increased COMP & progression in rheumatoid arthritis, concluding that the COMP level was independent of stage of disease, number of painful and swollen joints, duration of morning stiffness, disease duration in RA patients

Several studies has reported that increased concentrations of COMP were found in RA patients who developed rapid hip joint destruction compared with a matched normal population and its level in aggressive RA predicts fast damage of large joints, especially hip joints.\textsuperscript{194,240}

But contrary to this, couple of studies reported serum COMP level was not found to be a reliable predictor of future large-joint damage in patients with rheumatoid arthritis.\textsuperscript{241,242}

In another study by Welsing et al\textsuperscript{243} reported that in RA patients with advanced destruction of the joints COMP is decreased, though serum COMP levels might reflect the cartilage pathology of both large and small joints but the small joints are more often involved in the RA disease process.

Jacobson et al\textsuperscript{244} has shown that reported assessing COMP level alone or in combination with other factors in early stages of RA may be useful in the future to facilitate better prognostic action.
Meanwhile, Lindquist et al\textsuperscript{245} reported that the increased serum COMP level in early-stage RA was connected with the risk of development of destructive changes in hand and feet joints in RA patients.

Hummel et al\textsuperscript{246} has suggested that the serum COMP levels are highly specific markers for the cartilage degradation process in RA patients.

But Crnkic et al\textsuperscript{247} has revealed that COMP is not unique to cartilage and serum COMP as a marker reflecting processes not directly linked to the inflammation in rheumatoid arthritis.

Manek and Lane\textsuperscript{79} has shown that serum COMP level reflect increased cartilage turnover and may be used as a prognostic marker of cartilage degradation in patients with established rheumatoid arthritis.

Study by Larsson et al\textsuperscript{248} with experimental models supports the feasibility of COMP as a serum marker for cartilage involvement in arthritis.

A significant correlation between serum COMP level with clinical activity in RA patients has been demonstrated by Feyertag et al.\textsuperscript{249}

The studies by Hanan et al\textsuperscript{250} has revealed that the pro-inflammatory cytokine such as transforming growth factor $\beta$ upregulates synovial cell production of COMP and increased serum COMP levels correlate well with the presence of synovitis in RA patients.

Garcia et al\textsuperscript{251} reported that the serum osteocalcin can be used as an indicator of bone formation and turnover.

A study on RA patients by Deodhar and Woolf\textsuperscript{86} concluded that there is no unanimity about the levels of osteocalcin, marker of bone formation.
Orth et al\textsuperscript{211} has shown that the reduced serum osteocalcin values in RA reflect reduced bone formation.

Magaro et al\textsuperscript{252} has reported increased levels of osteocalcin in patients with active RA reflecting high bone turnover in these patients.

Angela et al\textsuperscript{253} proved that patients with severe RA produce low amounts of active osteocalcin and higher than expected amounts of inactive osteocalcin in the synovial fluid concluding osteoblast function may be abnormal in the osteoporosis of rheumatoid arthritis.

Gevers et al\textsuperscript{254} reported that because bone demineralization is a common finding in patients with rheumatoid arthritis, osteocalcin produced by osteoblasts it is often used as a marker for the bone formation process.

Eggelmeijer et al\textsuperscript{255} and Lems et al\textsuperscript{256} reported that the level of serum alkaline phosphatase and osteocalcin are within the normal range in RA patients.

Suzuki et al\textsuperscript{257} concluded that in post-menopausal women with rheumatoid arthritis there is an increase in the level of osteocalcin than in pre-menopausal women due to uncoupling of bone formation and bone resorption accelerated by disease activity.

But Vis et al\textsuperscript{258} showed that no significant association was found between changes in disease activity and levels of osteocalcin at any of the time interval in rheumatoid arthritis.

Harvey et al\textsuperscript{259} has demonstrated that YKL-40 may be a surrogate marker for inflammatory and degenerative joint diseases.
Morrison et al\textsuperscript{260} has concluded that YKL-40 may play a role in remodeling or degradation of extracellular matrix and in the inflammatory process thereby directly involved in the process of inflammation and joint tissue degradation in rheumatoid arthritis. So measuring the YKL-40 levels in serum may provide new information in arthritic disease activity along with other conventional markers.

Supporting this study, Johansen et al\textsuperscript{261} reported YKL-40 in blood and synovial fluid in patients with different rheumatoid diseases and its level in serum can be projected as a new biochemical marker for joint injury.

Best et al\textsuperscript{262} reported a positive correlation between YKL-40 and ESR in RA patients indicating that YKL-40 is also expressed by peripheral blood mononuclear cells in patients with rheumatoid arthritis.

Hakala et al\textsuperscript{78} pointed out that YKL-40 mRNA expression is found in inflamed synovial membrane from RA patients but not in non-inflamed synovial membrane concluding YKL-40 mRNA expression is high in cartilage from RA patients and undetectable in normal cartilage.

Verheijde et al\textsuperscript{161} observed that the YKL-40 derived peptides were selectively recognized by peripheral T cells in patients with rheumatoid arthritis.

Two different studies by Volck et al\textsuperscript{159} and Nyirkos and Golds\textsuperscript{151} showed that synovial cells obtained from the synovial membrane of RA patients at time of joint replacement secrete YKL-40 and the level of serum
YKL-40 has the potential to provide new information on disease activity and pathophysiology of the synovitis in rheumatoid arthritis.

In another study with early RA patients, Peltomaa et al\textsuperscript{263} explained that serum YKL-40 is an inflammatory marker correlating with disease activity, meanwhile the levels of YKL-40 correlated with laboratory and clinical markers of disease activity both at baseline and during follow-up of early RA patients.

In the same year another Italian study concluded that YKL-40 as a local prognostic marker of joint destruction since the serum levels of YKL-40 were enhanced in all patients with rheumatoid arthritis\textsuperscript{163}.

Studies by Maeda et al\textsuperscript{264} reported that most of the matrix metalloproteinases are produced in synovial lining cells and released into synovial fluid and degrade the articular cartilage matrix in advanced stages of rheumatoid arthritis.

Another study by Emery and Salmon\textsuperscript{265} suggested that synovial cell collagenase or matrix metalloproteinases are more important in the breakdown of the cartilage matrix than chondrocytes and it participates in the destruction of the entire cartilage surface, not just in a localized area such as the cartilage-pannus junction in rheumatoid arthritis.

The expression of MMPs in cartilage or synovial membrane was increased in rheumatoid arthritis\textsuperscript{266}.

Taylor et al\textsuperscript{267} demonstrated an increased serum levels of MMPs in tissue, synovial fluid and in the systemic circulation of patients with rheumatoid arthritis.
In another study by Lohmander et al\textsuperscript{268} a higher level of MMPs have been detected in the serum, synovial fluid and synovial membrane of patients with arthritis compared with normal controls.

Tetlow and Woolley\textsuperscript{269} reported that the high MMP levels in arthritis are thought to result from increased production by inflamed joints and from the sites of cartilage erosion in humans with rheumatoid arthritis.

The gene expression of MMP-1 and MMP-3 has been observed in the synovial lining layer, scattered cells in the sublining area and in activated synovial endothelial cells in RA patients by Yoshihara et al\textsuperscript{270}.

In another study by Walakovits et al\textsuperscript{271} concluded that MMP-1 and MMP-8 have been elevated levels in RA synovial fluid.

Clark et al\textsuperscript{84} has reported an elevated level of only MMP-1 has been found at in RA serum.

Woolley et al\textsuperscript{272} has demonstrated that collagenase was localized by immune-staining on the extracellular matrix components at the cartilage-pannus junction in rheumatoid arthritis.

A study by Harris\textsuperscript{22} confirmed that explants from human rheumatoid synovial tissue have been found to release large amounts of collagenase and increased collagenase activity has been more prominent in rheumatoid synovial fluid.

Two different studies in the same year by McCachren\textsuperscript{273} and Gravallese et al\textsuperscript{274} proved MMP-1 and MMP-3 are important in rheumatoid arthritis.
Green *et al*\(^\text{275}\) concluded that the serum levels of MMP-1, MMP-3 and tissue inhibitor of metallo proteinases-1 (TIMP-1) are independent predictors of radiographic damage in terms of total erosion score in rheumatoid arthritis.

Studies by Murphy *et al*\(^\text{276}\) showed that MMP-1, gelatinase A and matrilysin may have a role in the synovitis associated with rheumatoid arthritis.

According to Kevorkian *et al*\(^\text{277}\) baseline serum MMP-1 levels correlate with disease activity and predict functional and radiographic outcome in early untreated RA moreover, serum levels may be particularly helpful when traditional markers are less accurate like the patients with a normal CRP and who have non-erosive disease at presentation.

But two different studies by Ishiguro *et al*\(^\text{278}\) and Manicourt *et al*\(^\text{279}\) failed to show a correlation between MMP-1 levels and the progression of RA in terms of joint damage and systemic inflammation.

Roehr *et al*\(^\text{280}\) proved that the hyaluronic acid is a predominant component of the articular surface and synovial fluid. The tissue destruction in joints causes the accumulation of large quantities of free HA in RA synovial fluid.

According to Laurent and Hallgren\(^\text{281}\) increased levels of serum HA in patients with RA and the levels increased with disease state or stage.

Majeed *et al*\(^\text{282}\) reported that the serum HA level is raised in patients with early RA and correlates with clinical and laboratory measures of
disease activity suggesting a raised HA level early in disease may also predict future joint destruction.

Goldberg et al\textsuperscript{283} showed serum HA is showing a positive correlation with morning stiffness, which is a characteristic symptom of rheumatoid arthritis.

In one of the studies Nagaya et al\textsuperscript{284} found a high hyaluronidases activity in the synovial fluid and serum of RA patients, implying an abundance of small HA molecules in the RA synovium.

High levels of inflammatory cytokines such as IL-1 and TNF-α was observed in the synovial fluid of patients with RA, induce the production of HA from synovial B cells.\textsuperscript{285}

Contrary to this observation another study by Shigemori et al\textsuperscript{286} reported that the hyaluronic acid itself strongly induces the production of IL-1 from synovial cells thereby the increased levels of HA in synovial fluid or serum appear to be specific to inflammation in the synovial tissue.

Andersson and Fasth\textsuperscript{287} observed that the serum HA levels are low in normal subjects regardless of age and measuring methods and based on these findings suggested the increased serum HA level may be specific to inflamed synovial tissue in rheumatoid arthritis.

Although the HA levels are higher in the serum of RA than in healthy persons, the relatively low levels of HA in serum do not prevent antithrombin activity and thus cannot cause blood clots in the circulation by Partsch et al.\textsuperscript{288}
In another study, Xiaotian et al\textsuperscript{149} explained that low levels of HA significantly blocked the ability of antithrombin to inhibit thrombin thereby proving its involvement in the pathogenesis in rheumatoid arthritis.

Confirming the therapeutic role of HA Masuko et al\textsuperscript{141} reported that intra-articular injection of HA has been used in patients with inflammatory arthritis such as RA indicating that HA has beneficial effects on inflammatory arthritis.

Another clinical study by Kobayashi et al\textsuperscript{289} concluded that injecting HA into articular rheumatoid joints can ameliorate inflammation.

1.5 Lacunae in Current Literature

Rheumatoid Arthritis is a chronic disease with a significant impact on the population. It damages the synovium, cartilage and bone of the joints causing pain, impairment, and disability in patients. It is characterized by irreversible joint destruction which can be prevented by intervention at the early stages of the disease; hence early diagnosis of RA is important.

An extensive literature search has revealed that the biochemical markers of the synovium, cartilage and bone have not been pursued on RA patients in India and there is a need for study of new biological markers of rheumatoid arthritis. In particular, biomarkers that would allow reliable diagnosis and monitoring of the early stages of the disease and permit early intervention to potentially prevent pain, joint destruction and long-term disability, are highly desirable.

In the present study it is proposed to measure these markers at the baseline and follow-up after a period of time. This study may indicate
whether these markers contribute to the pathogenesis and progression of disease and the importance of these markers will be studied by comparing with the traditional markers of disease activity.

**Significance of the study**

This study aims to observe whether the biochemical markers (COMP, HA, Osteocalcin, YKL-40 and MMP-1) are contributing to the pathogenesis and progression of the disease in rheumatoid arthritis patients and also to know whether these markers are as useful as the traditional markers of disease activity (RF and anti-CCP) in rheumatoid arthritis patients.

**1.6 OBJECTIVES OF THE STUDY**

1. To study the levels of serum biochemical markers of synovium-Hyaluronic acid, YKL-40 and MMP-1 in Rheumatoid arthritis and normal healthy individuals.

2. To evaluate the levels of cartilage marker-Cartilage Oligomeric Matrix Protein (COMP) and bone formation marker-Osteocalcin in RA and normal healthy individuals.

3. To investigate the markers of disease activity levels (RF, anti-CCP) in RA and normal healthy individuals and correlate them with above mentioned markers.

4. To correlate between the markers of synovium, cartilage and bone and with the conventional markers of disease activity.

5. Follow-up of all these markers after a period of one year in RA patients.