2 Review of Literature

2.1 Anatomy of human joints[34, 35]

Human body is one of the most advanced mechanical structures which are the result of very evolved and complex arrangement of bones and muscles. Locomotion or movement is characteristic features of the living things due to arrangement of joints with the framework called skeletal system. Articulation or joint is a point where bones connects with the skeletal system to provide characteristic movement depending upon the type of joint which gives the certain degree of movement to the specific part of the body. Anatomically joints are composite structures made up of bone enclosed with cartilage, synovium and ligament, this arrangement is necessary to bear weight and to give strength, support and mobility in one or more directions. As a person goes older, with the age joints become weak due to wear and tear and sometimes due to disease conditions. Joints are categorized in three different types according to the motion they allow and bones with which they joined: **Fibrous (immovable), Cartilaginous (semi-movable)** and **Synovial (freely movable)** joints. Synovial joints are most important joints which are the key joints to provide mobility by allowing load-bearing, low-friction, wear-resistant and smooth movement between articulating bone surfaces. There are six groups of synovial joints in our body which are categorized by the opposing bone surface at joints and the types of movement they permit, named as pivot, hinge, saddle, plane, condyloid and ball-and-socket joints. The major joint type and the basic anatomical features of the synovial joints are illustrated in **fig. 2.1**

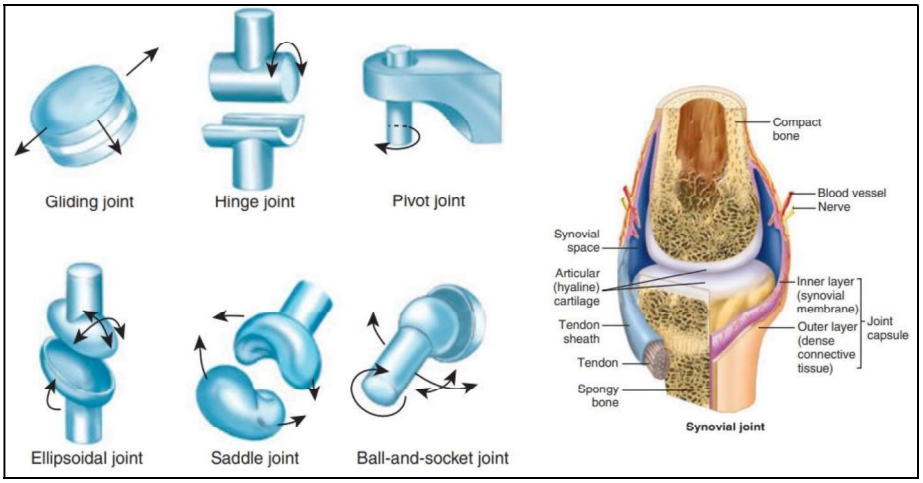


Fig. 2-1 Structure, type and arrangement of synovial joints

2.2 Structural features of human synovial Joints[35]

Synovial joints also denoted as **diarthrosis**, are the most important as well as most common joints of the body. A synovial joint is composite arrangement of **ligaments, muscles, tendons** and **cartilage**. The synovial joints have a synovial cavity which is the characteristic feature of these joints. This cavity has wall around the space formed by the connective tissue structure that is attached to each other just outside the area of bone's articulating surface and joints are also articulated with each other in this cavity. This capsule like cavity is termed as **articular capsule.**

The articulating surfaces of each bone is covered with a thin layer of hyaline cartilage to prevent the friction between bones at the synovial joint, this cartilages are termed as **articular cartilage**. These articular cartilages works as a coating over the individual bone surfaces and they are not continues unlike at a cartilaginous joint, so they are allowing the articulating bones to move smoothly against each other without damaging the underlying bone tissue. A thin membrane covers the lining and the inner surface of the articular capsule known as **synovial membrane**.

This cavity **(articular capsule)** is not hollow, the cells of this membrane secretes a thick, slimy fluid that provides lubrication to further reduce friction between the bones of the joint called, **synovial fluid.** The term synovia stands for "a thick fluid" which provides nourishment to the articular cartilage, as it does not contain blood vessels. This synovial fluid allows bones to move smoothly against each other within the joint cavity, and also gives freedom to joint movement. To give extra support to the synovial joints the articulating surfaces and the bones are connected together with strong fibrous connective bands termed **ligaments**, which prevent excessive or abnormal joint movements by limiting the range of their motions. On the basis of external and internal connectivity with the bone at articular surfaces these ligaments are further classified as **extrinsic ligament and intrinsic ligament**. Sometimes at the point of joint body needs an extra indirect support to give strength and stability to the connective bones, this support is given by the muscles and their **tendons**. These tendons are dense connective tissue structure that attaches a muscle to bone. When the forces acting on joint increases, the body automatically increases the overall strength of contraction of the muscles crossing that joint, thus allowing the muscle and its tendon to serve as a "**dynamic ligament**" to resist forces and support the joint.

Supportive structures of synovial joint

There are some other outer structures which are important to give the supporting structure to the synovial joints. These additional structures prevent the friction between bones of the joint and they are overlying muscles, tendon or skin. Some of the important supportive structures are as follows which play an important role in morphology as well as they can also involve in the disease progression.

- **Articular disc-** In body some synovial joints have fibro cartilage structures, located between the articulating bones some of them are called an **articular disc**, and other small and oval-shaped structures are called **meniscus**, which is larger and has C-shaped structure. One such articular disc is present in sternoclavicular joint (between distal ends of the radius and ulna bones) here these disc provide strong support to the bones to hold together. These discs also serve as shock absorption element and some of the discs contain fat pads to provide cushioning between the bones, like meniscus within the knee joint. At temporomandibular joint an articular disc gives smooth movements between the articulating bones.
- **Bursa**- At the point where skin, ligaments, muscles, or muscle tendons can rub against each other at joints of the body, a thin connective tissue sac filled with lubricating liquid is present to prevent the friction, this sac with fluid is called a bursae. A bursa can be **subcutaneous** (to allows skin to move smoothly over the bone), **prepatellar bursa** (over the kneecap) and **olecranon bursa** (at the tip of the elbow). They can also be present between a muscle and an underlying bone, or between adjacent muscles called as **submuscular bursa** (prevents rubbing of the muscle during movements). Examples include the subacromial bursa that protects the tendon of shoulder muscle as it passes under the acromion of the scapula, and the suprapatellar bursa that separates the tendon of the large anterior thigh muscle from the

  distal femur just above the knee.
- **Tendon sheath –** It has structural similarity with a bursa, but they are smaller in size. It is a connective tissue sac that surrounds a muscle tendon at places where the tendon crosses a joint. It contains a lubricating fluid that allows for smooth motions of the tendon during muscle contraction and joint movements.

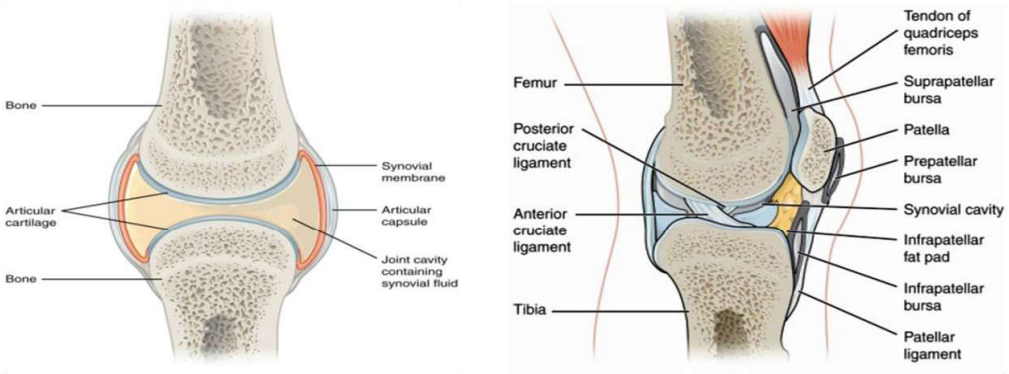


Fig. 2-2: Synovial joints and their arrangement with supportive structures

2.3 Anatomy of Rat joints[34, 36, 37]

In the present study rats were taken as the model animals for induction and experimentation of Rheumatoid Arthritis. The model species must show the similar anatomical and physiological changes occur in humans to mimic the disease for better insight of clinical presentation to support biomedical sciences. Rat and mouse are the frequently used model animals for induction of RA, due to similarities present in the anatomical joint involved in the disease. Although rats are **quadrupedal (Qp)** animals having involvement of all four legs for locomotion and humans have **bipedal (Bp)** locomotion but the studies suggests that both the species have involvement of similar neuronal systems for locomotion and the joints and bones also represents similarities in arrangement and function. The figure depicts the rat skeleton and arrangement of bones.

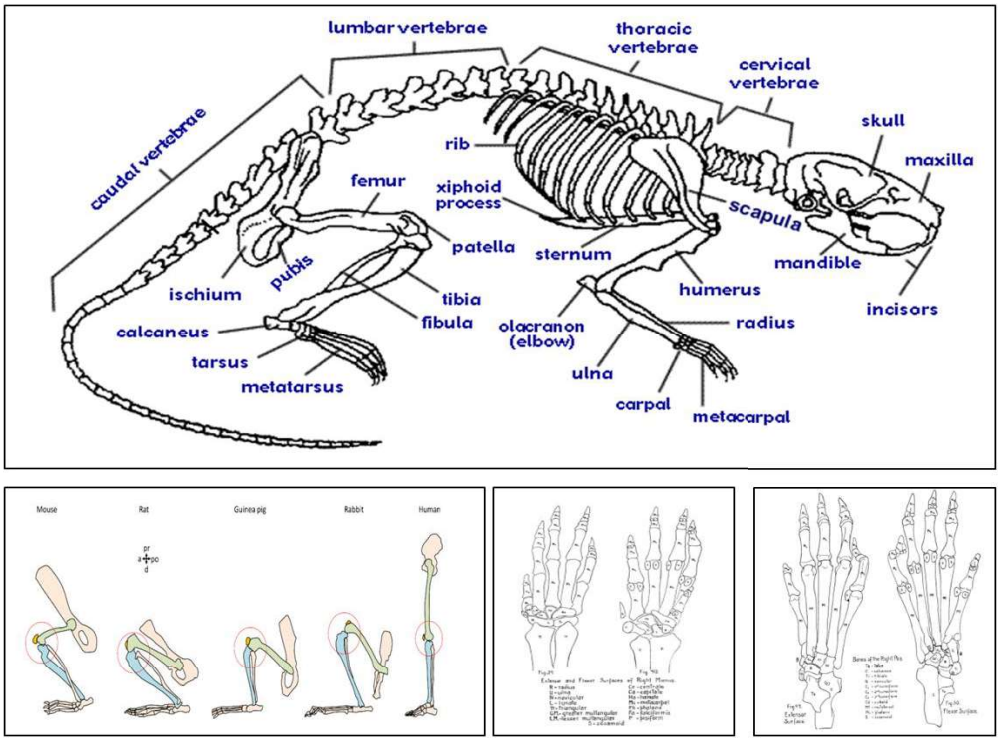


Fig. 2-3**:** Structure, type and arrangement of rat joints

As the present research is oriented for RA, the major joints involved in the pathogenesis of RA were studied broadly on anatomical and morphological aspects. Rats typically weigh 200-400 gm and have a life expectancy of 2-3 years and reach skeletal maturity at 3-10 months. The basic anatomy of rat knee is similar as human in some extent, which contains the tibia, the fibula, patella metatarsal, metapharyngeal joints with synovial cavity and cartilages having synovial fluid with well developed bursa and the nerve system involved in the pathophysiology of RA. Rats have forelimbs and hind limbs which reflects the morphological and anatomical features of human hand and feet respectively. The hind limbs are organ of interest in the selected disease; RA. Hind limb of rats has the tibia, the fibula and the femur which are persisting epiphyseal cartilages in the adult rats. The tibia and fibula are partially fused and the curvature of femoral condyles on posterior end with an intercondylar fossa. The lateral femoral and tibia condyles are larger than the medial one which is a similar factor with human this forms intercondylar area of the rat tibia and creates a distinct depression for weight bearing. **The rat proximal tibial shaft** is also present which lies in anterior convexity and distal part shows a slight posterior convexity which makes lightly S shaped in sagittal plane, here in middle portion a prominent pretibial muscle is present and Proximally, the tibia and the fibula are connected by a joint between a prominent posterolateral bony projection from the lateral tibial condyle and caput fibulae. **In the distal quarter** of the leg the tibia and

the fibula are fused. Further distally, near the talocrural joint, the two bones are again getting separated. A ligament is present here which connects the bony patella across the joint to the tibia. From this ligament an infrapatellar fat body extends into the femoral intercondylar fossa. At the upper border of the bony patella a cartilaginous patella extends proximally, forming the anterior wall of the suprapatellar bursa.

In the quadriceps muscle a tendon like ligament is present which bridges the knee joint with the bone. Rats also have calcified and noncalcified cartilages with a calcified cartilage which is much higher than human. Similarly to other small rodents, rat cartilage has a higher cellular density and high DNA content, which results in better regenerative capacity. The 3D arrangement of collagen fibers is more columnar in rat compared with the more leaf-like arrangement in man. The calcified cartilage zone in rodents also often contains large vacuolated chondrocytes and age related effects on rat joint also observed; rats maintain an open growth plate into old age the rate of growth increases between one and five weeks, then declines until skeletal maturity, which is achieved by 11.5-13 weeks after which bone growth rate is reduced till rat reaches age of 26 weeks after which growth virtually ceases in rats.

The rat knee has well-developed ossified menisci, the lateral of which is largest. Both menisci contain pyramid-shaped ossicles in their horns. The ossicles in the anterior horns are prominent and constantly present. The arrangement of the anterior and posterior cruciate ligaments is similar to that in man. The anterior horns of both menisci are attached to the tibial intercondylar area through ligaments, which cross each other in front of the ACL (Articular cartilage Ligament). Due to these similar anatomical features rats are the desirable models for toxicology testing of pharmaceutical compounds in RA, osteoarthritis, pain and cartilage damage diseases.

2.4 Joints involved in Rheumatoid Arthritis[38, 39]

Rheumatoid Arthritis (RA) is a systemic, chronic inflammatory disease with involvement of self destructive (autoimmune) components that affects principally the joints and sometimes many other organs and tissues throughout the body as well. More specifically the disease is characterized by a nonsupportive proliferative synovitis, which in time leads to the destruction of articular cartilage and progressive disabling arthritis. Inflammation associated with synovial membrane is primary cause for initiation of RA. Synovium contributes to cartilage and bone erosion and uncontrolled inflammatory process leads to deformity of the joints.[40].Most prominent effects of RA are seen in **Proximal interphalangeal (PIP)** and **Metacarpophalangeal (MCP)** joints. It produces symmetric arthritis, which affects

principally the small joints, Metatarsophalangeal (MTP) of hands and feet, ankle, knees, wrists, elbows and shoulders.

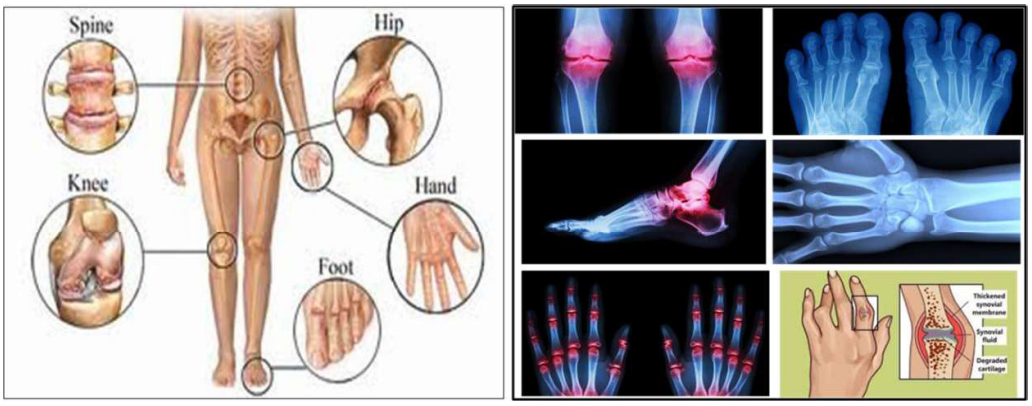


Fig. 2-4 Major joints involved in Rheumatoid Arthritis

2.5 Overview of RA

Rheumatoid arthritis (RA) is chronic systemic autoimmune disease in which body's immune system mistakenly attacks on joints, this causes inflammation that lines the inside of joints (Synovium) to thicken, resulting in swelling and pain in and around joints. Inflammation associated with synovial membrane is primary cause for initiation of RA. Synovium contributes to cartilage and bone erosion and uncontrolled inflammatory process leads to deformity of the joints.

2.5.1 Epidemiology[41]

On epidemiological account RA affects 1% of population worldwide with 0.5% to 1% prevention and in India the RA prevalence has been estimated to be 0.7%. RA can occur at any age, but is most likely to show up between ages 30-50. Disease involves younger people, elderly people and females. Females have two folding risks for the outbreak of illness, but the disease's extra-articular manifestations are more prevalent in men, causing impairment and mortality.

RA is a chronic multisystem disease of unknown cause. Although there is a variety of systemic manifestation, the characteristic feature of established RA is persistent inflammatory synovitis, usually involving peripheral joints in a systemic distribution. The potential of the synovial inflammation to cause cartilage damage and bone erosion and subsequent changes in joint integrity is the hallmark of the disease. Despite its destructive potential, the course of RA can be quite variable. Due to major intervention of auto immune responses, some patients

may experience only a mild oligoarticular illness of brief duration with minimal joint damage but most will have a relentless progressive polyarthritis with marked functional impairment. Manifestations of auto immunity are found in a large number of diseases. To classify a disease auto immune it is necessary to demonstrate that the immune responses to a self antigen causes the observed pathology. Initially the demonstration that antibodies against the affected tissue could be detected in the serum of patient suffering from various diseases was taken as evidence that these diseases had an autoimmune basis.

2.5.2 Causative factors, sign and symptoms of RA[2, 42, 43]

RA is considered as an autoimmune disorder with systemic, chronic inflammation affecting many tissues but principally attacking the joints to produce a nonsupportive synovitis that frequently progress to destroy cartilage and underlying bone with resulting disabling arthritis. Rheumatoid Arthritis results disability with progression and severity of disease in long term onset. Due to involvement of complex pathogenic molecule involvement the disease has different signs and symptoms which get aggravated in different stages of RA progression-

1) More than one painful, tender and swollen joints (high involvement of small joints at early stage).
2) Stiff joints in morning hours with tiredness and fatigue.
3) Symmetric progression of disease (The same symptoms on both sides of the body such as both hands or both knees).
4) At disease progression symptoms of symmetric progression affects wrists, knees, ankles, elbows, hips and shoulders which is major cause of disability.
5) The disease has autoimmune connectivity which also involves the major organ of body with or without physical sign and symptoms of joint involvement and cardiovascular disease progressed in such cases due to biomarker elevation in body.

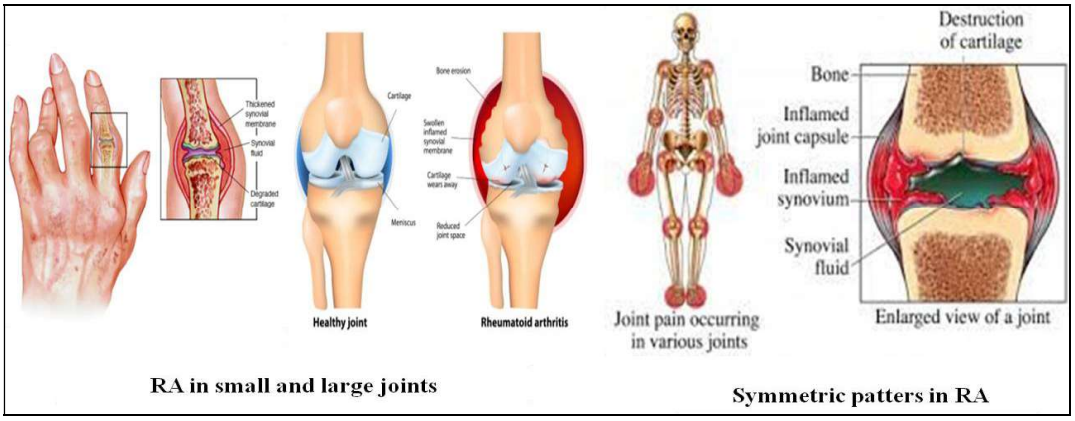


Fig. 2-5 Overview of Rheumatoid Arthritis

2.5.3 Pathogenesis of RA[44-46]

As per the pathogenesis and etiological accounts, Rheumatoid arthritis (RA) is a **chronic inflammatory disease** characterized by **joint swelling, joint tenderness,** and **destruction of synovial joints,** leading to **severe disability** and **premature mortality**.

In present scenario Rheumatoid arthritis is an open field for research, it is considered as an autoimmune disease with unknown etiology and presently the effective drugs with less toxicity have not been established. As several biomarkers involved in the initiation of the disease, Toll like receptors plays a vital role in initiating the cascade of events in the form of activation of Neutrophills and Macrophages which are the first guardian of the innate immune system. These cells have TLR epitopes on their surfaces which are responsible for the activation of the events for primary defense mechanism. On activation of TLR receptors proinflammatory cytokines and activation of T cell B cell and lymphocyte activation is occurs. In result endotoxemia, gut permeability microbial burden and cell infiltration are stimulated which results in impaired Immunity. This process is mediated by an interdependent network of **cytokines, prostanoids, and proteolytic enzymes**. Proinflammatory cytokines such as **interleukin (IL)-1 and tumor necrosis factor-alpha (TNF-α) and NF-κB** are central mediators in RA.

Rheumatoid synovium is characterized by the presence of a number of secreted products of activated lymphocytes macrophages and fibroblast. The local production of these cytokines and chemokine appears to account for many of the pathologic and clinical manifestation of RA. The earliest lesion of the disease generation is increase in lining of synovium due to biological insult of some protective mediators along with perivascular infiltration of mononuclear cells. In clinical intervention before onset of RA only myeloid cells are present,

but as the symptomatic disease progression occurs the presence of T cells confirms the disease onset with edematous synovium and villous projections in synovium cavity with hyperplasia and hypertrophy, micro vascular injury, thrombosis and neovascularization with the associated endothelial dysfunction.

The following Fig. 2.6 is depicted the cascade of pathogenic events in RA due to different factors which stimulates the release of biomarkers and their participation in sequential generation of the stable RA

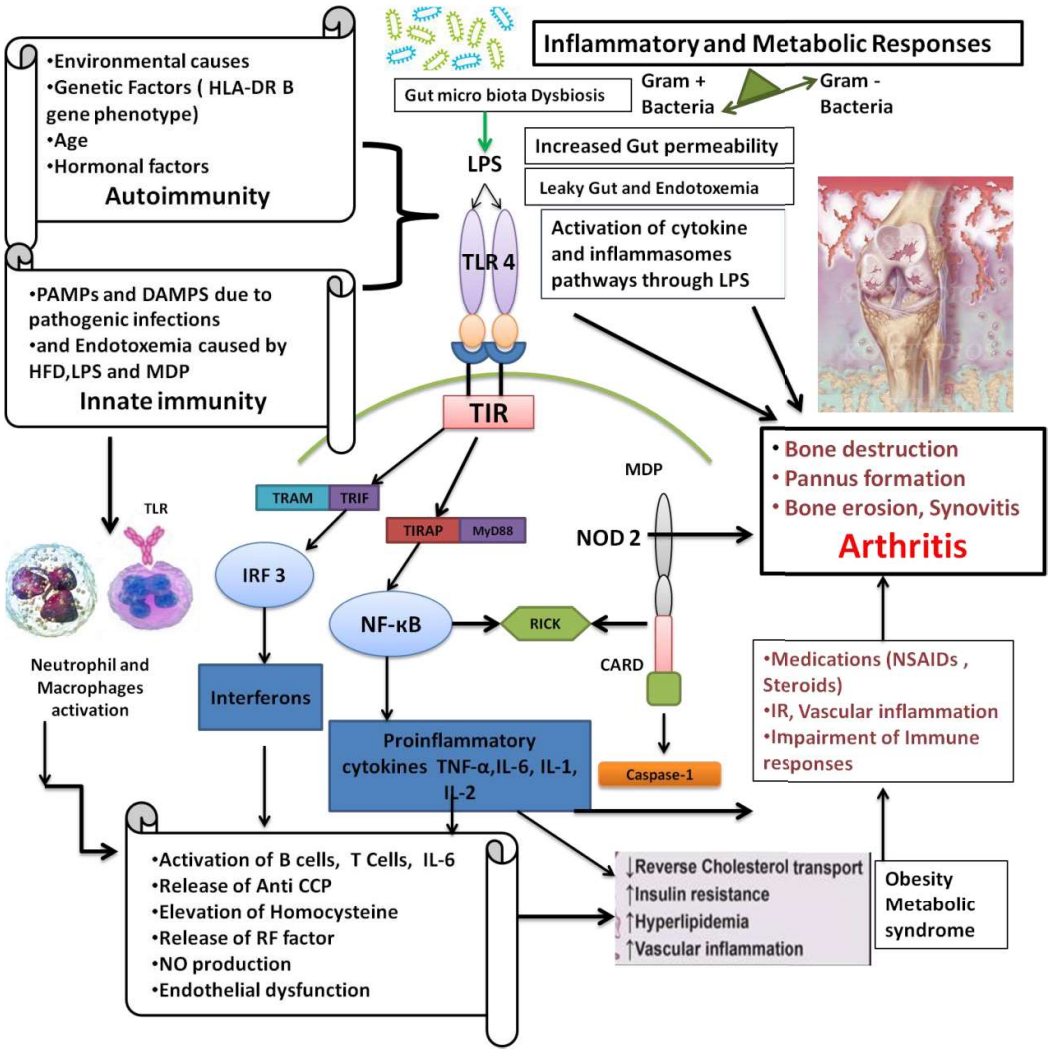


Fig. 2-6 Biomarkers and factors involved in pathogenesis of Rheumatoid Arthritis

2.5.4 Risk factors associated with aetiopathogenesis of RA[10, 47, 48]

Rheumatoid Arthritis is one of the diseases which are having a simple one line description of being chronic inflammatory disorder but it is the most incomprehensible conditions in terms of pathogenesis, marker involved and the person to person variations of the symptoms, severity and the unpredictability of RA in the form of extraarticular manifestations.

RA is connected with metabolic disorders, autoimmune triggers, and inflammatory pathways with genetic modifications as disease involves the complex pathophysiological events with clusters of signalling molecules generated in crosslinking of multiple pathways which categorized RA for having unclear etiology.

RA attributes the two main causative factors for initiation of risk of RA generation-

a) Traditional risk factors- These are the **environmental factors** which can be acquired by the person via natural factors like **aging, diet, surrounding causes, acquired faulty life style** and **habits**. The most prominent risk factors for occurrence of RA are **age, smoking and obesity** which accumulate the different pathogenic attacks in form of **metabolic dysbiosis, low physical activity and the epigenetic changes**.

Fig. 2-7 Environmental risk factors of RA

b) Nontraditional risk factors- These nontraditional factors are generally denoted as **host specific factors** or the factors which **attacks the immune system** of susceptible person by different factors viz; exposure to infectious agents like **Pathogen associates molecular patterns (PAMPs), Damage associated molecular patterns (DAMPs)** which directly

responsible for the immune responses in the form of the activation of the different markers associated with disease and these are the major cause for release of signal modulators. Another major host specific cause is **Genetic factors** in form of specific gene HLA-DRB activation are primary causes of RA aggravation. This gene can also get activated due to smoking and obesity in later phases of life

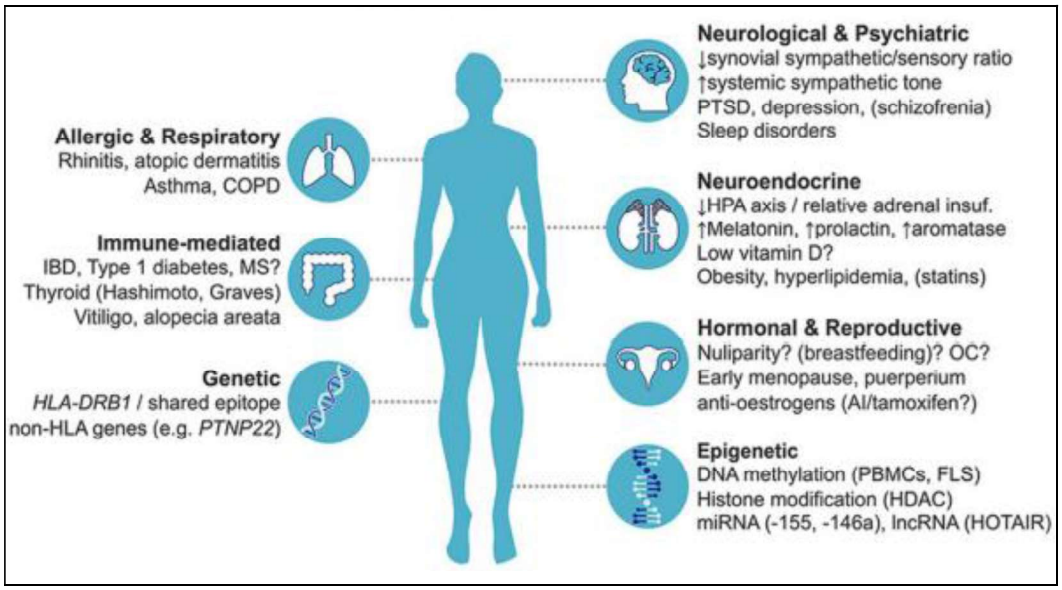


Fig. 2-8 Host specific risk factors of RA

2.5.5 Risk factors and their role in generation of RA

a) Environmental factors

1. Smoking: [49]

Cigarette smoking (CS) is known to be one of the major factors associated with the risk of developing RA. Active as well as passive smoking is one of the strongest risks associated with RA in terms of duration, intensity and exposure. Both the genders are equally affects with this condition but males are at higher side as per the statistics person smokes 1-10 pack/ year have 26% risk of RA development and the person smoking 20 packs are at 94% .

CS can be attributed as environmental factors but it is having genetic effects due to epigenetic changes in pivotal biomarker responsible for progression of RA; ***HLA-DRB-1,*** **PTPN, PDI and ACPA**. Moreover the patients taking DMARDs for treatment of RA also affects due to CS as the response of these therapy is hindered by CS and further joint destruction is occurs.

HLA-DRB-1(human leukocyte antigen) gene known for the major histocompatibility factor associated for susceptibility of RA (person having high expression of *HLA-DRB-1* gene are more prone for onset of RA). CS changes the alleles of *HLA-DRB-1* responsible for ACPA auto antibody production and RF production which are the bench mark of developing RA. These activities are highly expressed in seropositive persons.

Another link of CS and RA is generation of Tetrachlorodibenzo-p-dioxin (TCDD) which binds with aryl hydrocarbon receptor intracellularly associated with NF-κB signaling cascade. The stimulation of the kinases associated with this signalling stimulates and up regulates the cytokine production (IL-1b, IL-6, IL-8) which are the major inflammatory players in RA progression.

CS also contributes activation of PTPN22 via interaction with citrullinated α-enolase which is also one of the main genes responsible for inflammatory as well as autoimmune activation.

The final and the most important role of smoking is in increased production of ACPA.

These findings indicate that lung and other barrier tissue environmental stressors may via posttranslational changes in lung and other tissues to increase production of Peptidyl Arginine Deiminase type IV (PADI4) polymorphism responsible for quantitative or qualitative changes in mucosal protein citrullination involved in pathology of RA.

2. Occupational exposure and pollutants:

Exposure of air pollutant and other occupational exposure is also contributes in associated risk of RA. These pollutants are responsible for chronic inflammation and autoimmune activation which stimulate the production of proinflammatory cytokine, pulmonary inflammation and silica induced adaptive immune insult progress tissue damages cause risk of RA.Such as tobacco smoking these pollutants can also responsible for local lung and systemic inflammation as well as asthma, chronic bronchitis and cardiovascular illnesses.

3. Diet:[50, 51]

As per the general belief and universal law of healthy living we must follow fresh vegetable and fruit rich, low fat and carbohydrate, low sugar, low sodium and high protein dietary habits to maintain the homeostasis of body functions.

Obesity is one of the initiators of metabolic dysbiosis and as per the Ayurveda also RA is considered a metabolic disorder generated via disbalances of *vata* and *pita.* Like all other diseases RA can also be prevented with healthy life style (diet and physical activity). When we look in to the insight of pathophysiology associated with diet and RA, there are different clinical trials were performed (cross sectional and retrospective epidemiological studies) to check the effects of diet on RA progression which suggests that consumption of high sugar

and salt and the high fat rich components are responsible to generate high ROS and which can leads the activation of pro inflammatory cytokines and immune responses.

4. Metabolic dysbiosis:[52-54]

It is one of the newer concepts which show the direct connectivity of our diet in form of alteration of GUT flora (microorganism population responsible for maintenance of GUT health). The alteration of these micro organisms or the food rich in fat, sugar and salt when increase or decrease the colony of these tiny organisms the person will suffer with the condition called SIBO (small Intestinal Bacterial Overgrowth) which triggers the peristalsis movement, mucosal wall damage, pH change and ultimately receptor activation with are responsible for the inflammatory and immune responses to stimulate the disease conditions.

In patients which diagnosed with RA recently showed the alteration of microbial colonies and the presence of *Prevotella copri, Lactobacillus spp.* and *Clostridium spp.* and decrease *in Bacteroides spp.* and *Haemophilus spp.* which was not present in healthy population. This dysbiosis activates the T cells as well as the *Prevotella copri* peptides can sensitize the *HLA-DRB* gene to generate RA-specific Th1 and Th17 responses.

In RA particularly when GUT micro biota increases these secretes LPS which is a component of bacterial cell wall and it is an endotoxine when constant LPS secretion initiates the mucosal cell wall get damaged and endothelial dysfunction occurs which gives an passage to these microorganism to deposit in visceral organs through chemotaxis and they can give a growth bed to already inflamed or damaged cell to increase the productivity of inflammasomes. On the other hand the LPS directly activates the macrophages and neutrophil which initiates the immune responses when this LPS sensitizes the neutrophills this can attached to TLR receptors known for their inflammatory and immune responses via pattern recognition. These TLR receptors activate the MyDD and TRIF pathways which initiates the cascade of events to generate RA.

5. Age: [55]

RA is previously considered as the disease of elderly but the onset of disease can hit between ages 25-45 years. If the onset of disease occurs in 65 or before that the condition is called as early onset of disease which is connected with the severity of symptoms in patients. Generally the patients having early onset of disease are more prone for the extra articular manifestations and co morbid conditions. One of the interesting criteria of early and late onset of disease is that the females shows the late onset of disease in which symptoms may be severe but low chances of co morbid conditions but males are more prone to early onset of disease with co morbidity and mortality.

6. Gender:[56-58]

The most common statement in epidemiology of RA is females are more prone for the risk of RA than males and the ratio is 3:1 which an interesting fact is, but apart from the risk of the onset of RA in females the co morbid conditions are more prominent in males.

There are different data which shows this gender related variables regarding late onset of RA in females, high severity of disease, phenotype and low risk factors of extra organ manifestations.

Women have estrogen hormone as a guardian against the different disease preventions in the early childhood, pubic age and at maturity levels by controlling inflammatory as well as immune responses by controlling production of immunoglobulins. When menopause occurs the estrogen activity is declined which aggravates the sudden changes in body and it is responsible for the thyroid dysfunction, low calcium level and stimulates the rate of infections and inflammatory responses prevented in early ages due to estrogen. At this stage inactivity of estrogen and high rates of ESR present in female system suddenly worsen the inflammatory and immune attacks of RA which represents the higher disease severity and leads the higher incidences of disability.

The other theory is related with dual X chromosome (inheritance gene/sex chromosome) which is responsible for less infectious and longer life span of females. The X chromosome is found to be give prevention against inflammatory responses in both male and female but due to presence of dual X chromosome in female it was earlier hypothesized that women can be prone for both inflammatory and immune responses but studies showed that the different effects of paternal and maternal X chromosome (as females are cellular mosaic and have two cell lines working one of maternal gene and another one of paternal gene). These X chromosomes were not paired and function individually in both male and females and in developmental stage of female fetus one of the X chromosome get inactivated for life time but still they can regulates the genes. Y chromosome contains about 100 genes and it can regulate their function too, but X chromosome contains 1000 genes and regulates them in different manners. They are associated with immune system with regulatory genes CD40L, CXCR, OGT, FOXP3, TLR7, TLR8, IL2RG, BTK, and IL9R which are responsible for higher production of immunoglobulin in women than men. This regulatory system is responsible for auto immune responses in females which are more prone then males as the one X chromosome can regulate the most of inflammatory responses but pair of then

regulates more genes involved in auto immune responses and if a faulty gene is their it can be risk factor for auto immune responses. Another factor which may be responsible for increase in the risk of RA is microchimerism, which is presence of cells or DNA that travelled from fetus to mother during pregnancy. These cells may stay longer in the body of mothers and may cause autoimmunity. On the other side, HLA-DRB gene is more susceptible in females which are responsible for production of auto immune antibodies presenting RA.

7. Miscellaneous Factors:

Infections, Urbanization, low quality life, race, menopausal status and hormone use, oral contraceptive use, body mass index, physical activity, are some of the other factor which is responsible for RA generation. Along with these low body weight at the time of birth of child, smoking done by pregnant women can also put her child for congenital RA risk and short term lactation are other major risk factors for RA in adulthood to such children as well as for mother in its later phase of life. Oral contraceptives can provide protection against RA. Replacement hormone therapy seems to be useful for the activity of RA disease.

b) Genetic factors[49, 59-62]

Genetic factors can play dual role for prevention or for activation of the certain characteristic in body. As per the GWAS study RA is accounted as polygenic disease because risk of development of disease is heredity dependent. In RA which is denoted as auto immune disease, genetic factors influence the condition by 50-60%. The major player in this category is Human Leukocyte Antigen (HLA) which is auto reactive lymphocytes and they are one of the most important parts of central as well as peripheral immune system. In pathogenesis of RA, HLA region present on chromosome 6is responsible due to genes encoding expression of immune responses. These cell surface glycoproteins are categorized as HLA class I, HLA class II and HLA class III. Among which HLA II is expressed on antigen presenting cells (APCs) which includes macrophages, B cells, dendritic cells, T-helper cells and CD4+ cells.

In RA environmental factors affects to genetically susceptible persons to activate the immune responses by alteration of self peptides citrullination which initiates the generation of self destructing autoantibody (ACPA) which expressed in 75% of RA patients and this is the major factor which can distinguish RA with osteoarthritis. The role of ACPA in pathogenesis of RA is due to multiple cross linking with stimulation of neutrophills, Fcγ receptor activation on macrophages, increases TNF- α production, as well as generation of IgM which is responsible for generation of Rheumatoid Factor (RF). These multiple factors when

cumulates they increase the proinflammatory cytokine release and ultimately immune responses.

The ACPA has other multidirectional roles in RA pathogenic molecule sensitization by combination of environmental as well as genetic predisposition ultimately causing epigenetic changes in the genes. Shared epitopes (SEs), protein tyrosine phosphatase non-receptor type 22 (PTPN22), α1-antitrypsin, type I interferons are the genetic changes caused by ACPA. Apart from this epigenetic modifications like DNA methylation, histone acetylation and deacetylation, miRNA expression were also results with ACPA and environmental factors noxious agents, influence of pathogens such as *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans* (Aa) and *Epstein–Barr virus* (EBV).

These interplay of genetic and environmental factors led to the activation of Ca^{2+} and sensitize the catalysis of citrullination of targeted protein via Peptidyl-Arginine-Deiminase (PAD) in granulocytes and macrophages which is also one of the factors for generation of autoimmune responses.

Antigen Presenting Cells (APCs), and generation of PAMPs and DAMPS activates Toll-like receptors (TLRs) to connect inflammation with immunity as extra articular manifestations (specially in liver, kidney and heart).

Other then ACPA, Tyrosine phosphatase gene PTPN22 on chromosome 1, among other genes also affected with the HLA susceptibility in which mutation in this gene results the exchange of amino acids tryptophan with arginine and resulting the loss of function with increased regulation of the signaling of T-cell receptors during thymic choice which allows auto antigens present on T-cells to predisposed autoimmunity. The Fig…. shows the complications and different arms of ACPA in generation of RA.

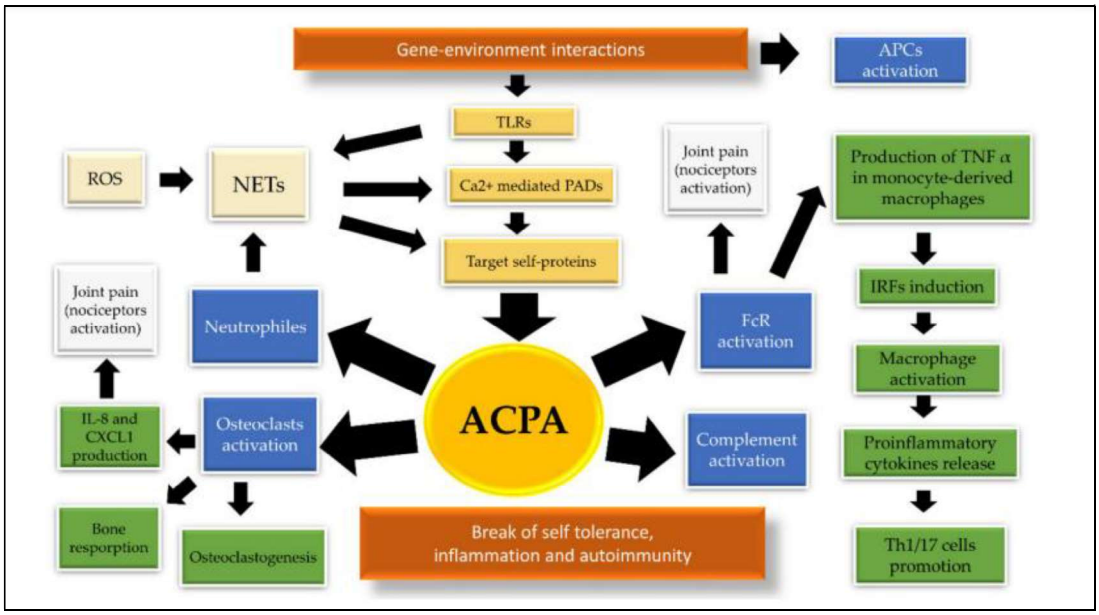


Fig. 2-9 Generation of molecular players by environmental and genetic interactions

2.5.6 Clinical stages of Rheumatoid arthritis[63, 64]

RA is a chronic condition which takes time to evolve as a lifelong disease. Stages in any chronic disease denote the changes and severity in response to various pathophysiological episodes in body and these events are called the stages of a disease. Here in RA clinically the disease is divided in four stages as per the outer inflammatory response as well as inner pathogenic changes. Despite the clinical ease for treating a patient it is important to emphasize that there is no current way to predict the progress from one stage of inflammation to the next and once established each can influence the other important features of this model with the following episodes-

The major pathologic events vary with time in RA which is accounted as chronic disease as time required to progress from one step to the next may vary in different patients and so as the pathogenic events operating the events once established may also persist simultaneously. The process of disease severity is chronic and reiterative, with successive events stimulating progressive amplification of inflammation.

In advanced stages when body defense system activates memory T cells and B cells in response to inflammasomes therapeutic incorporation of anti inflammatory and anti cytokine therapy may be capable of suppressing disease manifestations but not preventing recrudescence of disease activity once therapy is discontinued, with all these available information in etiological complexity of RA the clinical stages are divides as-

i) Stage 1 (Early stage of RA): Inflammation is the primary response in RA due to accumulation of macrophages and mononuclear cells in inner lining of synovial membrane. Activation of macrophages leads a cascade of cytokine (T cell, B cell, CD40 cells, TNF-α and Interleukins) production which further produce autoantibodies (Anti-CCP/ACCP) locally. These events activate a variety of cells in the synovium, bone, and cartilage to produce effector molecules that can cause tissue damage characteristic to **chronic inflammation.** The clinical response of this chain of events results **joint pain, swelling, and stiffness.**

ii) Stage 2 (Moderate stage of RA): The etiology of disease become more severe due to **involvement of autoantibody** responses which leads the nitric oxide production and endothelial dysfunction these sequential progression of cytokine and pro inflammatory mediators worsen the inflammation of the synovial tissue as well as cartilage damage. **Loss of mobility and restricted motion of joints** are the clinical symptoms of this stage.

iii) Stage 3 (Severe RA): Pannus formation is more prone in this stage as cartilage destruction proceeds towards the joint and bone destruction as well as erosion. In this stage clinical indications are **joint deformities and immobility** of joints due to **permanent pain, swelling and stiffness in joints and weakening of supportive muscle strength.**

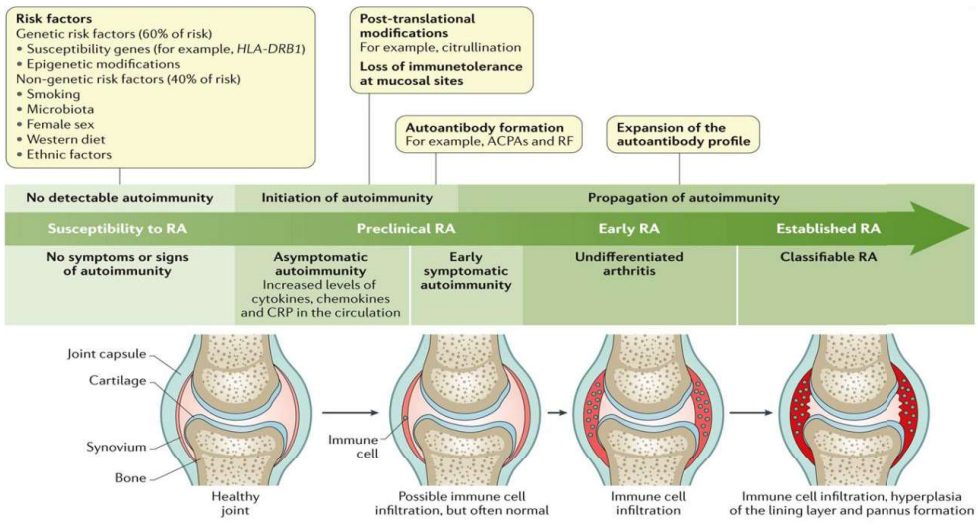


Fig. 2-10 Different stages of Rheumatoid Arthritis

2.5.7 Classification of Rheumatoid Arthritis

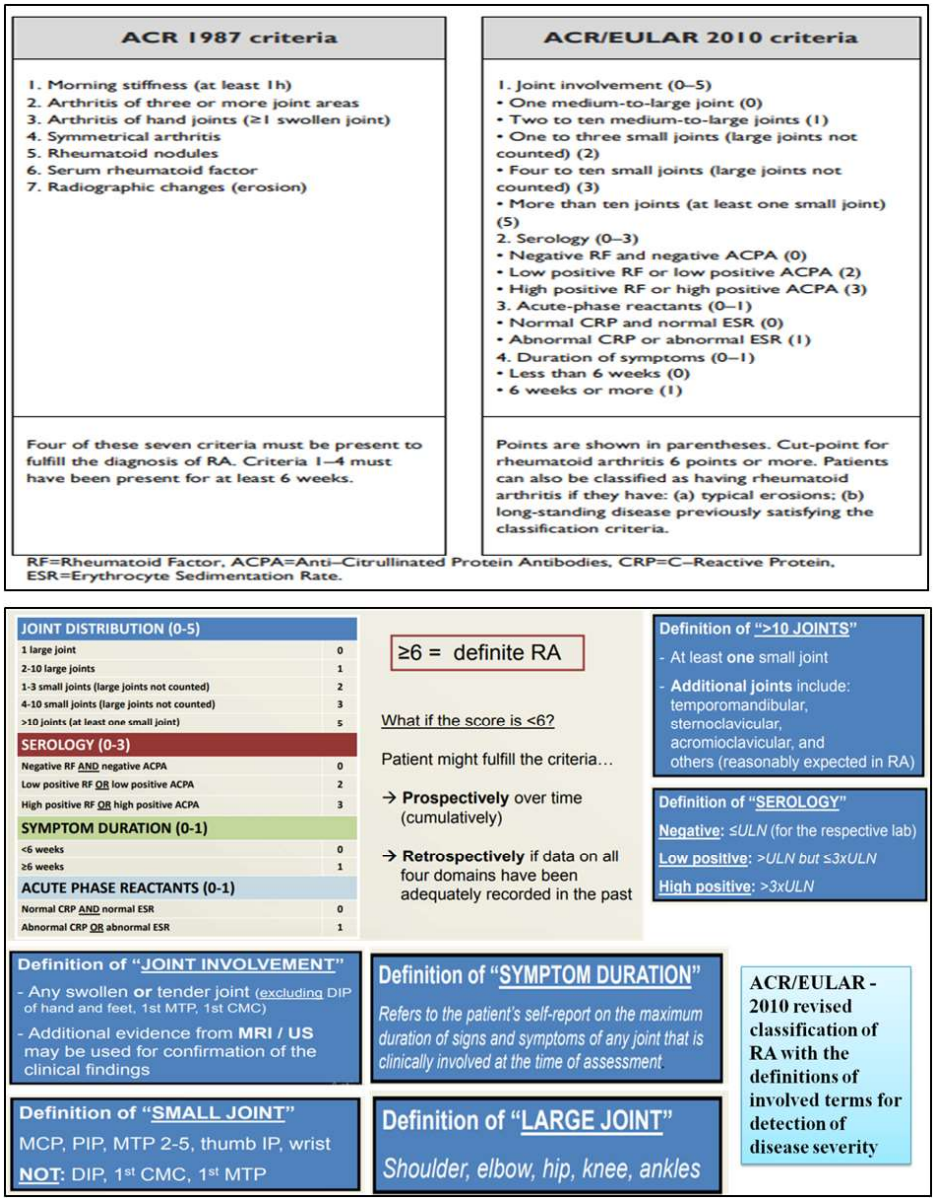
According to American College of Rheumatology (ACR) and European League against Rheumatism (EULAR)[43]

Intervention of autoimmune responses produces autoantibodies, such as Rheumatoid Factor (RF) and Anti–citrullinated protein antibody (ACPA/ ACCP) to precede the different clinical manifestation of RA in very early stages. Crosslinking of innate and adaptive immunity with overall systemic and articular inflammatory load drive the destructive progression of the disease with individualization, which is the basic ambiguity in setting a definite classification for disease. In some patients the genetic factors also involved and due to the unclear etiology for the stages the deformity can occur at any stage of the disease which can cause the impairment.

To set the standard and accepted means of definition the classification criteria were set after performing the clinical trials by two major research bodies in the field of Rheumatism; American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR). These criteria are well accepted as providing the benchmark for disease definition, but have a significant limitation in that they were derived by trying to discriminate patients with established RA from those with a combination of other definite rheumatologic diagnoses.

The goal of these committee was to develop a set of rules to be applied to newly presenting patients with undifferentiated synovitis that can be helpful for –Identification of the subset at high risk of chronicity and erosive damages in RA, these can be used as a basis for initiating disease modifying therapy and to keep the patient on the trek for not exclude the capture of patients later in the disease course as per the inclusive criteria.

The classification criteria was formerly given by the ACR in 1987 which was constituted to indentify the patients with established RA , but due to poor specificity and sensitivity this was not suitable for detection of early onset of RA. These criteria were further revised and updated by ACR/EULAR in year 2010 for better judgment of clinical events and to start the therapy for patients at earliest stage of onset of disease. These committees also provided the treatment guidelines for therapeutic agents developed for treatment in RA for each and every stage on the basis of different clinical trials performed on the basis of classification and treatment goals.



ACR 1987 criteria	ACR/EULAR 2010 criteria
1. Morning stiffness (at least 1h) 2. Arthritis of three or more joint areas 3. Arthritis of hand joints (≥1 swollen joint) 4. Symmetrical arthritis 5. Rheumatoid nodules 6. Serum rheumatoid factor 7. Radiographic changes (erosion)	1. Joint involvement (0–5) • One medium-to-large joint (0) • Two to ten medium-to-large joints (1) • One to three small joints (large joints not counted) (2) • Four to ten small joints (large joints not counted) (3) • More than ten joints (at least one small joint) (5) 2. Serology (0–3) • Negative RF and negative ACPA (0) • Low positive RF or low positive ACPA (2) • High positive RF or high positive ACPA (3) 3. Acute-phase reactants (0–1) • Normal CRP and normal ESR (0) • Abnormal CRP or abnormal ESR (1) 4. Duration of symptoms (0–1) • Less than 6 weeks (0) • 6 weeks or more (1)
Four of these seven criteria must be present to fulfill the diagnosis of RA. Criteria 1–4 must have been present for at least 6 weeks.	Points are shown in parentheses. Cut-point for rheumatoid arthritis 6 points or more. Patients can also be classified as having rheumatoid arthritis if they have: (a) typical erosions; (b) long-standing disease previously satisfying the classification criteria.

RF=Rheumatoid Factor, ACPA=Anti–Citrullinated Protein Antibodies, CRP=C–Reactive Protein, ESR=Erythrocyte Sedimentation Rate.

Fig. 2-11 RA classification criteria given by ACR/ EULAR 1987 and 2010

2.5.8 Pathogenesis of development of associated cardiovascular complications in RA[18, 65]

Rheumatoid Arthritis is an autoimmune disease progressed through crosslinking between inflammatory and immune responses. Being a lifelong disease RA has a major concern of both morbidity and mortality due to development of cardiovascular complications (CVD) in patients having RA with the amalgam of metabolic dysbiosis and pathophysiological drivers affecting immune as well as inflammatory responses.

There are two main reasons of occurrence of CVD in RA: The first being inflammation driven by TLR receptor activation, works as underpinning factor in both the diseases and second one is the side effects and drawbacks of existing therapies. TLRs are trigger factors highly expressed proteins in the synovial lining and sub lining layer of RA synovium and cardiac muscles. TLRs process endotoxemia, infiltrate the cells and stimulates cytokines like **TNF-α, IL-6, IL-8, and NF-ĸB** responsible for parallel damage in synovium and arteries due to endothelial damage and highly expressed T cell and B cells.

Besides the markers generating due to compromised immune system the most commonly prescribed allopathic drugs like NSAIDs, Steroids, Methotrexate (MTX) and Biologics in the form of oral, Injectables and topical preparation are also contributors in the progression of extra articular manifestations. All the set regimens are only systemic approaches to manage the condition and they are not targeting the causality of condition. Moreover RA is considered as protracted disease therefore the medication for management is also to be taken persistently for cure as well as symptom management. Due to such long period of time patients adherence to the therapy is also accountable due to absence of side effects of these medicaments. According to the European League Against Rheumatism (EULAR) and FDA the drug regimen (NSAIDs, DMARDs) therapies are having major side effects like nephrotoxicity, neurotoxicity and CVD complication which causes vascular damage in the form of endothelial damage, neovascularization and inflammation. The following Fig… depicts the all possible markers and pathways associated with cardiovascular complications associated with RA hypothesized in this study for model development.

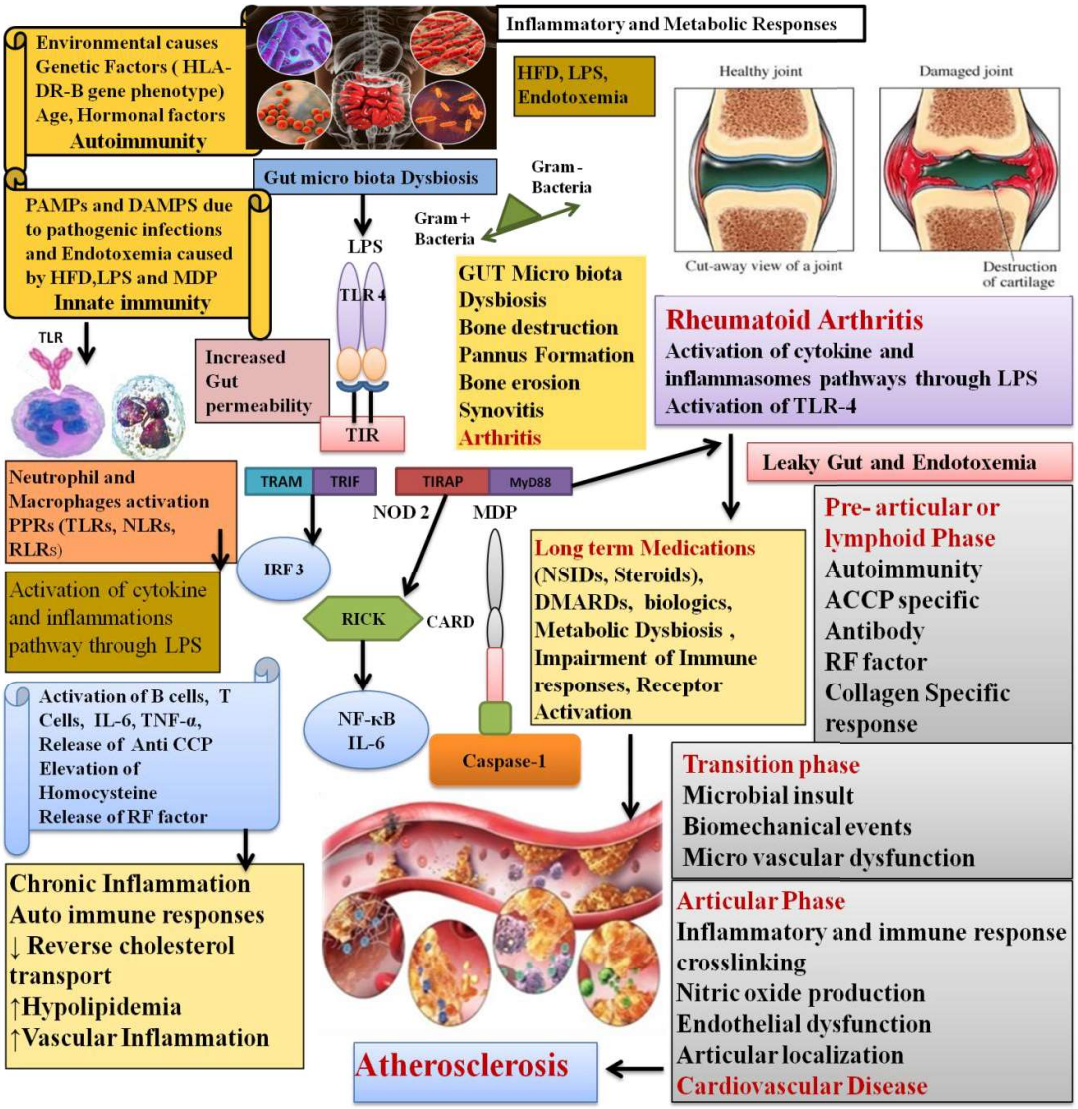


Fig. 2-12 Biomarkers and factors involved in pathogenesis of CVD in RA

2.6 Biomarkers and newer targets involved in pathogenesis of RA

The role of biomarkers in any disease progression has dual role; primarily they are the key initiators for progression of disease and on the other hand these biomarkers can be an important target for management of disease with therapeutic agents. RA has involvement of numerous markers and the possibility for disease management is also high still there are some obstructions in therapy management, which opens the arm for trial of different molecules for treating this disease.

2.6.1 Role of different biomarkers in pathogenesis of RA

The first response in the RA progression is ***"Inflammation which is a protective response intended to eliminate the initial cause of cell injury as well as the necrotic cells and tissues resulting from the original insult".*** Although inflammation helps clear infections and other noxious stimuli and initiates repair, the inflammatory reaction and the subsequent repair process can cause considerable harm.

I) Innate immune cells in RA

Innate immune responses are the primary factors which appear before clinical initiation of inflammatory responses. These cells (macrophages, lymphocytes, monocytes, dendritic cells, and osteoclast) are lineage differential cells involved in pathogenesis of RA.

a) Macrophages/ Monocyte: When pathogens enter the host body, they are phagocytosed by cells of innate immunity which primarily involved macrophages. These macrophages activates the release of chemokine and cytokines, which is a generic term reserved for secretary proteins (glyco proteins) that typically act on neighboring cells and attracts them towards the inflammatory site.

These macrophages and monocytes are prominently present in inflamed synovial membrane and they are responsible for the production and activation of-

Pro inflammatory cytokines such as Interleukins IL-1, IL-6, IL-15, IL-18, IL-23and IL-27 and TNF-α and chemoattactant group of cytokines IFN-γ

These polypeptides are the messenger molecules of immune system produced principally by lymphocytes, macrophages and endothelial cells. Cytokines (*cyto*= cell and *kinesis*=movement) play an important mediator role in inflammation and immune responses.

Cytokines also activates Monocyte activated Chemokine production in inflamed cells (CXCL8, CCL3), RANTES (CCL5) and CX3CL1 with IL-1, TNF-α, IFN-γ. These particular cytokine molecules involved in innate immunity and inflammatory responses. When foreign particles attacks to the body such as microbes or pathogenic molecules, these mediators gives signals to responsible cells for phagocytosis. The *M. Tuberculosis* has capacity to survive under these inflammatory rich environments and the bacterial flora is attracted by the damaged cells in synovial membrane which switches the further cascade of events to generate RA. These cytokines have transcription and post- translational activities and have cell contact dependent or independent signalling depending upon stimuli. When these cytokines stimulates the inflammatory responses in mesenchymal cells fibroblast macrophage interaction elicits the production of IL-6, GM-CSF, CXCL8 and stimulates the cartilage

degradation. Macrophages can also be activated by cell interaction with T cells. In response to this interaction macrophages produce MMPs, IL-1α and IL-1β. Moreover, stimulated T cells produce TNF-α and NK cells, upon cell-contact interaction.

In RA, cytokines like VEGF and IL-6 in synovium are responsible for activation of pro-antigenic factors as core initiators of Pannus formation, bone erosion, and cartilage damage. These cytokines initiates Angiogenesis for blood supply and bone invasion to maintain this Pannus. IL-1b, MMPs and TNF-a are responsible for cartilage degradation via synoviocytes.

These cytokines are also responsible for extra organ manifestations in RA viz; ***CVD complications, Osteoporosis, Fatigue and depression*** due to Acute-phase response (APR) which change the concentration of certain plasma proteins, such as CRP, hepcidin, serum amyloid A, haptoglobin and fibrinogen, following protein synthesis alterations within hepatocytes.

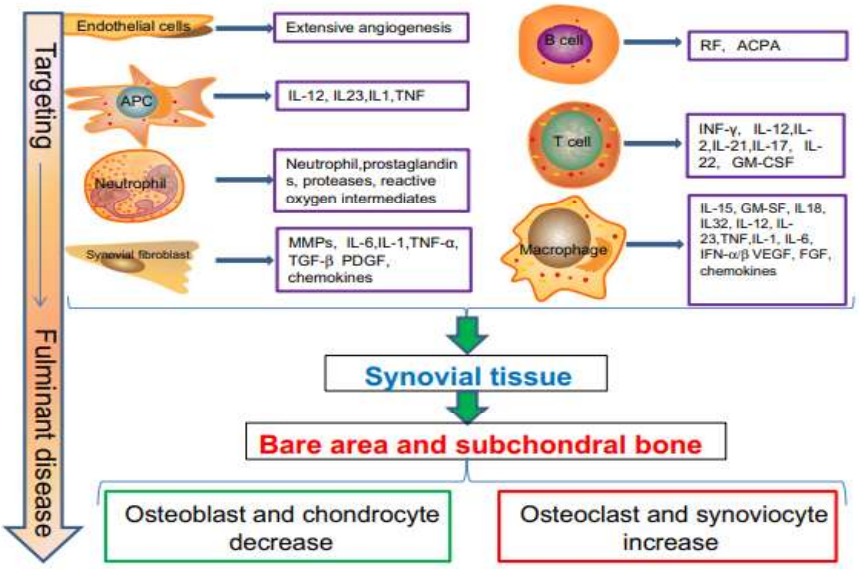


Fig. 2-13 Role of different biomarkers in pathogenesis of RA

b) Dendritic cells: DCs are non phagocytic cells that express high levels of class MHC and T- cell co stimulatory molecules. Mature DCs are present in T cell zone of lymphoid tissues and have the ability to present antigen to T cells, which is a prime factor for development of both the innate and adaptive immune responses. These DCs distinguishes naïve T cells from other antigen-presenting cells (APC) and they are involved in the maintenance of central and peripheral tolerance. They are also essential in the generation of primary antibody response, and are powerful enhancers of NK cell cytotoxicity. Myeloid DC (mDC) and plasmacytoid

(pDC) are the types of DCs which are present in synovial tissues. In RA, they secret pro inflammatory mediators, infiltrate synovial membrane towards antigens to contribute disease perpetuation. And finally they drive the generation of ectopic lymphoid tissue in synovial membrane and activate the autoimmune responses by presenting self-antigens to auto reactive T cells.

c) NK cells: NK cells are the important part of immune and innate immunity and they have role in adaptive immune responses also. These cells have dual action of protective as well as phagocytic responses. CD cells are different subsets of NK cells and these have capacity to secrete pro inflammatory cytokines with the interaction of different cells in synovial membrane responsible for pathogenesis of RA. NK cells can stimulate or suppress DCs and they secrete co stimulatory signals to T and B cells.

d) Osteoclast: These are the fusion cells of macrophages or monocytes participating in bone resorbing and remodeling. In development of RA macrophages undergo osteoclast differentiation by appropriate signalling provided by T cells and Fibroblast type synoviocytes cells (FLS). These FLS also activates Nuclear Factor NFκB ligand (RANKL) that drives osteoclast formation and T cells produce IL-17 which is an important cytokine in osteoclastogenesis. Pro-inflammatory cytokines such as TNF-α, IL-6, IL-17 and IL-1, which are abundant in RA synovial membrane, regulate RANKL expression driving osteoclast formation which is linked to bone damage in RA in the form of bone erosion at early stages of RA.

II) Adaptive immune cells in RA

Adaptive immune responses when merge with innate immune responses they generate autoimmune incidences. The genetic factors associated with RA are due to these autoantibody productions and the current focus of the RA management is also intended to mange T cell and B cells activated in RA as an adaptive immune response.

a) T cell Activation: Pathogenesis of RA has a strong evidence of incorporation of T cells generated in thymus via activation of $CD4^+$ cells. *HLA-DRB* gene has strong association by shared epitopes to present the antigens to activate PTPN22, CTLA4, CD40 and CD28 cells which are the key components of pathogenesis of RA. When *HLA-DRB* activated via environmental stimuli (smoking, PAMP, DAMP) the genetic predisposition occurs. T cells are responsible for activation of cytokine release as well as activation of T helper cells (Th-cells) in synovium via IFN-γ, IL-2, IL-12, IL-18, TNF-α and GM-CSF activation.

b) B cell activation: B cells developed in bone marrow and mature in spleen. When there is a pathogenic conditions in body due to immunological insult the B cells stops developing in immature stage and these transitional cells activate the T cells, cytokines, Igs CD cells and Th cells. B cells are source of relevant autoantibody production, they are abundant in synovial membrane and they can stimulate the T cells via APC in the synovial cavity and joints which makes them a major player in RA pathogenesis.

III) Auto antibodies in RA

RA is an autoimmune disease which has involvement of auto antibody generation due to altered body defense system. Rheumatoid Factor (RF) and Anti- CCP are the most prominent autoantibodies generated in RA and they have significant role in pathogenesis of RA.

a) ACPA/Anti-CCP activation: Anti-Citrullinated Protein Antibodies (ACPAs) are the auto antibodies generated in RA due to autoimmune response. In inflammatory conditions the process of citrullination is activated, which is conversion of arginine in to citrulline in presence of calcium-dependent enzyme Peptidyl Arginine-Deiminase (PAD). When this process occurs and due to post transcription modifications or enzyme alteration the shapes of these citrulline get changes which present their self as antigens and generates immune responses. ACPA is major diagnostic marker in RA and it is present in 60% cases of RA patients.

The association of ACPA and HLA-DRB is also important in diagnosis of RA in patients. There are two conditions of ACPA tests; Seropositive and Seronegative which is dependent upon the genetic expression of *HLA-DRB* gene and the metabolic dysbiosis which causes the epigenetic changes in the body. Gut micro biota alteration is also one of the reasons to activate this citrullination process associated with ACPA production.

b) Rheumatoid Factor: Rheumatoid Factor is also an autoantibody which is directed against IgG molecules and it has different role on different fractions of Fc molecules. IgE, IgM, IgA and IgG are the major isotypes where it binds and stimulates the immune responses. RF is also accounted as one of the major factors for diagnosis of Rheumatoid arthritis but the presence or absence of RF factor (Seropositive/ Seronegative) is dependent on expression of *HLA-DRB* gene. The stable RA patients can also show the negative RF values despite of disease in last stages if the *HLA-DRB* gene is not expressed in such patients and some patients which do not have RA can also show the presence of RF factor in their blood samples. The confirmation of onset of disease or the disease severity is dependent on the expression of ACPA in such patients.

c) GUT micro biota alteration (Leaky GUT): The concept of leaky GUT syndrome is associated with autoimmune response of body. The generation as well as reversal of autoimmune responses is dependent on the damage and healing of GUT lining. When intestinal lining get damaged the space between gut wall cell is increases and these enlarged spaces allows the protein molecules to absorb before their complete breakdown. These partially absorbed or unabsorbed proteins were sensed as antigen for defense system and in response to which immune system produces antibodies with the help of proteins derived from previous harmless food and human tissues have APCs which mimics some parasites, bacteria and fungi. These antibodies formed against leaky gut can incorporate in different tissues of body and they can initiates inflammatory responses when this similar food is consumed again. This chain of autoantibody production is persisting and the inflammation become chronic. If this inflammation occurs in joint tissues it can develop autoimmune arthritis if this attacks brain chronic fatigue syndrome (myalgic encephalomyelitis) can be occurs and if it targets blood vessels vasculitis is results as an autoimmune response.

d)Toll like receptor activation: TLR receptors link inflammation with Immunogenicity via metabolic dysbiosis .TLRs; the prime factors and highly expressed proteins in the synovial lining and sub lining layer of RA synovium and cardiac muscles.[66] TLRs process endotoxemia, infiltrate the cells with leaky gut and stimulates cytokines like TNF-α, IL-6, IL-8, and NF-ĸB responsible for pathogenesis.[13] If diet rich in high fat content is consumed by person the microbial flora secrete Lipopolysachcharide (LPS) which is an endotoxine which is known for activation of cytokine release in *in-vitro* and *in-vivo* conditions. The association of these LPS molecules with pattern recognition receptors (TLR and NLRP) is proven in previous studies. LPS promotes the TLR-4 binding with its co receptor MD-2 on epitopes present on defense cells (especially on macrophages) and initiates two major intracellular signaling pathways, MyD88-dependent and TRIF-dependent (MyD88-independent). The main role of the MyD88-dependent pathway (downstream of TLR4 is to induce the expression of inflammatory cytokines such as IL-6, IL-12, and TNFα, whereas the main role of the TRIF-dependent pathway is to induce up regulation of co stimulatory molecules and the expression of type I IFNs These two pathways seem to cooperate to maximize the expression of inflammatory cytokines.

2.7 Clinical diagnosis and treatment approaches for RA

As per clinical prospects Inflammation in synovial membrane of metacarpophalangeal (small joints of the fingers and feet) is major causative factor for initiation of RA. Pain, swelling,

loss of function and a morning stiffness (lasting more than one hour) are common complains of patients suffering with disease. Fatigue, malaise, weight loss, fever and depression are other constitutional symptoms which precede the onset of RA. In primary stage disease is diagnosed by ultrasound and MRI (magnetic resonance imagery) for detection of inflammation markers in the joints and the surrounding structures; radiography and MRI to detect complications in cartilage and bones. As disease progression occur hematological markers such as Anti- CCP (ACCP), RF- factor, ESR and C-RP becomes major criteria for diagnosis and interventions. RA differs clearly from person to person due to auto immunity and presence of genetic variants. The basic problem in the long term diseases like Rheumatoid is the continuation of the same therapy over the period of time. Being as an auto immune disorder there are some problems in the current treatment therapy in arthritis.

2.7.1 Sign and symptoms of RA and associated co morbid conditions

The disease has different signs and symptoms which get aggravated in different stages of disease progression-

- More than one painful, tender and swollen joints (high involvement of small joints at early stage).
- Stiff joints in morning hours with tiredness and fatigue.
- Symmetric progression of disease (The same symptoms on both sides of the body such as both hands or both knees).
- At disease progression symptoms of symmetric progression affects wrists, knees, ankles, elbows, hips and shoulders which is major cause of disability.
- The disease has autoimmune connectivity which also involves the major organ of body with or without physical sign and symptoms of joint involvement and cardiovascular disease progressed in such cases due to biomarker elevation in body.

2.7.2 Treatment algorithm in RA

To overcome increased evidences of progression of disease different organization and committees in the field of Rheumatology working toward the newer management options with the conventional therapeutic regime including NSAIDs (Ibuprofen, COX inhibitors), Steroidal regimen (glucocorticoids) and most established DMARDs (Methotrexate, and Biologics). ACR guidelines provide management of RA on the basis of onset, symptoms and stage of the disease. The algorithm is initiated with the general treatment of NSAIDs for management of pain and stiffness and the different stages where DMARDs can be added with

the existing therapy. In advances levels different newer approaches also be added according to patient need. Following are the markers which are targeted with the treatment options for management of RA.

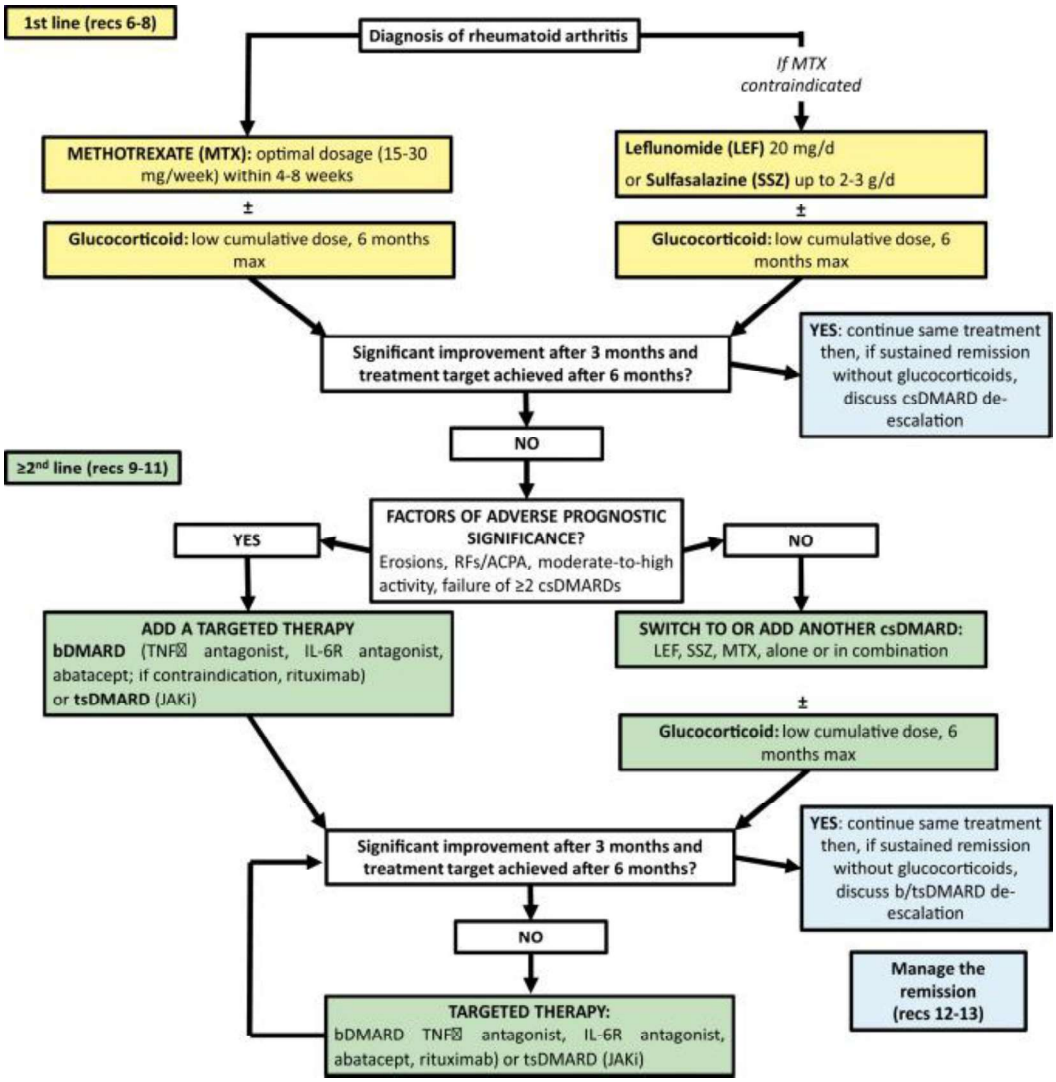


Fig. 2-14 Treatment algorithm in Rheumatoid Arthritis

2.7.3 Newer approaches in RA therapy

The RA being autoimmune disease have complex pathophysiological biomarkers. Involvement of the other organs; Lung, renal heart and skin are the major contributor in worsen the conditions of the RA patients. The prevalence of cardiovascular complications (CVD) in patients suffering from Rheumatoid arthritis (RA) is one of the major concerns

today which is associated with both morbidity and mortality because according to the European League Against Rheumatism (EULAR) and FDA the drug regimen (NSAIDs, DMARDs) of RA also contributes in progression of CVD. Endotoxemia, gut permeability, microbial burden and cell infiltration are stimulated due to impaired Immunity. Proinflammatory cytokines such as interleukin (IL-1, IL-6) and tumor necrosis factor-alpha (TNF-α) and NF-κB are central mediators in RA. The research in this line is objected to block these biomarkers to stop the pathogenesis of RA. The following table summarized the therapeutic candidates for management of RA.

Table 2-1Therapeutic candidates for management of RA

Classification	Drug	Mechanism of action	Potential Mechanism	Side effects
1. Conventional synthetic DMARDs				
Methotraxate	Analog of folic acid	Folate-dependent processes, Adenosine signaling; Methyl-donor production; Reactive oxygen species; Adhesion-molecule expression; Cytokine profiles Eicosanoids and MMPs		Increased liver enzymes, pulmonary damage.
Leflunomide/ Teriflunomide	Pyrimidine synthesis inhibitor	DHODH-dependent pathway; Leukocyte adhesion; Rapidly dividing cells; NF-kB; Kinases; Interleukins; TGF-β		Hypertension, diarrhea and nausea, hepatotoxicity
Sulfasalazine	Anti-inflammatory and immunosuppression	Cyclooxygenase and PGE2; Leukotriene production and chemotaxis; Inflammatory cytokines (IL-1, IL-6, TNF-α); Adenosine signaling; NF-kB activation.		Gastrointestinal, central nervous system, and hematologic adverse effect.
Chloroquine /Hydroxychloroquine	Immunomodulatory effect	Toll-like receptors, Lysosomotropic action Monocyte-derived proinflammatory cytokines Antiinflammatory effects Cellular immune reactions T cell responses, Neutrophils; Cartilage metabolism and degradation		Gastrointestinal tract, skin, central nervous system adverse effect and retinal toxicity.
2. Biological DMARDs (Antibody-based therapies)				
TNF-α targeted therapy	Infliximab Adalimumab Etanercept Golimumab Certolizumab pegol	TNF-α inhibitor	Phagocytosis and pro-inflammatory cytokines; Chemoattractant; Adhesion molecules and chemokines; Treg cell function; Function of osteoclasts, leukocytes, endothelial and synovial fibroblasts.	Infection (pneumonia and atypical tuberculosis) injection-site reaction. Hypertension Severe /anaphylactoid transfusion reaction.
B-cell targeted therapy				
Rituximab Ofatumumab Belimumab Atacicept Tabalumab	B cell depleting Inhibitors of B cell function	Fc receptor gamma-mediated antibody-dependent cytotoxicity and phagocytosis; Complementmediated cell lysis; antigen presentation; B cell apoptosis; Depletion of CD4+ T cells		Infection, hypertension, hypogammaglobulinemia,viral reactivation, vaccination responses Late-onset neutropenia Belimumab Inhibitors of B cell function Severe/anaphylactoid transfusion reaction
T-cell targeted therapy				
Abatacept Belatacept	CD28/CTLA4 system,	Auto antigen recognition; Immune cell infiltration; T cells activation.		Infection, malignancy.

	CD80/CD86		
Interleukin targeted Tocilizumab Anakinra Canakinumab Rilonacept Secukinumab Ixekizumab	IL-6 inhibition IL-1 inhibition IL-17 inhibition	Innate and the adaptive immune system perturbation; Acute-phase proteins Inflammatory responses; Matrixenzyme Mitochondrial function; Autophagosome formation.	Soft tissue and skin infections ,increase in serum cholesterol, decreases in neutrophil count, abnormal liver function,neutropenia, malignancy,nasopharyngitis, candidiasis, neutropenia,
Growth and differentiation factors			
Denosumab Mavrilimumab	RANKL inhibitor GM-CSF inhibitor	Maturation and activation of osteoclast Activation, differentiation, and survival of macrophages, dendritic cells, and neutrophils; T helper 1/17 cell; modulation of pain pathways.	Calcium and phosphate depletion, muscle cramps, cellulitis, and numbness
Small molecules JAK pathway			
Tofacitinib Baricitinib Filgotinib	JAK1 and JAK3 inhibitor JAK1 and JAK2 inhibitor JAK1 inhibitor	T-cell activation, pro-inflammatory cytokine production, synovial inflammation, and structural joint damage.	Zoster infection (advice is to vaccinate beforehand) and other potential side-effects should be monitored carefully through further study
Future drug and target			
Toll like receptors pathway	Bruton's tyrosine kinase,Transforming growth factor-beta	Phosphoinositide-3-kinase, Neuropathways, Dendritic cell	Not proven yet

These newer approaches are utilized to add QOL in RA patients with the advancement in therapeutic regimen. Fig: depicts the suitable target and their action on the markers responsible for the pathogenesis of RA.

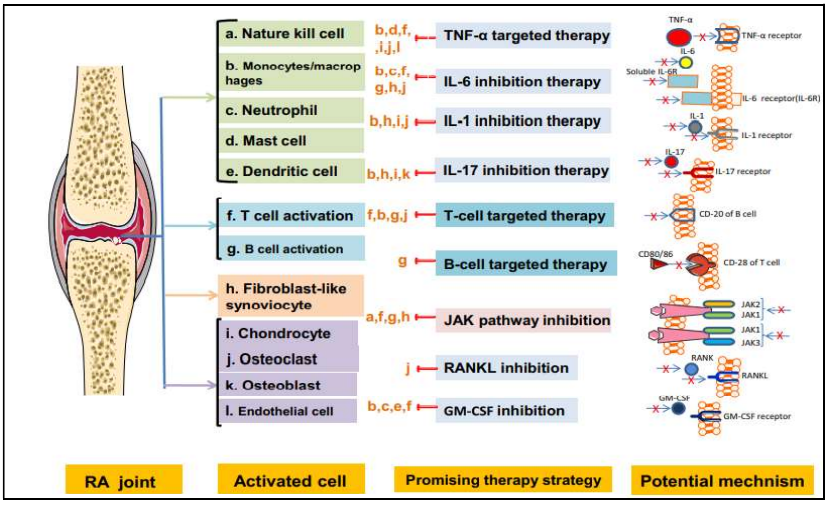


Fig. 2-15 Targets for newer therapy in RA

2.8 Animal models for Rheumatoid Arthritis [67-70]

Rheumatoid arthritis is a long-term, progressive, disabling autoimmune disease and the third leading cause of disability among chronic diseases associated with the mortality and co morbidity in all age groups RA can be attributed to numerous factors viz; complex involvement of metabolic, inflammatory and immune factors. Different models; Carrageen induced paw edema, Adjuvant induced RA, Priston induced RA, and collagen induced RA were proposed by different researchers to give insight into inflammation and progression of disease and for search of newer treatment options. These *in- vivo* models developed with different inducing agents which have different mode of actions for development of RA in rat and mouse.

In-vitro and genetically modified models are also available for induction of RA but they were not incorporated in this section as this study is designed to develop RA and associated complications in wistar rats for designing a better treatment option using select Neutraceuticals. Following Table give a brief account for the different available models for Rheumatoid Arthritis and their pathway of disease generation with similarities with human disease pathogenesis.

Table 2-2 *In-vivo* rat and mice models for Rheumatoid Arthritis with different inducing agents and their comparison with human disease resemblance

Sr. no.	Model	Similarities to human disease	Differences from human disease
1.	Adjuvant-induced arthritis (AIA) in rats	Symmetrical joint involvement, peripheral joints affected, persistent joint inflammation, synovial hyperplasia, inflammatory cell infiltration, marginal erosions, genetically regulated by MHC and non-MHC genes, responsive to most therapies effective in RA	Rapid explosive onset of highly erosive polyarthritis, monophasic course, involvement of axial skeleton, no Rheumatoid Factor, gastrointestinal, genitourinary tract and skin affected; periostitis, bony ankylosis and extra-articular manifestations not typical of RA
2.	Pristane-induced arthritis (PIA) in rats	Symmetrical joint involvement, peripheral joints affected, relapsing joint inflammation, synovial hyperplasia, inflammatory cell infiltration, marginal erosions, presence of Rheumatoid Factor, genetically regulated by MHC and non-MHC genes	Mild to moderately severe polyarthritis
3.	Pristane-induced arthritis (PIA) in mice	persistent joint inflammation, chronic progression of disease, synovial hyperplasia, inflammatory cell infiltration, marginal erosions, presence of Rheumatoid Factor, genetically regulated by MHC and non-MHC genes, Symmetrical joint involvement, peripheral joints affected	Mild polyarthritis with late onset of clinical disease

4.	Proteoglycan-induced arthritis in mice	persistent joint inflammation, synovial hyperplasia, inflammatory cell infiltration, marginal erosions, presence of Rheumatoid Factor and circulating autoantibodies to collagen type II, deposition of immune complexes, genetically regulated by MHC and non-MHC genes	Development of spondylitis
5.	Streptococcal cell wall-induced arthritis in rats	Symmetrical joint involvement, peripheral joints affected persistent and relapsing joint inflammation, synovial hyperplasia, inflammatory cell infiltration, marginal erosions, greater disease susceptibility in females, genetically regulated by MHC and non-MHC genes	No Rheumatoid Factor
6.	Oil-induced arthritis (OIA) in rats	Symmetrical joint involvement, peripheral joints affected mild persisting joint inflammation, synovial hyperplasia, inflammatory cell infiltration, marginal erosions, genetically regulated by MHC and non-MHC genes	Mild polyarthritis, absence of Rheumatoid Factor
7.	Avridine-induced arthritis in rats	Symmetrical joint involvement, peripheral joints affected, persistent joint inflammation, synovial hyperplasia, inflammatory cell infiltration, marginal erosions, greater disease incidence in females, genetically regulated by MHC and non-MHC genes	Moderate severity, monophasic, polyarthritis
8.	COMP-induced arthritis in rats	Symmetrical joint involvement, peripheral joints affected, persistent joint inflammation, immune responses to proteins in the cartilage, genetically regulated by MHC and non-MHC genes	No permanent destruction of joints, transient disease
9.	Collagen-induced arthritis (CIA) in rats	Symmetrical joint involvement, peripheral joints affected, Anti-collagen responses not present in many cases of RA persistent joint inflammation, synovial hyperplasia, inflammatory cell infiltration, marginal erosions, presence of Rheumatoid Factor and anti-collagen antibodies, genetically regulated by MHC and non-MHC genes, responsive to most therapies effective in RA	Anti-collagen responses not present in many cases of RA
10.	Collagen-induced arthritis (CIA) in mice	Symmetrical joint involvement, peripheral joints affected , persistent joint inflammation, synovial hyperplasia, inflammatory cell infiltration, marginal erosions, remission of joint inflammation during pregnancy but flaring up in the postpartum period, genetically regulated by MHC and non-MHC genes, responsive to most therapies effective in RA except NSAIDs	Greater incidence and severity in males, poor responses to NSAIDs

2.9 Need to develop a new model for RA and co-morbidity in RA

Rheumatoid arthritis is considered as an autoimmune disease with unknown etiology and presently the effective drugs with less toxicity have not been established. Animal models are small instrumentation for assessment and estimation of the etiology of RA involving synovial cell proliferation and fibrosis, Pannus formation, and cartilage and bone erosion[71, 72].This study is to establish a new model for Arthritis using Wistar rat as an experimental animal. The animal model developed hither to deals only with Rheumatoid Arthritis but in the proposed hypothesis this model has drive the replica of the CVD progression in RA patient. The basic inducing agent for the progression of RA are CFA and Collagen but in the later part of the experiment, animals were sensitized with high fat diet and LPS which are the key components for CVD disease progression through the toll like receptor activation. LPS is an

endotoxine obtained from the cell wall component of gram negative bacteria and it is an agonist of TLR 4 receptor[73]. As per the literature survey when the Toll like receptors are sensitized to the animal after four week diet modification (HFD) endotoxemia, gut permeability, microbial burden and cell infiltration occurs which results in impaired Immunity, obesity, Insulin resistance and hypertension, the common factors of leading cardiovascular complications[15, 74].This process is mediated by an inter dependent network of cytokines, prostanoids, and proteolytic enzymes. Proinflammatory cytokines such as interleukin (IL-1) and tumor necrosis factor-alpha (TNF-α) are central mediators in RA.