COMPARISON FOR DIAGNOSTIC UTILITY OF ANTI CYCLIC CISTRULLINATED ANTIBODY, ANTI KERATIN ANTIBODIES AND RHEUMATOID FACTOR IN RHEUMATOID ARTHRITIS PATIENTS IN A TERTIARY CARE HOSPITAL

DISSERTATION SUBMITTED TO
In partial fulfillment of the requirement for the degree of
DOCTOR OF MEDICINE IN MICROBIOLOGY
(Chair IV) M. D. (MICROBIOLOGY)
of

THE TAMIL NADU DR. M. G. R MEDICAL UNIVERSITY
CHENNAI- 600032

DEPARTMENT OF MICROBIOLOGY
TIRUNELVELI MEDICAL COLLEGE
TIRUNELVELI- 11

MAY 2018
BONAFIDE CERTIFICATE

This is to certify that the dissertation entitled “COMPARISON FOR DIAGNOSTIC UTILITY OF ANTI CYCLIC CITRULLINATED ANTIBODY, ANTI KERATIN ANTIBODIES AND RHEUMATOID FACTOR IN RHEUMATOID ARTHRITIS PATIENTS IN A TERTIARY CARE HOSPITAL ” submitted by Dr. AMBUJA SEKHAR to the Tamilnadu Dr. M.G.R Medical University, Chennai, in partial fulfillment of the requirement for the award of M.D. Degree Branch – IV (Microbiology) is a bonafide research work carried out by her under direct supervision & guidance.

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This is to certify that the Dissertation “Comparison For Diagnostic Utility Of Anti Cyclic Citrullinated Antibody, Anti Keratin Antibodies And Rheumatoid Factor In Rheumatoid Arthritis Patients In A Tertiary Care Hospital” presented herein by Dr. Ambuja Sekhar is an original work done in the Department of Microbiology, Tirunelveli Medical College Hospital, Tirunelveli for the award of Degree of M.D. (Branch IV) Microbiology under my guidance and supervision during the academic period of 2015-2018.

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DECLARATION

I, Dr. AMBUJA SEKHAR declare that, I carried out this work on “Comparison For Diagnostic Utility Of Anti Cyclic Citrullinated Antibody, Anti Keratin Antibodies And Rheumatoid Factor In Rheumatoid Arthritis Patients In A Tertiary Care Hospital” at the Department of Microbiology, Tirunelveli Medical College, I also declare that this bonafide work or a part of this work was not submitted by me or any others for any award, degree, or diploma to any other University, Board, either in India or abroad.

This is submitted to the Tamilnadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the rules and regulations for the M.D Degree (Branch IV) in Microbiology.

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Dear Dr. Ambuja Sekhar, MBBS., The Tirunelveli Medical College Institutional Ethics Committee (TIREC) reviewed and discussed your application during the IEC meeting held on 05.06.2016.

THE FOLLOWING DOCUMENTS WERE REVIEWED AND APPROVED

1. TIREC Application Form
2. Study Protocol
3. Department Research Committee Approval
4. Patient Information Document and Consent Form in English and Vernacular Language
5. Investigator's Brochure
6. Proposed Methods for Patient Accrual Proposed
7. Curriculum Vitae of the Principal Investigator
8. Insurance / Compensation Policy
9. Investigator's Agreement with Sponsor
10. Investigator's Undertaking
11. DCGI/DGFT approval
12. Clinical Trial Agreement (CTA)
13. Memorandum of Understanding (MOU)/Material Transfer Agreement (MTA)
14. Clinical Trials Registry-India (CTRI) Registration

THE PROTOCOL IS APPROVED IN ITS PRESENTED FORM ON THE FOLLOWING CONDITIONS

1. The approval is valid for a period of 2 year/s or duration of project whichever is later
2. The date of commencement of study should be informed
3. A written request should be submitted 3 weeks before for renewal / extension of the validity
4. An annual status report should be submitted.
5. The TIREC will monitor the study
6. At the time of PI's retirement/leaving the institute, the study responsibility should be transferred to a person cleared by HOD
7. The PI should report to TIREC within 7 days of the occurrence of the SAE. If the SAE is Death, the Bioethics Cell should receive the SAE reporting form within 24 hours of the occurrence.
8. In the events of any protocol amendments, TIREC must be informed and the amendments should be highlighted in clear terms as follows:
   a. The exact alteration/amendment should be specified and indicated where the amendment occurred in the original project. (Page no. Clause no., etc.)
   b. The PI must comment how proposed amendment will affect the ongoing trial. Alteration in the budgetary status, staff requirement should be clearly indicated and the revised budget form should be submitted.
   c. If the amendments require a change in the consent form, the copy of revised Consent Form should be submitted to Ethics Committee for approval. If the amendment demands a re-look at the toxicity or side effects to patients, the same should be documented.
   d. If there are any amendments in the trial design, these must be incorporated in the protocol, and other study documents. These revised documents should be submitted for approval of the IEC, only then can they be implemented.
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CERTIFICATE II

This is to certify that this dissertation work title “Comparison For Diagnostic Utility Of Anti Cyclic Citrullinated Antibody, Anti Keratin Antibodies And Rheumatoid Factor In Rheumatoid Arthritis Patients In A Tertiary Care Hospital” of the candidate Dr. Ambuja Sekhar with registration number 201514301 for the award of M.D. Degree in the branch of Microbiology (IV). I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion page and result shows 18 percentage of plagiarism in the dissertation.

Guide & Supervisor sign with seal.
Compared for diagnostic utility of anti-cyclic citrullinated antibody, anti-histin antibody and immunoglobulin factor in immunoenhanced antibody patients in tertiary care hospital.

1. Introduction: Rheumatoid arthritis is a chronic inflammatory disease of unknown etiology characterized by persistent inflammatory symptoms, usually involving small joints in symmetrical distribution. The disease is characterized by joint pain, swelling, and stiffness. Initial symptoms include pain, swelling, and stiffness of affected joints. Early diagnosis is crucial to prevent joint damage and improve long-term outcomes.

2. Early diagnosis is essential for preventing irreversible damage that frequently occurs. Early diagnosis often leads to better treatment outcomes, but it requires a high level of suspicion among healthcare providers. Early detection is crucial to prevent irreversible damage, which can significantly affect patients' quality of life.

3. Current diagnostic methods for rheumatoid arthritis include symptom assessment, physical examination, and laboratory tests. These methods are effective in identifying patients with rheumatoid arthritis, allowing early intervention and management. However, it's important to note that early diagnosis is crucial to prevent irreversible damage and improve long-term outcomes.
<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>RA</td>
<td>Rheumatoid arthritis</td>
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<tr>
<td>RF</td>
<td>Rheumatoid factor</td>
</tr>
<tr>
<td>CCP</td>
<td>Cyclic citrullinated peptide</td>
</tr>
<tr>
<td>AKA</td>
<td>Anti Keratin antibody</td>
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<tr>
<td>ACR</td>
<td>American College of Rheumatology</td>
</tr>
<tr>
<td>PIP</td>
<td>Proximal interphalangeal joint</td>
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<tr>
<td>MCP</td>
<td>Metacarpophalangeal joint</td>
</tr>
<tr>
<td>ESR</td>
<td>Erythrocyte sedimentation rate</td>
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<tr>
<td>CRP</td>
<td>C- Reactive protein</td>
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<tr>
<td>ACPA</td>
<td>Anticyclic citrullinated peptide antibodies</td>
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<tr>
<td>ELISA</td>
<td>Enzyme linked immunosorbent assay</td>
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<td>IF</td>
<td>Immunofluorescence</td>
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<td>MHC</td>
<td>Major histo compatibility</td>
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<td>PTPN22</td>
<td>Protein tyrosine phosphatase-22</td>
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<td>IL</td>
<td>Interleukin</td>
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<td>TNF</td>
<td>Tumor necrosis factor</td>
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<td>TCR</td>
<td>T cell receptor</td>
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<td>DC</td>
<td>Dentritic cells</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>EULAR</td>
<td>European League Against Rheumatism</td>
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<tr>
<td>IP</td>
<td>Interphalangeal joint</td>
</tr>
<tr>
<td>MTP</td>
<td>Metatarsophalangeal joint</td>
</tr>
<tr>
<td>ULN</td>
<td>Upper limit of normal</td>
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<tr>
<td>PAD</td>
<td>Peptidyl arginine deiminase</td>
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<td>IgM, IgA</td>
<td>Immunoglobulin</td>
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1. INTRODUCTION

Rheumatoid arthritis is a chronic multisystem disease of unknown etiology characterized by persistent inflammatory synovitis, usually involving peripheral joints symmetrically. Cartilaginous destruction, bony erosions and joint deformity are hallmarks. The propagation of RA is that, it is an immunologically mediated event in which joint injury occurs from synovial hyperplasia, lymphocytic infiltration of synovium and local production of cytokines and chemokines by activated lymphocytes, macrophages and fibroblasts.

RA occurs in 0.5% - 1.0% of the populations; women are more affected, around three times than men; prevalence increases with age, onset most frequent in fourth and fifth decade. Typically a symmetric polyarthritis of peripheral joints with pain, tenderness, and swelling of affected joints; morning stiffness is common; proximal interphalangeal (PIP) and metacarpophalangeal (MCP) joints frequently involved; joint deformities may develop after persistent inflammation.\(^1\)

Early diagnosis of rheumatoid arthritis is essential for prevention of irreversible damage that frequently occurs. This has been relied upon clinical criteria, physical examination, serological markers, radiological findings.\(^2-6\) Currently available diagnostic methods for Rheumatoid arthritis includes acute phase reactant and autoantibodies ie ESR, CRP, but their diagnostic value is poor and the
inflammatory activity can be low with erosions developing in spite the absence of significant inflammation\textsuperscript{[7-10]} Early recognition is necessary as significant group of patient develop irreversible joint damage \textsuperscript{[11]}, also to prevent the risk associated with treatment. \textsuperscript{[12]}. The diagnosis is based on American College of Rheumatology (ACR) 1987 revised criteria \textsuperscript{[13]}. The patient is considered RA if they have at least four out of the seven criteria for six weeks, of which the only laboratory test included is the RF. The sensitivity and specificity of RF for the diagnosis of RA has been reported in the range of 50-80\% and 70-80\%, respectively\textsuperscript{[14-15]} . With around 5\% false positivity in the general population, RF is found in many patients with other diseases of infectious or autoimmune origin. Consequently, a search for better diagnostic markers, especially those with improved specificity for RA, ensued.

Advanced search for a new Diagnostic method that helped in early diagnosis and identifying the disease progression yielded the detection of autoantibodies which included anticyclic citrullinated peptide antibodies (ACPA) and antikeratin antibodies (AKA)\textsuperscript{[16-18]}. Due to the inflammation, deamination or citrullination occurs in tissues causing release of enzymes. Of which Fibrin is one of the citrullinated antigen that induces anti cyclic citrullinated peptide antibody in Rheumatoid patients by an antigen mediated B cell activation. This antibody became an important surrogate marker for diagnosis and prognosis of Rheumatoid
arthritis because of probability in predicting the erosive consequence of the disease, leading on to development of undifferentiated arthritis. As it has a high level accuracy in detection rate with specificity of 95-98% and a sensitivity of 67-80%. The 2010 RA Classification criteria included detection of ACPA as a key item for diagnosing the disease [19].

A recent potential marker for diagnosis is antikeratin antibody. It appears to fulfill the requirement for a serological marker for RA with a prevalence range in patient from 16-68%[20-23]. The underlying mechanism behind the formation of AKA may not be conclusive but it has been proposed that the structurally altered keratin tend become immunogenic during the formation of subcutaneous nodules[24]. Antikeratin antibody seems to be more specific for Rheumatoid arthritis with a specificity of 98% [25]. In a recent study it was found that 60.5% patients with severe radiographic damage were Antikeratin antibody positive. [21]
2. AIMS AND OBJECTIVES

- To determine the sensitivity and specificity of IgM RF Latex agglutination, Anti CCP antibody ELISA and Anti Keratin antibody by Immunofluorescence for early diagnosis of Rheumatoid arthritis
- To compare the utility of Anti CCP antibody ELISA, with IgM RF Latex agglutination and Anti Keratin antibody Immunofluorescence for the early diagnosis of Rheumatoid arthritis
Rheumatoid arthritis (RA) is a chronic inflammatory disease of unknown etiology marked by involvement of symmetric, peripheral polyarthritis. It presents as the most common form of chronic inflammatory arthritis, mostly leading to joint damage and physical disability. As it is also considered a systemic disease, RA may result in a variety of extraarticular manifestations, including fatigue, subcutaneous nodules, lung involvement, pericarditis, peripheral neuropathy, vasculitis, and hematologic abnormalities.

Though Rheumatoid arthritis is primarily considered a disease of the joints, abnormal systemic immune responses are evident and can cause a variety of extra-articular manifestations. This clearly show that RA has features of a systemic disease that can involve many organs. In some cases, autoantibody production with the formation of immune complexes that fix complement contribute to these extra-articular findings. The synovium is the primary target, although the unique structure of its vascular bed could provide an environment that is ideal for innate and adaptive immune responses.

Although the precise causes of RA remain uncertain, environmental and genetic influences clearly participate. Clues have been provided by detailed immunogenetic studies and the observation that underlying autoimmunity antedates onset of arthritis by up to a decade. The roles of small-molecule
mediators of inflammation (e.g., arachidonic acid metabolites), autoantibodies, cytokines, growth factors, chemokines, adhesion molecules, and matrix metalloproteinases (MMPs) have been carefully defined. Synovial cells can exhibit behavior resembling a localized tumor that invades and destroys articular cartilage, subchondral bone, tendons, and ligaments. Irreversible loss of articular cartilage and bone begins soon after the onset of RA, and early interventions can probably improve long term outcomes. Increased appreciation of how comorbidities, especially cardiovascular disease and accelerated atherosclerosis, can affect mortality has also led to attempts to suppress synovial and systemic inflammation.

3.1. Etiology rheumatoid arthritis

Despite the intensive research over decades, the etiology remains a mystery. There are 3 areas that are interrelated and ideal for research

i) Host genetic factor

ii) Immunoregulatory abnormalities in autoimmunity

iii) Triggering or persistent microbial infection

3.1.1. Environmental factors and genetic set up

Cigarette smoke, bacterial products, viral components, and other environmental stimuli can contribute to these responses. This process probably occurs often in
normal individuals but is self-limited. The risk with smoking is strong in men with Rheumatoid factor associated with disease and those who have antibodies to citrullinated cyclic peptide. In Europe, investigations reported that coffee consumption is a risk factor for developing Rheumatoid arthritis, while in North America, it is postulated that the risk may be limited to decaffeinated coffee. The increased use of oral contraceptive pills has been associated with decreased in incidence of Rheumatoid arthritis, due to the presence of high amount of estrogen in the oral contraceptive pills and which in turn is responsible for the protective effect.

In individuals, a predetermined propensity for immune hyper-reactivity or autoreactivity might lead to a different outcome. The genome of these individuals might encode for a variety of genes implicated in RA including class II major histocompatibility complex (MHC) genes, protein tyrosine phosphatase-22 (PTPN22), cytokine promoter polymorphisms, signal transduction gene polymorphisms, population-specific genes (e.g., PADI4 in Japanese or Koreans), and other undefined genes.

3.1.2. Role of immunity

When autoreactive T cells are allowed to escape deletion, abnormal T cell selection occurs, along with it, post transcriptional modifications of proteins like citrullinated of arginine residue happens due to environmental stress. This takes
place in mucosal surfaces or the synovium. This normally occurs as an uneventful one in healthy individuals, but in people with a propensity for RA can develop antibodies against these modified proteins with formation of rheumatoid factors (RFs) and anticitrullinated protein antibodies (ACPAs). Synovial innate immunity activation can increase vascular leakage in the synovium, secretion of chemoattractants that recruit immune cells to the joint for the processing of antigens by dendritic cells. Antigen presentation can happen in the synovial germinal centers or, more commonly, in central lymphoid organs after the laden dendritic cells migrate via the lymphatics. Through interactions with the T cell B cell receptor and co-stimulatory signals, naïve T cells can then be activated. T cells can help, but B cells produce pathogenic antibodies and migrate to the joint, where they can power other cells through the production of cytokines such as interleukin (IL)-17 or via cell contact mechanisms which do not require a specific antigen. We still don’t know what transforms subclinical inflammation to symptomatic arthritis, this process can take up to a decade before it reaches completion. Ultimately, a destructive phase starts, which consists of an antigen-dependent and -independent mechanisms and that is mediated by mesenchymal elements such as fibroblasts and synoviocytes. Osteoclasts causes bone erosions subsequently, whereas proteolytic enzymes produced by synoviocytes in the pannus or synovial fluid neutrophils causes cartilage dissolution. Anti-inflammatory mechanisms such
as soluble TNF receptors, suppressive cytokines, cytokine binding proteins, protease inhibitors, lipoxins, antioxidants, antiangiogenic factors, and natural cytokine antagonists are not present in sufficient concentrations to truncate the inflammatory and destructive process. The only way to suppress this response is through therapeutic interventions that either modulate pathogenic cells or neutralize the effector molecules produced by the rheumatoid process, or restore tolerance.

3.1.3. Genetic factors

Although the etiology of RA remains unknown, many studies suggest that the interaction of environmental and genetic factors is responsible. Genetic susceptibility has been noted as an important consideration and RA seems to be more common in monozygote twins of about 30% and dizygotic around 5%. The fact that concordance is not higher provides us a fact that that other influences such as the environment, epigenetics, or microchimerism from maternal-fetal transfer might be as important as or even more important than the genetic component. The risk for a fraternal twin of a patient with RA is also high (≈2% to 5%). Around 10% of patients with RA will have an affected 1st degree relative and this incidence is so high and it is approximately 4 times the expected rate in 1st degree relative of individual with disease associated with presence of auto antibodies rheumatic factor One of the best-studied and perhaps most influential genetic risk factor is the
class II MHC haplotype of an individual. Certain MHC complex class ii alleles have also been associated with increased frequency as in those who are affected, HLADR1 is found in HLAR4 negative patients is found to be associated with population of ethnic groups like Israel, India, Arab

Early studies have suggested that 70% patients with RA express HLADR4. This association is strong for individual who develop RA associated with anti cyclic citrullinated antibody. This HLADR4 subtypes is due to few aminoacid differences in the 3rd hypervariable regions of the HLADR B chains. HLADR1 shows this similar aminoacid sequence, as do several other HLA alleles which have been more recently associated with RA in certain population. Therefore this shared epitope is een among evere MHC II appear to predispose to RA

Also homozygosity for the aminoacid sequence, especially if present in the HLA DR4 molecules, have been correlated with disease severity including more destructive joint disease, subcutaneous nodules, extra articular manifestations like rheumatoid lung disease and Felty syndrome

The crucial region for the shared epitope on HLA DR molecules appear to also a combining site for the T cell antigen receptor. As MHC class ii molecules present the process antigen to the TCR and CD4 T lymphocytes, it appears as an abnormal antigen specific cellular or humoral immune response is inherent to etiology of RA. The nature of the antigen either self or foreign my remain unknown, also
include type ii collagen microbial antigen or heat shock protein and immunoglobins. Other genes are also need for RA and gene mapping studies are in progress

3.1.4. Associated factors

Rheumatoid arthritis appears as an autoimmune disease, similar to the MHC class ii complexes associated disorders, autoantibodies to Fc of Ig G molecules or Rheumatoid factor are present in patient blood and synovial tissue of 80% of RA patient. These patients are considered seropositive. Raised titre of serum rheumatoid factor, considerably of IgM isotype, are associated with severe joint disease with extra articular manifestations.

Despite the strong association of Rheumatoid factor with rheumatoid arthritis, they are clearly not considered a causative agent. Rheumatoid factor also occurs in other disorders where there is chronic antigen stimulation like bacterial endocarditis, Tuberculosis, syphilis, kala azar, viral infection iv drug, cirrhosis. Rheumatoid factor also seen in healthy individuals especially with increasing age.  

*PTPN22* and *PADI4* increases the risk in some racial and ethnic groups, but not all. Based on Genome-wide screening, it has been implicated that least 35 genes, many of which are involved with immune function. However, most have a relatively modest contribution and the susceptibility polymorphism is only a 1.1- or 1.2-fold increase. Genes can combine with each other and cause an increased of
45-fold as conferred by a combination of \textit{HLA-DR}, \textit{PTPN22}, and the \textit{TRAF1-C5}\textsuperscript{(26)}. This combination is seen in less than 1\% of individuals with RA. The RA-associated alleles identified to date contribute approximately 40\% of total genetic susceptibility.

\textbf{3.1.5. Inflammatory factors in the synovium}

The rheumatoid synovium is a prototypic inflammatory effector site that has given us information about the autoimmune, inflammatory, and destructive mechanisms\textsuperscript{(27)}. From these studies therapeutic targets have been elucidated and used successfully to suppress disease in clinical rheumatology practice. Synovial tissue from normal joints consists of a lining layer of macrophages and fibroblasts from one to three cells thick, associated with a loose, vascularized, connective tissue sublining layer. The lining layer of macrophages and fibroblasts in inflamed RA synovial tissue is hyperplastic, and the sublining is infiltrated by inflammatory cells. In inflammation, vascular endothelial cells are activated, and angiogenesis and high endothelial venule development are evident. Foci of activated dendritic cells (DC), T cells, and inflammatory macrophages are recruited adjacent to vessels. B cells infiltrate RA synovial tissue and, in a subset of patients, form distinct germinal centres associated with follicular DC. Although neutrophils are infrequent in the tissue, they are abundant in inflamed synovial fluid.
3.1.6. The at risk patients

The European League Against Rheumatism (EULAR) Study Group for Risk Factors for RA has published guidelines focusing on the preclinical and very earliest clinically apparent stages of disease (Gerlag DM, Raza K, van Baarsen LG et al). Specific phases of RA are proposed, and each stage of disease is described by a terminology. This is gives a framework to define the temporal relationship between genetic, environmental, and immuno-inflammatory factors that bestow risk for RA, and will emphasize the development of risk stratification and prediction models in the future. Genetic and environmental risk factors are important components of risk stratification.
3.1.7. More on immunity

The cells contributing to the earliest events of pathways of innate immunity are now better appreciated. The idiosyncratic features and aberrations of adaptive immune responses in RA patients are being revealed, and the cartilage destruction and bone erosion caused by end organ effector pathways have now been identified. To apply targeted therapies that induce rapid and sustained remission without compromising host defence (e.g. through antigen-specific therapy) will be a major challenge in the future, coupled to well-tolerated regimens for drug tapering and withdrawal. As insights into methods of risk stratification evolve, primary prevention will become the next major goal. This will best be realized through:

- the full spectrum of disease subtypes to be appreciated, an understanding of disease pathogenesis at a molecular and cellular level and can be used more efficiently to predict outcomes and the best therapies for both individual patients and those at highest risk of developing disease
- insights into the inherited and acquired perturbations of immunity and inflammation and their impact on the disease over time
- detailed knowledge of the clinical and biological remission state (immunological tolerance) and how to measure it.
3.1.8. Pathology

RA is a condition which affects the synovial tissue, the underlying cartilage and bone. The synovial membrane, that is the covering most of articular surfaces, tendon sheaths, and bursae, is usually a thin layer of connective tissue. In joints, it faces the bone and cartilage, thereby bridging the opposing bony surfaces and inserting at periosteal regions at close proximity to the articular cartilage. It consists primarily of two cell types—type A synoviocytes (macrophage-derived) and type B synoviocytes (fibroblast-derived). The synovium consists more number of fibroblasts that helps in production of the structural component in the joints, including collagen, fibronectin and laminin, along with other extracellular constituents of the synovial matrix. There are layers called sublining layer which mainly consists of blood vessels and some populations of mononuclear cells embedded in a loose network of connective tissue. The synovial fluid, which is an ultrafiltrate of blood, seeps into the joint cavity via the subsynovial lining tissue, which is across the synovial membrane. The major constituent are hyaluronidase and lubrin, where hyaluronin is a glycosaminoglycan that provides the viscosity to the synovial fluid, thereby along with lubrin, helps to lubricate the surface of the articular cartilage.

The pathologic trademark of Rheumatoid arthritis is mainly the synovial inflammation and proliferation with focal bone erosion and thinning of articular
cartilage. Prolonged inflammation causes the formation of hyperplasia of synovial lining, resulting in pannus, which is nothing but a thickened cellular membrane of granulation reactive fibrovascular lesions that invades the underlying cartilage and bone. There is a molecule called Cadherin 11, which is an organizing molecule of the synovial membrane, which bestow the invasive nature of fibroblasts like synoviocytes. The inflammatory infiltrate consists of T cell, B cell, plasma, dendritic cells, mast cells and a few granulocytes, as most of the infiltrate is composed of 30-50% of the T cells and the remaining is composed of other cells. The topographical arrangement of these cells is intricate and may vary among individuals with Rheumatoid arthritis. Promotion of formation of new blood vessels in the synovial sublining by the growth factors secreted by synovial fibroblasts and macrophages, satisfy the increasing demands for oxygenation and nutrition required by the infiltrating leucocytes and expatiated synovial tissue. Osteoclasts mediates damage to the mineralised cartilage and subchondral bone. They are multinucleated giant cells with an expression of CD 68, tartrate resistant acid phosphatase, cathepsin k and the calcitonin receptor. They appear at the pannus – bone interface, eventually forming a resorption lacunae. These lesions localize where synovial membrane inserts into the periosteal surface at the edges of bones close to the rim of articular cartilage. This phenomenon explains the bone
erosions that usually developing at the radial sites of metacarpophalageal joint, juxtaposed to the insertion sites of all synovial membrane and tendons.

There is also periarticular osteopenia that occur at joint with severe active inflammation. It mainly leads to thinning of trabeculae along the metaphyseas of bones, due to inflammation of the bone marrow cavity.

The above lesions can be received as signal alterations in the bone marrow contiguous to the inflamed joints on MRI scans. These MRI features are mainly due to water richness with a low fat content and highly vascularised inflamed tissue. These marrow lesions form the antecedent of bone erosions.

The cortical layer which is relatively thin and susceptible to penetration by the inflamed synovium is formed by the cortical layer that separates the bone marrow from the invading pannus. The lesions on bone marrow are associated with an endosteal bone response due to accumulation of osteoblasts and deposition of osteoid, as seen in MRI. Therefore the joint pathology in Rheumatoid arthritis in recent times include the bone marrow cavity. There occurs bone loss due to osteoporosis leading to trabecular bone thinning throughout the body. The articular cartilage composes of collagen, proteoglycan and other proteins and it is avascular. It is arranged into four regions, namely superficial, middle, deep and calcifies cartilage zones. The major constitute that is unique for these layer include chondrocytes. Initially cartilage was thought to be an inert tissue, but now it is
considered as an highly responsive tissue that reacts to inflammatory mediator and mechanical factors, which alter the balance between cartilage anabolism and catabolism.

In Rheumatoid arthritis, the initial cartilage degradation are juxtaposed to the synovial pannus. There is generalized loss of proteoglycan, mostly in the superficial zone of cartilage matrix adjacent to the synovial fluid. Perichondrolytic zone and its associated regions consist of degraded cartilage.

### 3.1.9. Pathogenesis

#### 3.1.9.1. Macroscopic appearances of synovitis in rheumatoid arthritis

The gross pathologic changes that are typical of rheumatoid arthritis (RA) is the end result from chronic synovial inflammation. Classically, the surface of the synovium becomes hypertrophic and oedematous, with an elaborate system of prominent villous fronds that expand into the joint cavity. The macroscopic appearances of synovitis may be readily quantified at arthroscopy, which provides an easier access to human synovial tissue. This has opened up new opportunities for those engaged in the study of arthritis. Even in the earliest phases of disease synovial tissue can now be selected from many sites within large and small joints, enhancing studies of aetiology, prognosis, and response to treatment. Contemporary imaging modalities, such as magnetic resonance imaging and ultrasonography, possess the capability of further characterizing and
quantifying macroscopic synovial inflammation, joint effusion, cartilage integrity, and bone erosion (32;33)

3.1.9.2. Microscopic appearances

The synovium in RA is hypertrophic and oedematous. There is marked hyperplasia of the lining layer, and accumulation of many cell populations, including T-cells, plasma cells, B-cells, macrophages, neutrophils, mast cells, natural killer (NK) cells, and dendritic cells in the sub-lining layer.

The lining layer

The foremost cellular components of the lining layer are fibroblast-like synoviocytes (FLS) and macrophages. These cell populations liberate an array of proinflammatory cytokines and their inhibitors, which may encourage further intraarticular perturbations. There is accumulation of the abundantly produced matrix metalloproteinases (MMPs), cysteine proteases, and other tissue degrading mediators in the synovial fluid which augment joint damage by directly interacting with exposed cartilage matrix. These features are seen early in the disease course. When tissue samples were obtained after a few days of onset of symptoms, there is an increase in lining layer macrophage and prominent perivascular mononuclear infiltration (34), and in the clinically uninvolved joints (35), of patients with RA. In symptomatic joints, CD68+ macrophage accumulation in the synovium was more
prominent \(^{(36)}\). In synovial tissue obtained only 2 weeks after the onset of symptoms, abundant protease gene expression has also been observed, emphasizing the very early potential for joint destruction in RA \(^{(37)}\). The degree of joint damage can reflect the quantifiable immunohistologic features in the synovial lining layer \(^{(38;39)}\). This suggestion was highlighted in a recent study, which demonstrated that high levels of MMP-1 mRNA expression in the lining layer distinguished patients with more rapidly progressive erosive RA \(^{(40)}\).
The expansion of the synovial lining layer seen in rheumatoid arthritis from a thin 2–3-cell layer to a thick hypertrophic synovium
Note the change in cell morphology into fatter, more rounded cells reflecting the recruitment of type A macrophage-like synoviocytes.

The sub-lining layer

T-cells and plasma cells are prominent in the synovial sub-lining layer. Lymphocyte aggregates are observed in 50 to 60 per cent of patients with RA. These aggregates can be surrounded by plasma cells. In addition, macrophages and lymphocytes infiltrate the areas between the lymphocyte aggregates. In some patients, areas with granulomatous necrobiosis are apparent (41)Klimiuk et al. 1997). These areas are characterized by regions with fibrinoid necrosis lined by a collar of epithelioid histiocytes and granulation tissue. Fibrin deposition and
fibrosis can be observed. The macrophages often constitute the majority of inflammatory cells in the synovial sub-lining layer. Local disease activity is particularly associated with their number and with the expression of cytokines, such as TNF and IL-6 (42,43). The synovial sub-lining macrophages produce a variety of mediators of joint destruction (44,45).

Large numbers of T-cells are also present in the synovial sub-lining. There are two basic patterns of T-cell infiltration (26). First, perivascular lymphocyte aggregates can be found, which consist predominantly of CD4+ cells in association with B-cells, few CD8+ cells, and dendritic cells. The second pattern of T cell infiltration is the diffuse infiltrate of T-cells scattered throughout the synovium. A subset of the CD4+ T-cells in synovial tissue is activated. A possible biologic effect of activated perivascular T-cells in the synovium is the activation of migrating macrophage populations through direct cell contact. This mechanism is known to stimulate macrophage production of cytokines and MMPs in vitro (46,47). A factor in human serum, identified as apolipoprotein A-1 (apo A-1), was recently shown to inhibit contact-mediated stimulation of monocytes by activated T-lymphocytes in vitro (48). It was speculated that apo A-1 may play an important role in modulating T-lymphocyte-mediated effects in both acute and chronic inflammation. Many of the T-cells in synovial tissue are, on the other hand, in a state of hyporesponsiveness (26,49). Interdigitating dendritic cells, which are potent antigen
presenting cells, are located in proximity to CD4+ T-cells in the lymphocyte aggregates and near the intimal lining layer (50) (51); (52)

3.2. Clinical features

The incidence of Rheumatoid arthritis increases between 25 and 55 years of age, after which it plateaus until the age of 75 and then decreases. The presenting symptoms of Rheumatoid arthritis typically result from inflammation of the joints, tendons and bursae. Most common complaints include early morning joint stiffness lasting more than one hour that reduces with physical activity. The earliest involved joints are typically the small joints of the hands and feet. The initial pattern of joint involvement may be monoarticular, then oligoarticular or polyarticular (> 5 joints), usually in asymmetric distribution.

3.2.1. Small joints involvement

The joints that stand out as the most commonly involved once the disease is established are the wrists, metacarpophalangeal and proximal interphalangeal joints. There is flexor tendon tenosynovitis which forms the most common hallmark of Rheumatoid arthritis that leads to decreased range of motion, reduced grip strength and formation of trigger fingers. Chronic irreversible deformities occur due to progressive destruction of the joints and soft tissues. There occurs ulnar deviation due to subluxation of the MCP joints along with subluxation of the
proximal phalanx to the volar side of the hand. Hyperextension of the PIP joint with flexion of the DIP joint (swan neck deformity), flexion of the PIP joint with hyperextension of the DIP joint (boutonniere deformity) and subluxation of the first MCP joint with hyperextension of the first interphalangeal joint (Z deformity) may also occur from damage to the joint capsule and other soft tissues. Piano key movement of the ulnar styloid occurs due to inflammation of the ulnar styloid and tenosynovitis of the extensor carpi ulnaris and cause subluxation of distal ulna.

Although metatarsophalangeal joint involvement in the feet is an early feature of disease, chronic inflammation of the ankle and midtarsal regions usually comes later and may lead to pes planovalgus (flat feet).

**3.2.2. Large joint involvement**

In established disease large joints including the knees and shoulders, are often affected, although these joints may remain asymptomatic for many years after onset. Compressive myelopathy and neurologic dysfunction occur due to involvement of atlantoaxial involvement of the cervical spine. Neurologic manifestations are rarely a presenting sign or symptom of atlantoaxial diseases, but they may evolve over time with progressive instability of C1 on C2. There is a decline in prevalence of atlantoaxial subluxation in recent years and occurs now in less than 10% of patients. Unlike the spondyloarthritis, RA affects the thoracic and lumbar spine. Radiographic abnormalities of the temporomandibular joint occur commonly in
patients with rheumatoid arthritis, but they are generally not associated with significant symptoms of functional impairment.

3.2.3. Extraarticular involvement

Extra articular manifestations may develop during the clinical course of rheumatoid arthritis, even before the development of arthritis. Patients having a history of smoking most likely to develop extra articular disease, have an early onset of physical disability and test positive for serum Rheumatoid factor. Generally, the number and severity of extra articular features vary with the duration and severity of the disease. Several of these features may be related to extra articular foci of an immune response, based on evidence of independent and qualitatively different production of RF in the pleural space, pericardium, muscle and even meninges. Other unusual proteins and protein complexes in the circulation of patients with active rheumatoid disease include antiphospholipid antibodies, circulating immune complexes and cryoglobulins are the other unusual proteins and protein complexes in the circulation of patients with active rheumatoid arthritis. Extraarticular manifestations of RA are associated with excess mortality.
3.2.3.1. Rheumatoid nodules

Subcutaneous nodules occur in 30-40% of patients and more commonly in those with the highest levels of disease activity. When palpated, the nodules are generally firm; nontender; and adherent to periosteum, tendons, or bursae; developing in areas of the skeleton subject to repeated trauma or irritation such as the forearm, sacral prominences, and Achilles tendon. They may also occur in the lungs pleura pericardium, and peritoneum. Nodules are typically benign, although they can be associated with infection, ulceration and gangrene.

3.2.3.2. Sjogrens syndrome

Secondary Sjögren's syndrome is defined by the presence of either keratoconjunctivitis sicca (dry eyes) or xerostomia (dry mouth) in association with another connective tissue disease, such as RA. Approximately 10% of patients with RA have secondary Sjogren's syndrome.

3.2.3.3. Pulmonary

Pleuritis, the most common pulmonary manifestation of RA, may produce pleuritic chest pain and dyspnea, as well as a pleural friction rub and effusion. Pleural effusions tend to be exudative with increased numbers of monocytes and neutrophils. Interstitial lung disease (ILD) may also occur in patients with RA and
is heralded by symptoms of dry cough and progressive shortness of breath. ILD can be associated with cigarette smoking and is generally found in patients with higher disease activity, although it may be diagnosed in up to 3.5% of patients prior to the onset of joint symptoms. Diagnosis is readily made by high-resolution chest computed tomography (CT) scan. Pulmonary function testing shows a restrictive pattern (e.g., reduced total lung capacity) with a reduced diffusing capacity for carbon monoxide (DLco). The presence of ILD confers a poor prognosis. The prognosis is not quite as poor as that of idiopathic pulmonary fibrosis (eg, usual interstitial pneumonitis) because ILD secondary to RA responds more favorably than idiopathic ILD to immunosuppressive therapy. Pulmonary nodules may be solitary or multiple. Caplan's syndrome is a rare subset of pulmonary nodulosis characterized by the development of nodules and pneumoconiosis following silica exposure. Other less common pulmonary findings include respiratory bronchiolitis and bronchiectasis.

3.2.3.4. Cardiac

The most frequent site of cardiac involvement in RA is the pericardium. However, clinical manifestations of pericarditis occur in less than 10% of patients with RA despite the fact that pericardial involvement may be detected in nearly one-half of these patients by echocardiogram or autopsy studies.
Rarely, the heart muscle may contain rheumatoid nodules or be infiltrated with amyloid. Mitral regurgitation is the most common valvular abnormality in RA, occurring at a higher frequency than the general population

3.2.3.5 Vasculitis

Rheumatoid vasculitis typically occurs in patients with long-standing disease, a positive test for serum RF, and hypocomplementemia. The overall incidence has decreased significantly in the last decade to be less than 1% of patients. The cutaneous signs vary and include petechiae, purpura, digital infarcts, gangrene, livedoreticularis, and in severe cases large, painful lower extremity ulcerations. Vasculitic ulcers, which may be difficult to distinguish from those caused by venous insufficiency, may be treated successfully with immunosuppressive agents (requiring cytotoxic treatment in severe cases) as well as skin grafting. Sensorimotor polyneuropathies, such as mononeuritis multiplex, may occur in association with systemic rheumatoid vasculitis.

3.2.4 Hematologic

A normochromic, normocytic anemia often develops in patients with RA and is the most common hematologic abnormality. The degree of anemia parallels the degree of inflammation, correlating with the levels of serum C-reactive protein
(CRP) and erythrocyte sedimentation rate (ESR). Platelet counts may also be elevated in RA as an acute-phase reactant. Felty syndrome is defined by the clinical triad of neutropenia, splenomegaly, and nodular RA and is seen in less than 1% of patients, although its incidence appears to be declining in the face of more aggressive treatment of the joint disease. It typically occurs in the late stages of severe RA and is more common in whites than other racial groups. As opposed to Felty's syndrome, T-LGL may develop early in the course of RA. Leukopenia apart from these disorders is uncommon and most often due to drug therapy.

3.2.5. Lymphoma

Large cohort studies have shown a two- to fourfold increased risk of Lymphoma in RA patients compared with the general population. The most common histopathologic type of Lymphoma is a diffuse large B cell Lymphoma. The risk of developing Lymphoma increases if the patient has high levels of disease activity or Felty's syndrome.

3.2.6. Associated conditions

In addition to extraarticular manifestations, several conditions associated with RA contribute to disease morbidity and mortality rates. They are worthy of mention because they affect chronic disease management.

**Cardiovascular Disease** The most common cause of death in patients with RA is cardiovascular disease. The incidence of coronary artery disease and carotid
Atherosclerosis is higher in RA patients than in the general population. Furthermore, congestive heart failure (including both systolic and diastolic dysfunction) occurs at an approximately twofold higher rate in RA than in the general population. The presence of elevated serum inflammatory markers appears to confer an increased risk of cardiovascular disease in this population.

**Osteoporosis** Osteoporosis is more common in patients with RA than an age- and sex-matched population, with prevalence rates of 20-30%. Chronic use of glucocorticoids and disability-related immobility also contributes to osteoporosis. Hip fractures are more likely to occur in patients with RA and are significant predictors of increased disability and mortality rate in this disease.

### 3.3. Criteria for diagnosis

In 2010, a collaborative effort between the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) revised the 1987 ACR classification criteria for RA in an effort to improve early diagnosis with the goal of identifying patients who would benefit from early introduction of disease-modifying therapy. Application of the newly revised criteria yields a score of 0–10, with a score of ≥6 fulfilling the requirements for definite RA. The new classification criteria differ in several ways from the older criteria set. The new criteria include a positive test for serum anticyclic citrullinated peptide antibodies
as an item, which carries greater specificity for the diagnosis of RA than a positive test for rheumatoid factor. The newer classification criteria also do not take into account if the patient has rheumatoid nodules or radiographic joint damage because these findings occur rarely in early RA. It is important to emphasize that the new 2010ACR-EULAR criteria are “classification criteria” as opposed to “diagnostic criteria” and serve to distinguish patients at the onset of disease with a high likelihood of evolving into a chronic disease with persistent synovitis and joint damage. The presence of radiographic joint erosions or subcutaneous nodules may inform the diagnosis in the later stages of the disease.

<table>
<thead>
<tr>
<th>CLASSIFICATION CRITERIA FOR RHEUMATOID ARTHRITIS - SCORE</th>
</tr>
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<tbody>
<tr>
<td>1 Joint involvement</td>
</tr>
<tr>
<td>1 large joint( shoulder, elbow, hip, knee, ankle)</td>
</tr>
<tr>
<td>2-10 large joints</td>
</tr>
<tr>
<td>1-3 small joints ( MCP, PIP, Thumb IP, MTP, Wrists)</td>
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<tr>
<td>4-10 small joints</td>
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<tr>
<td>&gt;10 joints ( atleast 1 small joint)</td>
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<tr>
<td>0</td>
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<td>3</td>
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<tr>
<td>5</td>
</tr>
<tr>
<td>2 Serology</td>
</tr>
<tr>
<td>Negative RF and negative ACPA</td>
</tr>
<tr>
<td>Low positive RF or low positive anti-CCP antibodies (≤3 times ULN)</td>
</tr>
<tr>
<td>High positive RF or high positive anti-CCP antibodies (&gt; 3 times ULN)</td>
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<tr>
<td>0</td>
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<tr>
<td>2</td>
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<tr>
<td>3</td>
</tr>
<tr>
<td>3 Acute phase reactant</td>
</tr>
<tr>
<td>Normal CRP and normal ESR</td>
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<tr>
<td>Abnormal CRP ad abnormal ESR</td>
</tr>
<tr>
<td>0</td>
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<tr>
<td>1</td>
</tr>
<tr>
<td>4 Duration of symptoms</td>
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<tr>
<td>&lt; 6 weeks</td>
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<tr>
<td>&gt;6 weeks</td>
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<tr>
<td>0</td>
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<td>1</td>
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</table>
3.4 Why do we need a good diagnostic test

Most of the Rheumatoid arthritis patients may progress to severe joint damage leading to decline in physical functions, depression and disability. If not treated, about 20-30% may develop inability to work in 3 years\(^{(53)}\). In about 20 years with Rheumatoid arthritis, the physical functions may reduce to 60\%\(^{(54)}\). WHO has quoted that > 50 \% people with Rheumatoid arthritis terminate working within 10 years of disease onset, which causes a major economic imbalance and heavy impact on family( WHO). Also the mortality ratio is 2.26 times more with Rheumatoid patient as compared to general population\(^{(55)}\). But it has been shown that early diagnosis and management can prevent joint destruction in the early months of disease. Therefore early diagnosis of Rheumatoid arthritis becomes essential

3.5. Rheumatoid factor

These antibodies are immunoglobulins (Ig) that bind the Fc (constant region) of IgG. Several assays are available, including the classic Rose - Waaler test, which relies on the ability of rheumatoid factors to agglutinate sheep erythrocytes coated with anti - sheep immunoglobulin, and the latex agglutination test, in which latex particles coated with human IgG aggregate in the presence of IgM rheumatoid factor. An elevated rheumatoid factor has a definite but limited value as a diagnostic test for rheumatoid arthritis. The test is positive in 70 – 80\% of patients with rheumatoid arthritis and in some patients with other disorders, including other
arthritic conditions (such as lupus and Sjögren’s syndrome). Rheumatoid factor positivity is additionally seen in infections such as tuberculosis, hepatitis B, hepatitis C and syphilis.

3.6. Anti keratin antibodies in RF patients

Rheumatoid factor is present around 80-85% \(^{(20)}\) of the patients with rheumatoid arthritis, as it has both diagnostic and prognostic value. But in around 15- 20 %, the RF remain negative due to its poor specificity because of its association with large number of other diseases like chronic infections, other systemic autoimmune disorders. The recent addition certain antibodies like anti- keratin antibodies, anti RA 33 in RA patients gives a positive hope for further investigations. Of these AKA fulfills the requirements for a serological marker for RA, as its prevalence ranges in patients with RA from 16-68% \(^{(20-23)}\) in different ethnic populations. According to this study AKA was present in 2 of the 100 healthy controls and 47 of the 84 patients with RA. Thus the sensitivity of AKA for RA was 56 %, whereas its specificity was 98% as compared to the normal population.

3.7. Anti CCP antibody

Antibodies to proteins involved in epithelial cell differentiation, known as filaggrins, are found in patients with rheumatoid arthritis. Antibodies to cyclic citrullinated peptides (CCPs), which cross-react with anti-filaggrin antibodies. Citrullination is posttranslational modification of arginine to citrulline by the
enzyme peptidyl arginine deiminase (PAD). This process occurs naturally during inflammation, apoptosis and keratinization \(^{(56)}\). While fillagrin is not present in the synovium \(^{(57)}\), several citrullinated proteins, including fibrinogen and fibronectin, are present in RA synovium, and other citrullinated epitopes have been identified as targets of highly RA-specific autoantibodies \(^{(58-60)}\). In 1998, Schellekens and colleagues produced synthetic linear citrullinated peptides derived from human fillagrin, easily detected by ELISA with enhanced sensitivity and no loss of specificity \(^{(56)}\). To improve antigen composition and antibody recognition, a cyclic citrullinated peptide (CCP) was developed \(^{(61)}\). They are 90% specific for rheumatoid arthritis. These antibodies are measured by ELISA and are available in many immunology lab.

3.7.1. Pathophysiology of anti CCP

ACPA presence in the blood prior to clinical disease is associated with increased risk of developing RA\(^{(62,63,64)}\). Presence of these are associated with structural damage, radiographic progression and poor response to therapy \(^{(65,66-74,75)}\). Geneticists and epidemiologists hold ACPA-positive RA to be a homogeneous phenotype of severe RA. ACPA is strongly associated with the \(HLA-DRB1\) shared epitope \(^{(76)}\) and \(PTPN22\) \(^{(77,78)}\), strong genetic risk factors for RA, and smoking \(^{(79,80)}\), the strongest known environmental risk factor for RA. Smoking by individuals with inherited \(HLA-DRB1\) shared epitope genes may trigger RA-
specific immune reactions to citrullinated peptides, the generation of ACPAs and, ultimately, disease.\(^{(80)}\)

### 3.7.2 ACPA assay performance characteristics

Currently available assay kits use a substrate derived from the synthetic cyclic peptide described by Schellekens and colleagues\(^{(58,81)}\), with altered incubation time, volume and dilution of serum, type of conjugate and of enzymatic substrate, and thresholds for positive results\(^{(81-85)}\). In order to determine the diagnostic performance, manufacturers have used established RA patients meeting the 1987 ACR criteria\(^{(86)}\), and healthy individuals. Sensitivities range from 60-80\% and specificities from 85-99\%. These assays are based on identification of autoantibodies by ELISA, immunoenzymofluorimetry, depending on the amount of antibodies present in a non linear fashion. While changes in antibody concentration are reflected in a corresponding rise or fall in results, the change is not proportional in most assays (i.e. a doubling of the antibody concentration will not double the reactivity\(^{(87)}\)). Studies comparing different ACPA assays have concluded that the majority of assays are precise, with within-assay (intra-assay) coefficient of variations (CVs) for most available assays ranging from 4-19\% \(^{(87,88)}\).
3.7.3. Diagnostic utility

More than 300 studies have been published concerning the diagnostic accuracy of ACPA assays in RA diagnosis (65). These studies vary substantially in focus: some have addressed technical aspects, while others have compared the diagnostic accuracy in different populations of individuals (early or established RA; patients with other diseases or healthy controls). The studies are heterogeneous in their comparison of ACPA assay utility to other tests, including IgM, IgA and IgG RF (65) and their use of a gold standard for RA diagnosis (most often the existing 1987 American College of Rheumatology criteria for the classification of RA (89). In studies of early or undifferentiated RA, ACPA testing is generally more specific and equally sensitive to RF.

3.7.4. Comparing ACPA and RF

Given the substantial overlap between the diagnostic performance and utility of RF and ACPA for the diagnosis of RA, the marginal diagnostic value of adding one test to the other and the added value of performing both must be addressed. In particular, the challenge is to decide on the combination of assay or assays that offers superior performance for the identification of RA among patients presenting with early, undifferentiated inflammatory arthritis. Although correlated, RF and ACPA assays detect different underlying biological phenomena in RA, and thus
agreement between assay results is not static, but likely fluctuates during disease course. (90)

In cohorts containing both established and early RA, the performance characteristics of RF and ACPA are comparable and the sensitivity of both RF and ACPA is improved, (although the ranges of performance characteristics are large and data are mixed). A strategy requiring either ACPA or RF may improve sensitivity for both early and established RA. In one study, the presence of either ACPA or RF increased testing sensitivity for RA from 66% (ACPA) and 72% (RF) to 81%, with a good specificity of 91% (56). The specificity of requiring both to be present is comparable to that of ACPA alone. The addition of ACPA testing improved the sensitivity of the 1987 ACR criteria (which rely upon the presence of RF as one of the 11 possible criteria, 4 of which must be present) for the correct classification of early RA subjects (91). Adding ACPA results to the 1987 criteria increased sensitivity for early RA (≤ 6 month disease duration) from 25 to 44% and did not change the specificity of 86%. ACPA also played an important role in a rule developed by Van der Helm-van Mil and colleagues to predict which patients with undifferentiated arthritis would progress to RA (92). Five hundred and seventy patients with undifferentiated arthritis in the Leiden Early Arthritis Center were selected and reassessed at one year for RA development. The prediction rule consisted of nine variables: sex, age, location of symptoms, morning stiffness,
tender joint count, swollen joint count, C reactive protein, and RF and ACPA positivity. ACPA was one of the strongest predictors, and if positive, a subject received 2 points. A modified form of this prediction rule was validated in three cohorts of patients with recent onset undifferentiated arthritis and was found to have excellent discriminative ability to assess progression to RA. ACPA assays are increasingly available and affordable. The assays have good predictive validity as ACPA are associated with known genetic and epidemiologic risk factors for RA and therefore identify a population of RA patients with more severe, erosive joint disease that is at high risk for rapid joint destruction. Positive and negative results are highly correlated between current assays. International standardization of reporting units is underway and will facilitate inter-assay comparisons. ACPA assays offer a slight advantage over RF (including high titer RF and combined IgM, IgA and IgG RF levels) due to higher specificity. RF and ACPA are two different autoantibody systems that do not measure or reflect the same underlying biology. While there is substantial correlation between ACPA and RF seropositivity within patients, the ACPA assay may be especially valuable in predicting RA in patients who are RF-negative but nevertheless have a high probability of RA. If the role of the assay is to aid in the identification of patients developing RA among those presenting with early undifferentiated symptoms, a high-risk population with a high prevalence of disease (rather than
screening the general population), the positive predictive value of the ACPA assay is on the order of 95% \(^{(94)}\). ACPA assays have high specificity, high predictive validity, high specificity, apparent cost effectiveness and good reproducibility for the diagnosis of early RA. In prior studies, accepting \(\text{either ACPA or RF positive} \) assay results for the diagnosis of RA did not improve upon testing for RF alone and requiring both assays to be positive for diagnosis is a very specific, but not extremely, sensitive approach. Ultimately, the decision to use one or both tests depends upon the population tested, the indications for the testing, and the inherent trade-off between sensitivity and specificity.
4. MATERIALS AND METHODS

4.1 Study design
This is a cross sectional case control study

4.2. Study period
This study was conducted from June 2016 to May 2017

4.2. Study area
This study was conducted at the Department of Microbiology, Tirunelveli Medical College and Hospital, Tirunelveli.

4.3. Study population
Patients with arthritis along with fulfilled ACR criteria who attended the Rheumatology Clinic as cases and patients with symptoms of arthralgia as controls

4.4. Criteria of cases

Inclusion criteria:

1. Rheumatoid arthritis patients who fulfilled the ACR criteria
2. With and age of 18 years and above

Exclusion criteria:

1. Patients who were already been diagnosed with other connective tissue diseases
2. Pregnant women
4.5. Ethical clearance

Ethical clearance was obtained from the college ethical committee before the commencement of the study.

4.6. Consent

Informed consent was obtained from reliable informants of neonates who participated in the study.

4.7. Proforma:

The proforma was filled with the details like name of the patient, age, sex, In/Op number, complaints, duration of symptoms, ACR criteria fulfillment, and clinical diagnosis and other parameters relevant to the present study.
4.8 . METHODS

Blood samples were taken from 50 clinically positive rheumatoid patients and 50 undifferentiated arthralgia patients were tested for IgM RF by Latex agglutination, Anti CCP antibodies by ELISA and Anti Keratin antibodies by Immunofluorescence.

4.8.1. Sample collection and processing:

Ideal blood sample collected from the subjects is 2ml of blood was usually considered as the standard volume of blood adequate to provide serum for the tests

- Proper aseptic precautions were undertaken during blood specimen collection to avoid sample contamination.
- With clean gloved hands, preliminary aseptic precautionary steps like cleansing the venipuncture site with 70% ethanol and 2% tincture iodine and proper drying were followed.
- Then using a 2ml syringe with a 28G needle about 2-3 ml of blood was aspirated.
- Immediately and without changing or contaminating the needle 2 ml of blood is poured into the red capped serum separating vial and labeled appropriately.
- Sharps were disposed in a sharps container.
The collected samples were subjected to various laboratory studies.

4.8.2. Storage of serum sample:

Blood samples were centrifuged within 30 minutes of collection. Serum samples were immediately tested for IgM by latex agglutination method and then stored for Anti CCP ELISA and Anti Keratin antibody Immunofluoresence.

4.9. IgM Rheumatoid Factor detection by Latex Agglutination test:

All the 100 samples were tested for IgM RF detection by latex agglutination test with the help OF R.A TEST KIT from BEACON DIAGNOSTICS PVT. LTD, NAVSARI, INDIA

4.9.1. Principle

R.A test antigen consists of polystyrene latex particles coated with specially purifies Human Gammaglobulin. The suspension of coated latex particles agglutinate visibly when mixed with a serum containing Rheumatoid factor in concentration equal to or greater than the sensitivity mentioned as detectable by slide test method. In specimen negative for RF, the latex remains in a smooth suspension form in the test cell.

4.9.2. Reagents

The given IgM RF kit consists of the following items in order to perform the assay

1. R.F antigen mainly composed of polystyrene Latex particles coated with purifies human Gammaglobulin
2. Positive control –

3. Negative control

4. Disposable slides with 8 test cells.

5. Disposable mixing sticks.

6. Disposable plastic droppers with a rubber teat.

4.9.3. Storage

All reagents are stable at 2 – 8 °C till the expiry date mentioned on the individual labels. It should not be freezeed.

4.9.4. Procedure

**Qualitative slide test:**

Allow all reagents as well as sample to reach room temperature

1. Using disposable plastic dropper place one drop of test specimen in circled area of the glass slide provided in the kit.

2. Add one drop of Latex Gammaglobulin reagent to the above drop and mix with disposable applicator stick

3. Rock the slide gently back and forth for two minutes and examine for agglutination.

4. At the end of two minutes the results were read under bright light.

5. For positive and negative controls same procedure is followed
4.9.5. Interpretation of results:

- Strong Positive – Distinct coarse agglutination occurs within 0.5 minute.
- Weakly Positive – Fine agglutination usually taking full 2 minutes.
- Negative - No agglutination.

**RHEUMATOID FACTOR FOR RA**

**PLATE 3**
4.10. Anti Citrullinated Cyclic peptide antibodies detection by ELISA

All the 100 samples were tested for Anti CCP antibodies detection by ELISA with the help of BIOSYSTEMS S.A, COSTA BRAVA. SPAIN

4.10.1. Principle of the method:

Anti citrullinated protein antibodies in the sample bind to the antigen immobilized on the microwell surface. In a surface incubation, a conjugate of horseradish peroxidase – labeled immunoglobulin to human IgG binds to surface bound antibodies. Finally, 3,3’’,5,5’’- tetramethylbenzidine (TMB) with H2O2 is added to each well as enzyme substrate and after colour development, the enzymatic reaction is stopped with acid. The yellow product formed is measured in terms of absorbance units at 450nm, and it is proportional to the amount of antibodies present in the sample.

4.10.2. Materials provided:

- Concentrated washing buffer containing 50 ml concentrated phosphate buffered saline, sodium azide 15mmol/L
- Sample diluents containing 100 ml of Tris buffer and sodium azide 15 mmol/L
- Positive control of 1.5ml containing Human serum free of anticitrullinated protein, sodium azide 15mmol/L
• Negative control of 1.5ml containing Human serum free of anticitrullinated protein, sodium azide 15mmol/L
• Conjugate of 15ml containing Horseradish peroxidase – labeled polyclonal rabbit immunoglobulins to human IgG
• Substrate is 15ml of 3,3’,5,5’- tetramethylbenzidine (TMB)
• Stop solution of 15ml Phosphoric acid 4.5% Microplate with 12 modules of 8wells each coated with mutated citrullinated vimentin
• S1 – S6 Standards of 1.5ml each containing Serum with anti Citrullinated protein, sodium azide 15 mmol/L. Concentrations of antibodies are 0, 10, 20, 50, 150 and 500 U/ ml, as stated in the vial label. Caliberated against an Internal Reference Standard

4.10.3. Material required:

➤ Microtitre plate reader with appropriate filters (450 nm required with optional 620nm reference filter).
➤ Microplate washer
➤ 10, 50, 100, 200 and 1000 l adjustable single channel micropipettes with disposable tips.
➤ 50 -300 l multi-channel pipette and reagent reservoirs.
➤ Distilled water.
Vortex mixer.

4.10.4. Kit storage:
Store kit reagents between 2 and 8°C. Immediately after use remaining reagents should be returned to cold storage (2-8°C).

4.10.5. Preparation of wash buffer:
Dilute concentrated washing buffer with distilled water in the proportion 1/20. Mix thoroughly. About 50 ml of washing buffer are used per strip. Stable for 30 days at 2-8°C. All other reagents are provided ready to use.

ELISA Procedure:
1. Serum is collected by standard procedures. Dilute sample 1/100 with sample diluents. Use always fresh sample dilutions.
2. Allow the reagents and microwells to warm up to room temperature.
3. Open the microplate package and take out the required amount of wells.
4. Qualitative assay: Pipette 100µl of each standard S3, Positive control, Negative control and diluted sample into different wells. Pipette 100µl of sample diluents for the blank.
5. Place wells in a moist chamber and incubate for 30 minutes at room temperature.
6. Aspirate the contents and wash the wells 3 times with 300µl of washing buffer for at least 10 seconds

7. Pipette 100 µl of conjugate into all wells.

8. Place the wells in the moist chamber and incubate for 15 minutes at room temperature.

9. Wash the wells in the step 6

10. Pipette 100 µl of Substrate into all wells

11. Place the wells in the moist chamber and incubate for 15 minutes at room temperature

12. Pipette 100 µl of Stop solution into all wells and incubate for 5 minutes at room temperature

13. Read the absorbance of the contents of each well at 450nm using the S1 standard or the blank well for zero adjustment. The colour is stable for at least 30 minutes

4.10.6. Data calculations:

Calculate the absorbance of the cutoff as follows:

A450nm Cut off = S3 × 0.5

Calculate the Absorbance ratio as follows:
Absorbance ratio = \text{A450NM Sample} \over \text{A450nm Cut off}

When the absorbance values obtained are over the upper limit of the microplate reader range, samples should be further diluted with sample diluents and reassayed.

4.10.7. Reference value

Samples with concentrations above 10 U/ml or with absorbance ratio higher than 1.0 are to be considered positive.

Samples with concentration below 10 U/ml or with absorbance ratios lower than 1.0 are to be considered negative.
ANTI CYCLIC CITRULLINATED ANTIBODY ELISA FOR RA

PLATE 5
PLATE 6
4.11. Anti keratin antibodies detected by Indirect Immunofluorescence

All the 100 samples were tested for Anti Keratin antibodies detection by Indirect Immunofluorescence with the help of BIOSYSTEMS S.A, COSTA BRAVA, SPAIN.

4.11.1. Principle of the method:

Serum anti keratin antibodies bind to filaggrin present in rat esophagus sections. The antigen antibody complexes are detected by means of a fluorescein labeled anti human immunoglobulin G and visualized with the aid of a fluorescence microscope.

4.11.2. Materials provided:

- Slides of rat esophagus sections
- Phosphate buffer solution containing
- Phosphate buffer solution containing sodium phosphate 112.5mmol/L, potassium phosphate 30mmol/L, sodium chloride 1.15mmol/L, sodium azide 0.95g/L, pH 7.2
- Negative controls contain human serum, sodium azide 0.95g/L
- IgG FITC/EVANS is goat antihuman IgG conjugated with fluorescein isothiocyanate, Evans blue 0.01g/L, sodium azide 0.95g/L
- Mounting medium is Mowoi12%, Glycerol 30%, Tris 20mmol/L, sodium azide 0.95g/L
• Blotting paper

4.11.3. Material required:

• 10, 50, 100, 200 and 1000 l adjustable single channel micropipettes with disposable tips.
• 50 - 300 l multi-channel pipette and reagent reservoirs.
• Distilled water.
• Moisture chamber
• Wash tray
• Cover slips 24 ×60mm
• Fluorescence microscope equipped with a 495 nm excitation filter and a 525 emission filter for FITC visualization

4.11.4. Kit storage:

Store kit reagents between 2 and 8°C. Immediately after use remaining reagents should be returned to cold storage (2-8°C).

4.11.5. Preparation of wash buffer:

Dilute reagent PBS 1/10 with distilled water. It is stable for 1 week at 2-8°C.

i. Procedure:

1. Bring the reagent and samples to room temperature
2. Place 1 drop of the diluted sample or control on each slide well making sure that it is completely covered.
3. Incubate the slide for 30 minutes at room temperature into a moist chamber

4. Drain sample drops off by gently tapping the inclined slide. Avoid cross contamination of the sera

5. Rinse gently the slide with PS

6. Wash thoroughly the slide by immersing in a washing tray filled with PBS for 5 minutes. Change PBS and repeat wash

7. Carefully dry off the slides by using the blotting paper provided. Keep the tissue section moist along the procedure

8. Place 1 drop of IgG FITC A on each well. Incubate the slide for 0 min at room temperature into a moist chamber

9. Wash and dry

10. Place several drops of mounting medium on the slide and cover with a cover slip avoiding the formation of air bubbles

4.11.7. Reading

Examine the slides under fluorescence microscope. Sera showing linear laminated staining along the confined squamous epithelium of rat esophagus at the recommended dilution should be considered positive. When above specific staining is not observed, the result should be considered negative for these antibodies
ANTI KERATIN ANTIBODY IMMUNOFLUORESCENCE FOR RA

PLATE 8
PLATE 9

TEST SERUM POSITIVE
PLATE 10

TEST SERUM NEGATIVE
5. RESULTS

5.1. Age wise distribution of the Rheumatoid arthritis patients:

Out of the 100 samples tested, there were totally 8 males and 92 females, of which majority were in the 40 – 49 age group with values of 4% and 36% respectively. The mean age of males was 54.0±14.1 years and females was 45.2±11.4 years. The difference of mean age between the two gender was not statistically significant (P>0.05). The mean age of total subject was 45.9±11.6 years with range of 16 to 75 years. (Table 1 & Figure 1)
**Table-1:**

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>MALE</th>
<th>FEMALE</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>0.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>20-29</td>
<td>0.0</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>30-39</td>
<td>0.0</td>
<td>14.0</td>
<td>14.0</td>
</tr>
<tr>
<td>40-49</td>
<td>4.0</td>
<td>32.0</td>
<td>36.0</td>
</tr>
<tr>
<td>50-59</td>
<td>2.0</td>
<td>24.0</td>
<td>26.0</td>
</tr>
<tr>
<td>60+</td>
<td>2.0</td>
<td>10.0</td>
<td>12.0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>8.0</td>
<td>92.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>54.0±14.1</td>
<td>45.2±11.4</td>
<td>45.9±11.6</td>
</tr>
<tr>
<td>Significance</td>
<td>P&gt;0.05</td>
<td></td>
<td>Range=75-16=59 years</td>
</tr>
</tbody>
</table>
Figure. 1

Age wise distribution of the Rheumatoid arthritis patients

- <20: 2%
- 20-29: 12%
- 30-39: 8%
- 40-49: 14%
- 50-59: 36%
- 60+: 26%
5.2. **Sex wise distribution of the Rheumatoid arthritis patients**

Out of the 100 samples tested, more number of females were present, around 92%, compared to the male population which was only 8%. The mean duration of males was 21.0±11.5 months and females was 44.2±41.2 months. The mean difference of duration between the male and female was not statistically significant (P>0.05). *(Table 2 & figure 2)*

<table>
<thead>
<tr>
<th>Duration (Months)</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;12</td>
<td>0.0</td>
<td>22.0</td>
<td>22.0</td>
</tr>
<tr>
<td>12-23</td>
<td>4.0</td>
<td>20.0</td>
<td>24.0</td>
</tr>
<tr>
<td>24-59</td>
<td>4.0</td>
<td>18.0</td>
<td>22.0</td>
</tr>
<tr>
<td>60-95</td>
<td>0.0</td>
<td>12.0</td>
<td>12.0</td>
</tr>
<tr>
<td>96-120</td>
<td>0.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Total</td>
<td>8.0</td>
<td>92.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>21.0±11.5</td>
<td>44.2±41.2</td>
<td>42.4±40.1</td>
</tr>
<tr>
<td>Significance</td>
<td>P&gt;0.05</td>
<td></td>
<td>Range=120-2= 118 months</td>
</tr>
</tbody>
</table>
Sex wise distribution of the Rheumatoid arthritis patients

- <12: 20%
- Dec-23: 22%
- 24-59: 22%
- 60-95: 12%
- 96-120: 24%

Figure 2.
5.3. Large joint involvement along with sex wise distribution in Rheumatoid arthritis patients

Table 3.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Affected</th>
<th>Not affected</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>6.0</td>
<td>2.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Female</td>
<td>38.0</td>
<td>54.0</td>
<td>92.0</td>
</tr>
<tr>
<td>Total</td>
<td>44.0</td>
<td>56.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Among the 100 patients tested, more common large joint involvement was assessed, mostly females were affected around 38%, commonly the wrist joint. The remaining 56% of them were devoid of any major joint involvement. The gender male and female did not have any statistically significant association with incidence of large joints (P>0.05). (Table 3 & Figure 3)
Large joint involvement along with sex wise distribution in Rheumatoid arthritis patients
5.4. Small joint involvement along with sex wise distribution in Rheumatoid arthritic patients

Table-4:

<table>
<thead>
<tr>
<th>Gender</th>
<th>Affected</th>
<th>Not affected</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>0.0</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Female</td>
<td>22.0</td>
<td>70.0</td>
<td>92.0</td>
</tr>
<tr>
<td>Total</td>
<td>22.0</td>
<td>78.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Df 1

Sig P>0.05

Out of the 100 patients around 22% females were affected, mostly the interphalangeal joints and males did not exhibit any small joint involvement. The incidences of small joints involvement were not statistically significantly associated with either of the gender namely male and female (P>0.05). (Table 4. & Figure 4 )
Small joint involvement along with sex-wise distribution in Rheumatoid arthritis patients

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affected</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Not affected</td>
<td>8</td>
<td>70</td>
</tr>
</tbody>
</table>
5.5. Duration of symptoms wise distribution in Rheumatoid arthritis patients

Table 5

<table>
<thead>
<tr>
<th>Duration (Months)</th>
<th>MALE</th>
<th>FEMALE</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;12</td>
<td>0.0</td>
<td>22.0</td>
<td>22.0</td>
</tr>
<tr>
<td>12-23</td>
<td>4.0</td>
<td>20.0</td>
<td>24.0</td>
</tr>
<tr>
<td>24-59</td>
<td>4.0</td>
<td>18.0</td>
<td>22.0</td>
</tr>
<tr>
<td>60-95</td>
<td>0.0</td>
<td>12.0</td>
<td>12.0</td>
</tr>
<tr>
<td>96-120</td>
<td>0.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Total</td>
<td>8.0</td>
<td>92.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>21.0±11.5</td>
<td>44.2±41.2</td>
<td>42.4±40.1</td>
</tr>
<tr>
<td>t</td>
<td>1.113 df=48</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Out of the 100 patients tested for RA, most of them were females and the impact of duration of symptoms on the clinical output along with the sex was compared. Most of the patients belonged to the 96 – 120 months duration of symptoms. The mean duration of males was 21.0±11.5 months and females was 44.2±41.2 months. The mean difference of duration between the male and female was not statistically significant (P>0.05). (Table 5 & Figure 5)
Duration of symptoms wise distribution in Rheumatoid arthritis patients

- <12: 20%
- Dec-23: 22%
- 24-59: 22%
- 60-95: 24%
- 96-120: 12%
5.6. Evaluation of RF By Latex Agglutination In Detection Of Rheumatoid Arthritis

Table 6.

<table>
<thead>
<tr>
<th></th>
<th>TRUE POSITIVE (a)</th>
<th>FALSE POSITIVE (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40</td>
<td>12</td>
</tr>
<tr>
<td>FALSE NEGATIVE (c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>38</td>
</tr>
</tbody>
</table>

- **Sensitivity** of RF by IgM latex agglutination: \( \frac{A}{A+C} \times 100 = 80\% \)
- **Specificity** of RF by IgM latex agglutination: \( \frac{D}{D+B} \times 100 = 76\% \)
- **PPV** of RF by IgM latex agglutination: \( \frac{A}{A+B} \times 100 = 76\% \)
- **NPV** of RF by IgM latex agglutination: \( \frac{D}{D+C} \times 100 = 79\% \)
5.7. Evaluation Of Anti CCP Antibody By ELISA For Detection Of Rheumatoid Arthritis

Table 7

<table>
<thead>
<tr>
<th>TRUE POSITIVE</th>
<th>FALSE POSITIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) 10</td>
<td>(b) 0</td>
</tr>
<tr>
<td>FALSE NEGATIVE</td>
<td>TRUE NEGATIVE</td>
</tr>
<tr>
<td>(c) 40</td>
<td>(d) 50</td>
</tr>
</tbody>
</table>

- Sensitivity of Anti CCP antibody ELISA: \( \frac{A}{A+C} \times 100 = 20\% \)
- Specificity of Anti CCP antibody ELISA: \( \frac{D}{D+B} \times 100 = 100\% \)
- PPV of Anti CCP antibody ELISA: \( \frac{A}{A+B} \times 100 = 100\% \)
- NPV of Anti CCP antibody ELISA: \( \frac{D}{D+C} \times 100 = 55\% \)
5.8. Evaluation of Anti-Keratin antibody by Immunofluorescence in detection of Rheumatoid Arthritis

Table 8

<table>
<thead>
<tr>
<th>TRUE POSITIVE (a)</th>
<th>FALSE POSITIVE (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>FALSE NEGATIVE (c)</td>
<td>TRUE NEGATIVE (d)</td>
</tr>
<tr>
<td>40</td>
<td>43</td>
</tr>
</tbody>
</table>

- Sensitivity of Anti Keratin antibody IF: \( \frac{A}{A+C} \times 100 = 20\% \)
- Specificity of Anti Keratin antibody IF: \( \frac{D}{D+B} \times 100 = 86\% \)
- PPV of Anti Keratin antibody IF: \( \frac{A}{A+B} \times 100 = 58\% \)
- NPV of Anti Keratin antibody IF: \( \frac{D}{D+C} \times 100 = 51\% \)
5.9. Evaluation of three tests namely the RF by IgM Latex agglutination, Anti CCP antibodies By ELISA and Anti Keratin antibodies by Immunofluorescene

From the below data , it is evident that anti CCP ELISA has the maximum specificity and positivity. Even though AKA is at close to anti CCP in specificity, the positive predictive value is way too less than that of anti CCP. The conventional RF has good sensitivity than anti CCP and anti Keratin. ( Table 9 & Figure 9)

Table 9

<table>
<thead>
<tr>
<th>SL NO</th>
<th>CATEGORY</th>
<th>LATEX</th>
<th>ELISA</th>
<th>IF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sensitivity</td>
<td>80%</td>
<td>20%</td>
<td>20%</td>
</tr>
<tr>
<td>2</td>
<td>Specificity</td>
<td>76%</td>
<td>100%</td>
<td>86%</td>
</tr>
<tr>
<td>3</td>
<td>Positive predictive value</td>
<td>76%</td>
<td>100%</td>
<td>58%</td>
</tr>
<tr>
<td>4</td>
<td>Negative predictive value</td>
<td>75%</td>
<td>55%</td>
<td>51%</td>
</tr>
</tbody>
</table>
Figure 9.
5.10. Comparison of testing efficiency of three tests (Latex agglutination, ELISA, IF) by McNemar test

On comparing RF and anti CCP, RF had the overall better diagnostic value in screening the disease. Anti CCP and anti keratin were almost equal but anti CCP was much better due to its high positivity. Anti CCP due to its better value as compared to AKA and RF can be used in the early diagnosis of the disease in order to prevent the erosive complications (Table 10 & Figure 10)

Table 10

<table>
<thead>
<tr>
<th>SL NO</th>
<th>CATEGORY</th>
<th>SENSITIVITY</th>
<th>SPECIFICITY</th>
<th>NPV</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RF and Anti CCP</td>
<td>100 %</td>
<td>54 %</td>
<td>100 %</td>
<td>20.8 %</td>
</tr>
<tr>
<td>2</td>
<td>Anti CCP and AKA</td>
<td>75 %</td>
<td>85.5 %</td>
<td>95 %</td>
<td>30 %</td>
</tr>
<tr>
<td>3</td>
<td>AKA and RF</td>
<td>58 %</td>
<td>57%</td>
<td>87 %</td>
<td>22 %</td>
</tr>
</tbody>
</table>
Figure 10.

[Bar chart showing data for AKA and RF, Anti CCP & AKA, and RF & Anti CCP with ppv, npv, specificity, and sensitivity indicated]
6. DISCUSSION

The study was conducted to assess the sensitivity and specificity of three tests namely, IgM RF, anti CCP, anti Keratin, in Tirunelveli Medical College and Hospital. The necessity for the comparison of these three tests was important in order to find out which test was ideal in identifying the late erosive complications at the earliest.

According to this study the mean age of the disease was (45.9 ±11.6), this value is almost equal to the study conducted by Eman Sh. Al- Obeidy et al in 2012 which had a mean age of 41.9± 12.5 in Iraqi women however Al-Haidary study in 2003 showed that the average of age is 42.1, which was almost comparable to this result [95]. While it was to some extent lower than that of Anaya, et. al. [96] and Pascual et.al [97] who observed that the mean age was 47±12.7 years for the Colombian RA woman and 49 ± 2.5 among Spanish patients respectively. The lower mean of the age probably is due to the fact that the life span of Indians are lowers than that for European.

There is a higher prevalence of RA among women rather than men with a mean value of 44.2+- 41.2, which may be due to the hormonal differences between them and in turn, theirs effects on the immune responses.
The significance of duration of the disease and its association with the joint damage in male and females, with a mean duration being 21.0±11.5 and 44.2±41.2 respectively. The mean difference of duration between them was not statistically significant. In a study conducted by Ahmed bolad et al, they found that the median duration of disease was significantly longer in the patients who developed erosions.

In the present study, RF has more sensitivity in diagnosing the disease in the initial screening process. The high positivity was observed to be 76%, which means it has good ability to point out whether the positive cases have disease. While other studies showed 88% in Nebraska, 66% in Germany, 70.2% in Colombia and 72.2% in North America and 80% in Southern Spain of the RA cases as mentioned by [96,98,99,100] respectively. The high RF positivity in these study was probably related to use ELISA technique, which is highly sensitive one than Latex agglutination test that has been used in this study.

In this study, the specificity of Anti CCP antibodies were highest and also their positivity was maximum of all the three test. This shows that it is indeed one of the best test to specifically point out the diagnosis also that the positive patients have more chance of complications. Anti-CCP antibodies are important for diagnosis in RA because they are as sensitive as and more specific than the IgM RFs in early and fully established diseases. In addition, they 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 RA \[101,102,96\]. Anti-
CCP antibodies and RF are superior to several genetic markers in predicting the
diagnosis of RA from undifferentiated arthritis in early arthritis patients \[97\]. In
addition, the combination of anti-CCP antibodies and IgM-RF has been found to
have a high positive predictive value for RA \[98\]. Most of these studies had a normal
group as the control population, like our study and a few other studies used
patients with other rheumatic diseases as the control group \[103,104\].

In a study done by B.L. Sharma et al, AKA was present in 2% of the healthy
controls and 56% patients with RA. Thus sensitivity of AKA for RA was 56%,
whereas its specificity was 98%, whereas in this study, the specificity was around
86%, which was more than the study conducted in European countries. AKA was
found to be more specific in contrast to RF which in turn has a specificity of only
76%. Further, RF is also present in other connective tissue diseases, whereas
AKA is not present in these diseases.

On comparing all the three tests, it is clear that RF by all means is the most
sensitive test for the purpose of screening in a large population, but in order to
clinch the diagnosis, Anti CCP is better for the diagnosis of the disease. AKA is
also by large a better test than RF but it has a low positivity compared to anti CCP.
The present study was conducted at Tirunelveli Medical College and Hospital, Palayamkottai, Tirunelveli district, Tamil Nadu from June 2016 to May 2017 to assess the sensitivity and specificity of IgM RF by Latex agglutination, anti CCP antibody by ELISA, anti Keratin antibody by Immunofluorescence.

A total of 100 serum samples were collected for this study, 50 from Patients with arthritis along with fulfilled ACR criteria who attended the Rheumatology Clinic as cases and 50 samples from patients with symptoms of arthralgia as controls.

IgM RF was done by Latex agglutination, with positive controls satisfactory, showing coarse clumps on agglutination. Anti CCP ELISA was done after following all precaution and the values were calculated appropriately. In AKA IF, presence of linear fluorescence lining was seen to prove it confirmatory.

Out of the 100 samples, 52 positive for IgM RF, 10 were positive for Anti CCP antibody and 17 positive for AKA FOR Rheumatoid arthritis.

Among the three assays, IgM RF was found to be highly sensitive (80 percent), whereas ELISA and AKA had an equal sensitivity and Anti CCP
antibody was found to be highly specific (100 percent) in detecting Rheumatoid arthritis, whereas AKA has equal specificity with RF.

- Statistically the testing efficacy of anti CCP antibody by ELISA in detecting the RA patients was higher that of other two tests.

- The sex wise, age wise, distribution of the disease was compared and showed that females of age group 40 – 49 were mostly affected.

- Joint wise involvement and relation with duration of symptom was compared and showed no significant changes as it needs to be compared with future complications.

- On summarizing, anti CCP antibodies may serve as a better diagnostic marker than RF and AKA in Indian population.
8. CONCLUSION

This study establishes the value of anti CCP antibodies in the diagnosis of RA and the advantage of this test in comparison with the AKA and IgM RF tests. Anti CCP antibodies are highly specific marker for RA in several diverse group of patient groups. The low sensitivity of this test indicates that a negative anti CCP antibody does not necessarily exclude the disease, but its high specificity means that positive result can proportionally increase the probability that the patient will have RA. It also has the potential to identify the patients who have significant ongoing disease activity, ensue more damage and who will probably benefit from an early belligerent treatment. A significant number of these patients do not have rheumatoid factor, and my not otherwise have been expected to develop severe aggressive disease it is also found that these anti CCP antibodies, tend be more stable, decline with treatment and also not seen in other arthritic diseases. Due to their presence in serum years before the onset of the disease, these antibodies shall be able to provide an early insight in pathogenesis of the disease.
9. BIBLIOGRAPHY

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PROFORMA

NAME: 

AGE/ SEX : 

OP/IP NO: 

WARD: 

DOA: 

DOD: 

COMPLAINTS:

DIAGNOSIS:

JOINT INVOLVEMENT:

- One large joint
- 2-10 large joints
- 1-3 small joints (with or without involvement of large joints)
- 4-10 small joints (with or without involvement of large joints)
- >10 joints (atleast one small joint)

DURATION OF SYMPTOMS

- < 6 WEEKS
- > 6 WEEKS

ROUTINE INVESTIGATIONS: 

SPECIAL INVESTIGATIONS:

Hb: 

RF: 

CRP: 

ANTI CCP Ab: 

ESR: 

ANTI KERATIN Ab: 

S. URIC ACID:
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