PREVALENCE AND CLINICAL UTILITY OF ANTI-CCP IN PATIENTS WITH INFLAMMATORY ARTHRITIS AT KENYATTA NATIONAL HOSPITAL.

A DISSERTATION TO BE SUBMITTED IN PART FULFILLMENT OF REQUIREMENTS FOR THE DEGREE OF MASTERS OF MEDICINE IN INTERNAL MEDICINE OF THE UNIVERSITY OF NAIROBI.

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I certify that this dissertation is my own original work, and has not been presented for a degree at any other university.

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To my parents Elijah and Jerusha who taught me the value of humility.
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I thank God for the gift of life, peace and sustenance.

I would like to thank my supervisors Dr. G.O.Oyoo, Professor E.O.Amayo and Dr.A.Amayo for their selfless and tremendous support and guidance throughout the study period.

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I am grateful to the staff of both the department of Clinical medicine and Pediatrics laboratories for ensuring that samples were well handled and processed to produce credible results for the study subjects.

Am also thankful to Roche, Torrent, Orchid, Sun and Novartis Pharmaceutical companies for their material and financial support for the project.

Finally I convey my deepest gratitude to my wife Beatrice and sons Trevor and Ryan and my friends who stuck with me through those difficult times.
LIST OF ABBREVIATIONS

ACPA-Anti-citrullinated protein antibody
ACR-American College of Rheumatology.
AFA-Antifilagrin antibody
AKA-Antikeratin antibody
Anti-CCP-Anti-citrullinated cyclic peptide
Anti-RA33-Anti-Rheumatoid Arthritis 33
ARF-Acute renal failure
CRF-Chronic Renal Failure
DM-Diabetes Mellitus
DMARDS-Disease Modifying Anti-Rheumatic Drugs
EDTA-Ethylene Diamine Tetra Acetate
ELISA-Enzyme linked immunosorbent assay
ESR-erythrocyte sedimentation rate
GALS-Gait, arms, legs and spine
HCV-Hepatitis C virus
HPT-Hypertension
IgE-Immunoglobulin E
IgG-Immunoglobulin G
IgM-Immunoglobulin M
JIA-Juvenile Idiopathic Arthritis
KNH-Kenyatta National Hospital
MCP-Metacarpophalangeal
MOPC-Medical Outpatient Clinics
MTP-Metatarsophalangeal
NSAIDS-Non Steroidal Anti-inflammatory Drugs
PAD—Peptylarginine Deaminase
PI-Principal Investigator
PIP-Proximal Interphalangeal
RA-Rheumatoid Arthritis
RF-Rheumatoid Factor
ROC-Receiver Operating Characteristics
SLE-Systemic Lupus Erythematosus
SPSS-Statistical Package for Social Scientists
UA-Unspecified Arthritis
UON-University of Nairobi
CONTENTS

Title...................................................................................................................i
Declaration.......................................................................................................ii-iii
Dedication..........................................................................................................iv
Acknowledgements..........................................................................................v
Abbreviations.................................................................................................vi-vii
Contents..........................................................................................................viii-ix
List of tables..................................................................................................x
List of figures.................................................................................................x
Abstract..........................................................................................................xi-xii

1.0 Introduction and Literature Review........................................................1-12

2.0 Research Question..................................................................................13

3.0 Justification of the study........................................................................13

4.0 Objectives................................................................................................13-14

5.0 Materials and methods............................................................................14-19

5.1 Study design.............................................................................................14

5.2 Study location.........................................................................................14

5.2 Study population....................................................................................14

5.3 Patient selection.....................................................................................14

5.4 Inclusion criteria..................................................................................14

5.5 Exclusion criteria..................................................................................14

viii
**LIST OF TABLES**

Table 1. Sensitivity and specificity of various studies on Anti-ccp ......................... 8
Table 2. Table showing presence and predictive potential of Anti-ccp ....................... 10
Table 3. Normal ESR measurements in regard to age .................................................. 18
Table 4. socio-demographic characteristics of study patients ...................................... 21
Table 5. Kappa and PPV for RF ..................................................................................... 25
Table 6. Kappa and PPV for Anti-ccp ............................................................................ 25
Table 7. Kappa agreement for RF and Anti-ccp .......................................................... 27
Table 8. Family history of RA ...................................................................................... 28
Table 9. Clinical characteristics of RA/UA .................................................................... 29

**LIST OF FIGURES**

Figure 1. Flow chart of the study patients ................................................................. 20
Figure 2. Age distribution of the study subjects ......................................................... 21
Figure 3. Rheumatoid Arthritis prevalence ................................................................. 22
Figure 4. Prevalence of RA and UA in the study group ............................................. 22
Figure 5. Prevalence of RF and Anti-ccp .................................................................... 23
Figure 6. Prevalence of Anti-ccp among RA and UA patients ..................................... 24
Figure 7. Distribution of Anti-ccp and ACR criteria ................................................ 24
Figure 8. Prevalence of RF among RA and UA patients .......................................... 25
Figure 9. Receiver Operating Characteristic Curves ............................................... 26
Figure 10. Anti-ccp levels and joint counts ............................................................... 29
ABSTRACT

Introduction

Rheumatoid arthritis (RA) is a debilitating condition that causes serious morbidity and mortality. Diagnosed early, the condition can be treated with appropriate therapy so as to either stop or slow the progression of the disease. However, early diagnosis of RA can be difficult as the disease may initially be indistinguishable from other forms of arthritis. American College of Rheumatology (ACR) criteria for the diagnosis of RA is such that by the time the patient fulfills this criteria the disease has caused serious irreversible morbidity hence the need to get a criteria that might pick the disease early enough. A new serological marker, Anti-cyclic citrullinated peptide antibodies (Anti-ccp) are highly specific for RA and have been used to confirm early diagnosis of RA when the features for the condition are indistinguishable from other arthritis.

Objective

The objective of this study was to determine the prevalence and clinical utility of Anti-ccp antibodies in patients with inflammatory arthritis at presentation to KNH medical clinics.

Design

This was a cross-sectional descriptive study.

Setting

The study was conducted at KNH MOPCs between the month of October 2008 to February 2009.

Results

A total of 134 patients with arthritis were screened. 1 declined consent. 133 patients were eligible and consented to participate in this study. The entry point for further analysis was a raised erythrocyte sedimentation rate (ESR) and 95 patients fulfilled this criteria and hence had inflammatory arthritis and their samples were further analysed. 64 patients had RA as per the ACR criteria. 31 patients who did not satisfy the ACR criteria were classified as unspecified arthritis (UA).
The mean age of the patients studied in the RA and UA as classified by the revised ACR criteria was 44.7 and 41.2 years (p=0.356) respectively.

The overall prevalence of Anti-ccp antibodies in the population was 47.4% in comparison to that of rheumatoid factor (RF) that was prevalent at 36.8%. The prevalence of Anti-ccp antibodies in patients with RA was 62.5% as compared to 16.1% of those who had UA (p=0.000). The prevalence of RF in patients who had RA was 50% as compared to 9.7% for those who had UA (p=0.000). The male to female ratio of subjects studied was 1:11. The calculated sensitivity and specificity for Anti-ccp was 62.5% and 83.9% respectively. 20% of patients who were RF negative had their sera test positive for Anti-ccp antibodies. 5.26% of patients who did not satisfy the ACR criteria were Anti-ccp positive. The number of joints involved positively correlated with the ACR and Anti-ccp levels.

**Conclusion**

Anti-ccp antibodies are more prevalent in this cohort of patients with inflammatory arthritis than RF hence its reliability in early diagnosis of RA. It was also concluded that ACR characteristics correlated well with Anti-ccp and RF. Inflammatory arthritis is more prevalent in females than males and affects the middle aged. A greater percentage of patients with inflammatory arthritis were Anti-ccp positive but did not satisfy ACR and were negative for RF. Patients who never satisfied the ACR criteria and were thus classified as UA can still have RA because of positive Anti-ccp. The sensitivity of Anti-ccp was higher than that of RF. About 5.26% of patients who were classified as UA and did not satisfy the ACR criteria were positive for Anti-ccp.
1.0 INTRODUCTION AND LITERATURE REVIEW

The emphasis in the management of Rheumatoid Arthritis is early diagnosis and intervention. The hypothesis on which this approach is based is that a window of opportunity exists where therapy has a disproportionate impact on outcome(1,2). It would appear logical to introduce therapy prior to irreversible damage(3).

An inherent problem with earlier assessment is accurate disease classification. Pathognomonic features of RA such as deformity and nodules are related to chronicity and are absent at presentation. The American College of Rheumatology(ACR) classification criteria for RA(4) include such parameters, making them insensitive when applied early(5,6). Also routine laboratory investigations such as acute phase markers and X-rays may be normal in up to 60 and 70% of patients respectively(7). Current best practice for early diagnosis of RA is reliant on the history and examination findings, with supplementary additional investigations.

Rheumatoid Factor(RF) has been widely used as a screening test for patients with arthritis. Although RF is prognostically useful, as it correlates with functional(8) and radiographic(9) outcomes in both RA and early inflammatory arthritis(10), as a diagnostic test it performs poorly, with low sensitivity and moderate specificity(11). Used in isolation, RF has little diagnostic utility, but has retained its place in practice because of its prognostic ability and the lack of an alternative test.

More recently a highly specific autoantibody system has been described for RA, in which patients develop antibodies to modified(citrullinated) arginine residues, and this has resulted in the development of the Anti-cyclic citrullinated peptide(Anti-ccp) antibody test, which has a sensitivity of 68% and a specificity of 97%(12,13). Further development yielded novel peptides and a second generation test. The Anti-ccp2 test has improved sensitivity (80% comparable to Immunoglobulin M-RF(IgM-RF)(70-75%) and equivalent specificity(>98%)(14). Moreover, 35-40% of RF-negative patients are Anti-ccp antibody positive. Anti-ccp antibodies have also demonstrated prognostic utility with regard to radiographic outcomes(15-17).
One of the most common autoimmune diseases is rheumatoid arthritis (RA), affecting 0.5-1% of the population(18). This systemic disease is marked by chronic inflammation of synovial joints, which leads to destruction of cartilage and bone and eventually to disability of the patient. Though not directly life-threatening, RA severely affects the quality of life of a patient and also has major economic consequences for society.

Currently, the classification of RA relies mainly on the criteria described by the American College of Rheumatology (ACR)(19). These criteria, originally formulated 50 years ago and last adjusted in 1987, include a combination of clinical signs(morning stiffness, soft tissue swelling, rheumatoid nodules), laboratory data(rheumatoid factor) and or radiographic evidence of joint involvement. While these criteria provide an overall 91-94% sensitivity and 75% specificity for diagnosing RA from other rheumatic diseases, diagnosis is based primarily on clinical symptoms of an already established and sometimes advanced disease(20). Since these parameters are often only sufficiently fulfilled when the damaging effects of the inflammatory process are already in progress, this set of criteria is not very suitable for the early diagnosis of RA(21).

An ideal strategy for RA diagnosis would involve sensitive and specific laboratory markers that detect RA early in its course, differentiate RA from other rheumatic diseases and provide prognostic information regarding an individual's disease progression. Treatment could therefore be started early and might help to diminish progression to severe erosive arthritis(22).

Of patients with RA, 65-85% have evidence of circulating RF, IgM or Immunoglobulin G(IgG) antibodies directed against the Fc portion of IgG(3). Immunoglobulin A(IgA) and Immunoglobulin E(IgE) can sometimes be detected.

Unfortunately, 3-5% of general population also have low levels of circulating RF and this level increases to 10-20% by age 65(18,20). The majority of these patients however will not develop RA. As RF can be detected in numerous other rheumatologic and inflammatory disorders, it is somewhat limited in its usefulness as a sole laboratory test for the diagnosis of RA. While RF
has proven useful in providing some prognostic information regarding disease severity, the need for an additional and prognostic marker for RA is evident(23).

Two promising antibodies with high specificity for RA were identified in the 1960s and 70s: antiperinuclear factor (APF) and anti-keratin antibodies (AKA) which can be isolated in many patients with RA. However, the tissue based immunofluorescence assays required for their detection severely limit widespread clinical utility of these tests(24).

In 1995, a French research group demonstrated that APF/AKA recognize epitopes on filagrin, an epidermal intermediate filament associated protein(25). Filagrin subunits are cleaved from a (pro)filagrin precursor, they are dephosphylated, and then a fifth of arginine residues are converted to citrulline by the enzyme phosphatidylarginine deaminase (PAD)(26).

These citrulline containing proteins, however, became a logical target for the development of novel assays in the detection of RA associated antibodies. In 1988, a Dutch research group led by Walther van Venrooj published experiments using an array of synthetic citrullinated peptides, some of which proved to be highly specific substrates for RA antibodies(24). Similar results were obtained using recombinant filagrin(25). Conformationary stable cyclic variants of these synthetic peptides were soon created, which improved the binding of RA associated antibodies without decreasing the test performance(27). The term anti-cyclic citrullinated peptide was adopted to describe antibodies detected by this assay. This test was subsequently commercialized into a second generation Anti-ccp2 Enzyme Linked Immunosorbent Assay (ELISA) with an overall improved sensitivity(27,28).

Data compiled from 13 studies demonstrated that Anti-ccp antibodies had a sensitivity of 61.5% and a specificity of 94.5% while IgM RF had a comparable sensitivity of 63.8% and a specificity of 77.4%(22).

Anti-ccp antibodies are often found in patients with erosive or polyarticular symptoms(29,30). Anti-ccp antibody formation precedes the development of clinical symptoms in some patients with RA and multiple studies have now demonstrated that the presence of anti-ccp antibodies can provide valuable prognostic information regarding the aggressiveness of disease progression(31).
While the presence of Anti-ccp antibodies may support a diagnosis of RA, this information should be considered along with the overall clinical context and ACR diagnostic criteria(19). Anti-ccp antibody testing may be particularly useful in diagnosing cases of RF negative RA as well as differentiating RA from other rheumatic diseases. The presence of these antibodies early in disease development opens a window of opportunity for early diagnosis and treatment of RA.

1.2 RHEUMATOID ARTHRITIS ASSOCIATED ANTIBODIES

RA is associated with several autoantibodies which can serve as diagnostic and prognostic markers(32). These include:

a) Rheumatoid factor

b) Antifilagrin antibodies (AFA)/Anti-citrullinated protein antibodies (ACPA)

i) antikeratin antibodies (AKA)

ii) antiperinuclear factor (APA)

iii) antibodies to citrullinated peptides (Anti-ccp)

iv) anti-Sa antibodies-targeting citrullinated vimentin

c) Anti-RA33

They all may possibly precede the onset of clinical RA by a variable duration of time.

1.3 RHEUMATOID FACTOR

Antibodies are directed against the Fc portion of IgG molecule. Studies done have shown polyreactive RF with binding specificity for substrates other than IgG such as nuclear components. The polyreactive RF is usually of IgM variety with low affinity. RF has considerable heterogeneity in rheumatic diseases.
RF can be detected in 80% of the patients but these antibodies are found in several other diseases as well as 10-30% healthy controls lowering its specificity for RA. Furthermore the test may take many years to become positive and therefore has low sensitivity for early RA (32).

The fact that the test is also positive in other inflammatory conditions such as Sjogren's syndrome, infections (bacterial and viral) and hematological conditions (cryoglobulinemia and plasma cell disorders) makes it have a low specificity for confirmation of early RA (34).

IgM RF, the isotype most typically detected, is seen not only in RA but also in various other conditions. IgA RF, may be a better indicator of T-cell dependent affinity matured antibodies directed to particular Fc-gamma epitopes relevant to RA than IgM RF, but it has never gained wide interest. The combined detection of IgM and IgA RFs in serum is a strong indicator of RA.

1.4 ANTI-FILLAGRIN ANTIBODIES

Antibodies system that has the greatest clinical potential for RA are antibodies directed to citrulline containing epitopes.

Citrulline is a non-standard amino acid as it is not incorporated into protein synthesis. It is however generated by posttranslational modification of arginine residues by PAD enzymes. Conversion of arginine into citrulline involves the replacement of an amine group by an oxygen atom on the side chain of this amino acid with loss of a positive charge. This process occurs in the synovium and is a target of the autoimmune system.

Auto antibodies against citrullinated antigens have been detected and used for diagnostic purposes for many decades via the well-known APF and AKA tests. Supported by the fact that APF and AKA share many features and are reactive with native filaggrin, these auto antibodies are now designated as antifilaggrin antibodies (35).

Using the laborious and inconvenient immunofluorescence assay, roughly 50% of RA sera can be scored AFA-positive. These tests also lack interlaboratory standardisation. A key finding was the discovery that the reactivity of these AFA was completely dependent on the presence of citrulline residues (present in mature filaggrin but not in profilaggrin). Since then, two
approaches for detecting autoantibodies to citrullinated epitopes have been taken: a protein-based and a peptide-based approach.

1.5 ANTI-CCP

These are antibodies detected by enzyme linked immunosorbent assay (ELISA) and constitute early specific markers for RA (36). The second generation ELISA for Anti-ccp achieves a 98% specificity and a sensitivity of as high as 80% which is a major improvement over the RF test (37). Anti-ccp antibodies can be detected in 50% of patients with early RA at a time when RF is negative allowing for improved diagnosis and early specific treatment. Many series in the literature point to associations with erosive, more severe and progressive disease in patients positive for Anti-ccp (38).

Anti-ccp antibodies are rarely found in other clinical conditions such as viral hepatitis C, Lyme disease, Graves disease, Systemic Lupus Erythematosus (SLE) and Sjogrens syndrome. Children with Juvenile Rheumatoid Arthritis (JRA) do not benefit from this test because its sensitivity is only 0.2-3%. Positive results are seen in the polyarticular RF positive subset of children that evolves to the erosive adult form of RA (39).

Many authors now suggest anti-CCP test should greatly improve accuracy for RA disease classification if added to the modified 1987 criteria of ACR.

Screening for citrulline-specific RA reactivity has been performed with several proteins, including both purified naturally occurring citrullinated proteins and in vitro-citrullinated proteins. For these purposes, mainly filaggrin, fibrinogen and myelin basic protein have been used. Although most of these proteins are arginine-rich, there is obviously a limit to the number of citrullinated epitopes associated with a certain protein. A complication of the use of natural antigens is that it is difficult to obtain reasonable amounts in sufficient purity in a reproducible way. Batch-to-batch variation also compromises standardization when in vitro citrullination is used to generate the antigen. Insufficient purity of the antigen lowers the specificity of the test because reactivities directed to other components (e.g. the PAD enzyme, the non-citrullinated part of the antigen, other contaminants) may be detected as well. The use of proper (non-
citrullinated) controls is therefore very important, as indeed has been noted by Vittecoq and colleagues\(^{(40)}\).

Using various technologies, assays with various sources of filaggrin have been developed, allowing sensitivities up to 60%. Using citrullinated fibrinogen, Nielen and colleagues\(^{(41)}\) reported a similar sensitivity (56%) for a cohort of early arthritis patients.

The use of synthetic citrullinated peptides for anti-citrullinated protein antibody detection can overcome many of the complicating factors of the protein-based approach. Synthetic peptide production and purification is cheap and easily standardized, and via peptides one can synthesize an unlimited pool of defined epitopes. Citrulline residues can be incorporated during synthesis of the peptides, leading to a homogeneous preparation of citrullinated molecules.

A major breakthrough came with the development of an ELISA that used filaggrin-based citrullinated peptides. The reactivity of RA sera was completely dependent on the citrulline residue(s) present, since the same peptides in which the citrulline was replaced by another amino acid were not antigenic. The variation in reactivity patterns against different citrullinated peptides clearly showed that the Anti-ccp response in RA is polyclonal. When a filaggrin-based cyclic peptide (cyclization increased the sensitivity) was applied in the first generation Anti-ccp 1 test, a sensitivity of 68% was obtained with very high specificity for RA (98%). Though better than the protein-based methods, the sensitivity was not as high as that of the routinely used RF test. Because filaggrin is not present in the synovium, dedicated libraries of citrulline-containing peptides were screened with RA sera to select for superior epitopes. This culminated in the Anti-ccp2 test, which displays a sensitivity of up to 80% without loss of specificity.\(^{(42)}\)

### 1.6 ANTI-CCP AS DIAGNOSTIC MARKERS

Diagnostic markers of disease ideally fulfill three requirements:

- (i) good sensitivity, to detect a high percentage of patients;
- (ii) good specificity, to limit false-positive results as much as possible; and
- (iii) early presence, to facilitate early diagnosis.\(^{(43)}\)
Over the last decade many studies have investigated the diagnostic performance of the anti-CCP test. Those using the Anti-ccp1 test have been reviewed by van Boekel and colleagues. Increasing data on the improved second-generation Anti-ccp test show that the Anti-ccp2 test result is a good diagnostic parameter for (early) RA.

1.7.1 SENSITIVITY AND SPECIFICITY

The first large cohort studies of Anti-ccp2 as a diagnostic marker showed that it combines RF-like sensitivity with almost absolute specificity for RA. These multi-centre studies showed that Anti-ccp2 antibodies, just like RF, are present in about 80% of established RA patients. In the healthy control group and the non-RA disease controls, the Anti-ccp2 test was only positive in maximally 1 and 5%, respectively.

The corresponding percentages of the RF (over 10% of healthy controls and more than 20% of disease controls) were markedly higher.

Several recent independent studies confirmed these sensitivity/specificity data for CCP2. The table below summarizes the sensitivities and specificities as obtained by individual studies for Anti-ccp2 in patients with RA.

<table>
<thead>
<tr>
<th>Study</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suzuki and colleagues</td>
<td>77%</td>
<td>98%</td>
</tr>
<tr>
<td>Dubucquoi and colleagues</td>
<td>65%</td>
<td>96%</td>
</tr>
<tr>
<td>deRyke and colleagues</td>
<td>73%</td>
<td>98.5%</td>
</tr>
</tbody>
</table>

Taken together, these studies show that the Anti-ccp2 test at least equals the RF level for sensitivity, but combines this with far better specificity. The fact that around 40% of RF-
seronegative patients appear to be Anti-ccp positive substantiates the additional diagnostic potential of Anti-ccp.

The Anti-ccp test also enables clinicians to effectively distinguish RA patients from other arthritic diseases in cases where the RF is not always discriminative. One of the first examples of such a role in differential diagnosis comes from patients with SLE. Mediwake and colleagues(40) showed that Anti-ccp (in this case Anti-ccp1) can be used to distinguish RA patients from SLE patients who present with erosive polyarthritis, which is often accompanied by RF seropositivity. Another disease that can readily be misdiagnosed because it often reveals RA-like arthropathies is chronic hepatitis C virus (HCV) infection, which is often accompanied by a positive RF. Wener et al.(41) reported a good discriminative ability of Anti-ccp2 over RF in a group of randomly selected HCV patients (44% RF+, none Anti-ccp2+). These data were confirmed by Bombardieri and colleagues(44)

Whereas RF was detected in 15% of the HCV patients (37% in case of joint involvement), no Anti-ccp2 positivity was seen in these patients. The value of Anti-ccp for use in differential diagnosis was also shown when comparing RA patients with polymyalgia rheumatica patients. Taken together, these data clearly outline the diagnostic strength of the Anti-ccp test for RA.

1.8 EARLY PRESENCE AND PREDICTIVE POTENTIAL OF ANTI-CCP

Because RA patients at their first visit to the clinician often do not fulfill the criteria for the diagnosis/classification of RA, an early detectable, highly predictive marker would greatly help the clinician in reaching a diagnosis. Obviously, the sensitivity and specificity of such a marker should be as high as possible. Recently, two studies, both making use of dated samples from RA patients who were former blood donors, reported the presence of Anti-ccp antibodies prior to the appearance of the first clinical symptoms of arthritis. Samples from 72 blood donors were characterized by Nielen and colleagues(39) for positivity of IgM-RF or Anti-ccp1.

Both serological markers were detectable long before the disease became clinically overt. In some patients, Anti-ccp1 was found up to 14 yr prior to the first clinical symptoms of disease. The same was found for 39% of the patients 5.3 yr (median) before the first visit to the clinic.
IgM-RF was also found in pre-disease samples, but not as far back (up to 10 yr) and in a smaller percentage of patients (23% at a median study of 3.3 years).

The second study, with a similar set-up, detected Anti-ccp2 and RF up to 10 yr before clinical disease in pre-disease blood samples of 83 RA patients. Anti-CCP2 positivity gradually increased in the years prior to the first clinical symptoms and had reached a positivity of 70% at the time the patients visited the rheumatology clinic for the first time. The sensitivity for detecting RF auto-antibodies in these predisease samples was slightly less than for Anti-ccp2.

From these studies it is clear that the production of Anti-ccp and RF autoantibodies is an early process in RA development, and that their presence is predictive for the development of this disease.

Recent data from several longitudinal studies confirm the predictive ability of Anti-ccp2 for RA development. van Gaalen and colleagues (45) used serological markers to predict which of the patients attending an early arthritis clinic, who were classified as undifferentiated arthritis (UA), would progress to RA within the next few years. Follow-up data of 318 patients with UA clearly showed the predictive potential of anti-CCP autoantibodies for the development of RA.

Thus, the combined early presence and predictive ability of anti-CCP may find an important clinical application in the design of treatment strategies.

**Table 2**

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample size</th>
<th>%with RA at 1yr</th>
<th>%with RA at 3yr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van Galen and co.</td>
<td>318</td>
<td>Anti-ccp2+ 75%</td>
<td>93%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anti-ccp2_ve -</td>
<td>25%</td>
</tr>
<tr>
<td>Vittecoq and co.</td>
<td>314</td>
<td>Anti-ccp2+ 90%</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anti-ccp2_ve -</td>
<td>-</td>
</tr>
</tbody>
</table>
1.9 ANTI-CCP ANTIBODY AS A PROGNOSTIC MARKER

Various studies have addressed the prognostic value of Anti-ccp antibodies. Though the Anti-ccp test has only recently become widely available, several studies have already demonstrated its ability to predict the development of erosive RA. An increasing number of studies with Anti-ccp2 confirm the prognostic potential. First, in a cohort of 379 early RA patients, Forslind and colleagues showed that Anti-ccp2 positivity at baseline (55%) predicts radiological damage and progression at 2 yr follow-up. Similar results were obtained by Kastbom and colleagues(44).

In their study, Anti-ccp2 positivity at baseline predicted disease activity at the 3-yr follow-up. In addition, Rönnelid et al.(45) showed that Anti-ccp2-positive early RA patients developed worse clinical disease and greater radiological damage within a few years in comparison with Anti-ccp2-negative patients.

1.10 RECENT STUDIES ON ACR/ANTI-CCP

Epidemiology Research Unit, University of Manchester, UK carried a study to evaluate whether the 1987 ACR classification criteria for RA in patients newly presenting with inflammatory polyarthritis (IP) predict persistent, disabling, or erosive arthritis. 486 patients with early IP referred to the Norfolk Arthritis Register were studied(46). The 1987 ACR criteria were applied at baseline, and assessed for their ability to identify patients referred to hospital for whom the diagnosis of RA was recorded by the hospital physician; patients at 3 years with persistent synovitis; moderate or greater disability; and erosions.

At baseline, 323 (67%) patients satisfied the ACR criteria in the classification tree format. The sensitivity of the criteria was good, ranging from 77 to 87% depending on the outcome. The specificities were poor, and thus the overall discriminatory ability showed little improvement over random probability. Among patients newly presenting with IP, the 1987 ACR criteria for RA had a low ability to discriminate between patients who developed persistent, disabling, or erosive disease and those who did not. They concluded that an alternative criteria are required for studies investigating early RA.(47)
In a study done in University of Nijmegen, The Netherlands whose objective was to find out the prevalence of autoantibodies directed towards citrullinated peptides in patients with or without RA and a group with early arthritis, Anti-ccp was found to be extremely specific for RA(98%) with a reasonable sensitivity(68%). In the early arthritis group, Anti-ccp was 96% specific for RA.

In comparison with IgM RF, Anti-ccp ELISA had a significantly higher specificity(96% for Anti-ccp versus 91% for IgM RF(p=0.016) at optimal cut off values. Sensitivity of both tests was moderate at 48% for Anti-ccp and 54% for IgM RF(p=0.36). Anti-ccp and IgM RF had a higher PPV(91%,p=0.013). They concluded that Anti-ccp ELISA is very useful for diagnostic and therapeutic strategy in RA of recent onset.

A study carried in Milan Italy to find out prevalence and clinical significance of Anti-ccp in both JIA and RA in adults was able to come up with a prevalence of 1.8% (2/109) and 63%(19/30) in children and adults respectively. In the pediatric rheumatic control group, none of the patients with juvenile onset SLE was positive for Anti-ccp further confirming its discriminative ability. Their calculated specificity ranged from 95-100%.

In a study carried out in Milan Italy in a cohort of early arthritis patients and followed up for three years, none of the baseline clinical parameters and acute phase reactants could identify patients who developed erosions, but 91% of patients who were RF positive and 96% who were Anti-ccp positive ended up with erosive disease.

A combined analysis of publications concerning more than 2000 patients with early undifferentiated arthritis found a prevalence of 23% of Anti-ccp antibodies at baseline. Prevalence increased to 51% in more than 1000 patients who fulfilled the ACR criteria on follow up for 18 months. Anti-ccp positive patients had more severe radiological destruction during the disease course.
2.0 RESEARCH QUESTION

What is the prevalence and clinical utility of Anti-ccp antibodies in patients with inflammatory arthritis at presentation to KNH?

3.0 STUDY JUSTIFICATION

Early diagnosis of RA is difficult as it is initially indistinguishable from other inflammatory and rheumatic diseases. The current diagnostic criteria based on ACR do not provide for early diagnosis.

Anti-ccp antibodies have been found to be very highly specific for RA and their early presence in patients even several years before the disease is clinically manifest makes it a suitable serological test to confirm early diagnosis.

No study has been done in our setup to determine the prevalence and clinical utility of Anti-ccp antibodies in our group of patients.

This study will form a basis for further longitudinal studies regarding the use of Anti-ccp in our setup.

RF is a serological test that has been relied on by the ACR criteria for decades. Its sensitivity and specificity for RA is low compared to that of Anti-CCP hence the need for a study on this test to see how it compares with RF in our patients.

4.0 OBJECTIVES OF THE STUDY

4.1 BROAD OBJECTIVE

The main objective of the study was to determine the prevalence and clinical utility of Anti-ccp antibodies in patients with inflammatory arthritis at presentation to the KNH.

4.2 SPECIFIC OBJECTIVES

1. To determine the prevalence of Anti-ccp antibodies in patients with inflammatory arthritis.
2. To determine the prevalence of RF in patients with inflammatory arthritis.
3. To determine the proportion of patients with inflammatory arthritis who satisfy the ACR criteria.
4. To compare the Anti-ccp antibody and RF prevalence in patients with inflammatory arthritis at KNH.

5.0 METHODOLOGY

5.1 Study design

This was a cross-sectional study.

5.2 Study location

The study was carried out at Kenyatta National Hospital medical outpatient clinics (MOPC).

5.3 Study population

Patients aged 18 years and above referred to the medical clinics with arthritis.

5.4 Patient selection

5.4.1 Inclusion criteria

1. Patients referred to medical clinics with arthritis.

2. Patients who have given signed informed consent.

3. Age limit was 18yrs and above.

5.4.2 Exclusion criteria

1. Patients who had acute febrile illnesses (proven viral/bacterial).

2. Patients who were known to have other autoimmune diseases such as SLE/Sjogrens syndrome.

3. Patients known to have gout and septic arthritis.
5.5 Case definition/variables

**Arthritis** - patient with inflammation of the joint, swelling, pain, stiffness and tenderness.

**Inflammatory arthritis** - patient with arthritis that is worse in morning, associated with morning stiffness and improves with activity with an elevated ESR.

**Rheumatoid arthritis** - patient with signs and symptoms that satisfy the ACR criteria for RA. At least four elements of the criteria to be fulfilled.

**Unspecified arthritis**: patient with non-specific signs and symptoms of arthritis not satisfying the classification criteria for RA.

**Gout** - patient known to have had characteristic crystals in the joint fluid and or more than one attack of acute arthritis.

**Septic arthritis** - patient known to have an infection (bacterial) in the joint cavity.

**Variables**

**Elevated ESR**
- >15mm/hr and >20mm/h for men and women under 50yr of age respectively
- >20mm/hr and >30mm/h for men and women older than 50yrs respectively

**Anti-ccp:**
- positive above 5iu,
- negative below 5.0iu according to the manufacturers’ instructions

**RF:**
- positive above 50iu
- negative below 50iu.
5.6 Screening and recruitment

In the medical clinics, all new patients referred with arthritis were screened for recruitment into the study.

Consecutive sampling was used to recruit patients with inflammatory arthritis until the desired number of patients for the study was achieved.

5.7 Sample size estimation

Taking the prevalence of Anti-ccp antibodies in previous studies to be between 23-63%(51) and targeting a 95% confidence interval and a degree of precision of 10% the formula below was used to calculate the sample size.

\[
\text{Sample size} = \frac{z^2 (p)(1-p)}{c^2}
\]

Where:-

\(z=\) is the standard normal deviate for 95% confidence interval which is equal to 1.96.

\(p=\) average prevalence of Anti-ccp antibodies which is equal to 43%.

\(c=\) the precision expressed as a decimal which is equal to 0.1.

Substituting these values in the above formula, the minimum sample size required was 94 patients.
5.8 Clinical methods

5.8.1 Patient Evaluation

The investigator and his assistant introduced themselves to the selected participants, the purpose of the study was explained and after assurance of confidentiality consent was obtained.

An investigation-administered questionnaire was administered through face to face interview and filled out by the interviewer.

The investigators obtained such data that consisted of socio demographics including age, gender, marital status, place of residence, and occupation. Disease history was obtained to include duration of illness, when first diagnosed and family history.

5.8.2 Clinical Evaluation

Physical examination was carried out using the Gait Arms Legs Spine(GALS) screening system. ACR criteria was then applied to classify patients into RA and UA. The number of joints swollen and deformed were recorded. Patients were also examined to find out whether they had rheumatoid nodules, deformity of the upper and lower limb joints.

5.9 LABORATORY METHODS

5.9.1 Specimen Collection and Handling

Consenting patients had about 5mls of venous blood drawn from the forearm using a non heparinized needle and syringe and this blood was allowed to stand at room temperature to get serum for Anti-ccp, RF antibodies. 2ml of blood was collected in an Ethylene Diamine Tetra Acetate(EDTA) bottle for ESR estimation which was done immediately. RF was also done immediately. Serum for Anti-ccp test was frozen and was done as a batch at the end of the study period.
5.9.2 Laboratory Analysis

**RF:** This was measured in the Department of Medicine laboratory by use of agglutination method.

**Anti-CCP antibodies:** This was carried out in the department of Pediatrics Laboratory by use of Axysm Machine that uses automated ELISA for the detection of IgG and IgA anti-CCP. Results were interpreted thus:

Negative..................<5.0 IU,  Positive..........................>5.1IU.

**ESR:** This was measured in the Department of Medicine laboratory using the Winthrop method. The normal limits were interpreted as shown in the study variables.

5.9.3 Quality Assurance:

Strict internal quality control was performed to ensure that accurate results were obtained.

5.10 Data management and analysis

All data was collected on the study proforma and entered into a computer database. The data was then cleaned and verified. Statistical analysis was done using statistical package for social scientists (SPSS) version 15.0 software. Data was summarized into means, ranges, ratios and then presented in form of pie charts and tables.
5.10.1 Data Analysis

Patients were categorized as RA and UA using the ACR criteria. The patients baseline characteristics such as gender, age were analyzed for the two groups. The patients were also further categorized as Anti-ccp positive or negative in each subgroup. This applied also to RF.

The family history of RA was compared with Anti-ccp positivity. Similarly this applied to RF. The prevalence of inflammatory arthritis from the patients sampled was obtained. The proportions of UA to that of RA was also done. The number of joints involved was compared to the Anti-ccp positivity and that of RF.

The proportions of patients positive for Anti-ccp in the RA was compared to that positive for Anti-ccp in the UA group.

The Kappa statistic was used to calculate the Kappa agreement for the two tests. Cross tabulation tables were used to get sensitivity and specificity for both RF and Anti-ccp using ACR criteria as the gold standard.

6.0 ETHICAL CONSIDERATIONS

1. The study was carried out after approval from the department of medicine (UON) and KNH ethics committee.

2. Patients were included in the study after being explained the purpose of the study and giving a signed informed consent.

3. Participation in the study was voluntary and patients were at liberty to withdraw from the study without any prejudice.

4. The patients right to privacy was respected.

5. Study results were given to the primary care physicians to allow for provision of better health care and confidentiality was maintained.
7.0 RESULTS
7.1 FLOW CHART OF THE STUDY SUBJECTS

Figure 1
7.2 Socio-demographic characteristics of the patients

The study population was 95 patients selected from a group of patients referred with arthritis to MOPC. The patients' age ranged from 18 to 82 years and had a mean age of 43.5 years. The age distribution is as shown in figure 2.

Table 4: Socio-demographic characteristics of the study group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean/Frequency (%)</th>
<th>N = 95</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years (Mean)</td>
<td>43.5</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>8 (8.4)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>87 (91.6)</td>
</tr>
</tbody>
</table>

The sex distribution of the study population was 91.6% female and 8.4% male giving a M:F ratio of 1:10 in the study population. (table 4)

Figure 2: Age distribution for the study group
7.3 Rheumatoid arthritis (RA)

Using the ACR criteria, rheumatoid arthritis (RA) was identified in 67.4% of the patients.

As illustrated in figure 4, there was no difference between male and female groups of patients in terms of prevalence of RA and UA (P=0.759). In addition, mean age was not statistically significant between the RA (44.7 years) and UA patients (41.2 years), P=0.356.
7.4 RF and ANTI-CCP

The prevalence of Anti-ccp (47.4%) was higher than that of RF (36.8%), $P=0.05$

Figure 5: Prevalence of RF and Anti-ccp
7.4.1 ANTI-CCP in RA and UA patients

62.5% of RA patients were Anti-ccp positive compared to 16.1% of the UA patients. (P=0.000), (Figure 6).

![Figure 6: Prevalence of Anti-ccp among RA and UA patients](image)

Figure 6: Prevalence of Anti-ccp among RA and UA patients

![Figure 7: Distribution of Anti-ccp titres versus RA and UA patients](image)

Figure 7: Distribution of Anti-ccp titres versus RA and UA patients.
As shown in figure 7, the titres of Anti-ccp were markedly higher for patients who satisfied the ACR criteria than those who did not. However there were few patients who had higher titres of Anti-ccp who were in the UA.

7.4.2 RF in RA and UA patients

As illustrated in figure 8, a half of RA patients were RF positive. In UA patients, 9.7% were RF positive (p=0.000).

![Figure 8: Prevalence of RF among RA and UA patients](image)

7.4.3 Anti-ccp and RF versus ACR criteria - table 5

<table>
<thead>
<tr>
<th>RF</th>
<th>ACR</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RA</td>
<td>UA</td>
</tr>
<tr>
<td>Positive</td>
<td>32 (33.7%)</td>
<td>3</td>
</tr>
<tr>
<td>Negative</td>
<td>32</td>
<td>28 (29.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>64</td>
<td>31</td>
</tr>
</tbody>
</table>

Kappa – 32.5%, PPV – 91.4%
Sensitivity-50%, Specificity-90.3%
Table 6

<table>
<thead>
<tr>
<th>Anti-ccp</th>
<th>ACR</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RA</td>
<td>UA</td>
</tr>
<tr>
<td>Positive</td>
<td>40 (42.1%)</td>
<td>5</td>
</tr>
<tr>
<td>Negative</td>
<td>24</td>
<td>26 (27.4%)</td>
</tr>
<tr>
<td>Total</td>
<td>64</td>
<td>31</td>
</tr>
</tbody>
</table>

Kappa - 40.0%, PPV-80%

Sensitivity -62.5%, Specificity 83.9%

![ROC Curve](image)

1 - Specificity

Diagonal segments are produced by ties.

Figure 9-ROC curves
### 7.4.4 ANTI-CCP versus RF

The number of patients who were negative for both Anti-ccp and RF were 48 giving a negative concordance rate of 50.5% for the study population. The number of patients who were positive for both Anti-ccp and RF was 33 giving a positive concordance rate of 34.7%.

In the total study population, the total number of patients who were RF was 60. Twelve (12) (20%) of these patients were Anti-ccp positive. 32 of these patients who were RF negative satisfied the ACR criteria for RA. Of the 32 who satisfied the ACR criteria for RA, 10 (31%) were Anti-ccp positive. Hence a greater percentage of patients would miss treatment for RA yet they have RA.

### Table 7

<table>
<thead>
<tr>
<th>RF</th>
<th>Anti-ccp</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>33 (34.7%)</td>
<td>2</td>
</tr>
<tr>
<td>Negative</td>
<td>12</td>
<td>48 (50.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>50</td>
</tr>
</tbody>
</table>

**Kappa = 70%**

The Kappa agreement for the two tests is high.
7.5 The Patients with UA who were Anti-ccp positive

Five patients who were classified as UA using ACR criteria had their sera test positive for Anti-ccp. Three of those patients were RF positive. Three of them had negative family history for RA. The mean duration of illness was 126 weeks which was higher than the mean (61.1 weeks) duration for the total study population. The mean Anti-ccp titre was 127.64iu compared to the mean for the whole study population that was 59.68iu. The mean joint count was 10.4, similar to the mean found for patients who were classified as RA. The mean age of this group of patients was 45.4 years and hence comparable with the mean for the study population.

7.6 Family history of RA.

Family history of RA was present in 25.3% of all patients. Family history of RA was reported among 25% and 25.8% of the RA and UA patients respectively hence was not significantly associated to presence or absence of RA (P=0.932).

Table 8: Family history of RA.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency (%)</th>
<th>N = 95</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family history of RA</td>
<td>24</td>
<td>(25.3)</td>
</tr>
</tbody>
</table>

7.6.3 Duration of illness

The range of duration of illness was 2-260 weeks with a mean duration of illness of 62.8 weeks ± 54.8SD. The mean duration of illness was higher by about 10 weeks among the RA patients than the UA patients. However, the difference between the two means was not significant (P=0.433).
Table 9: Clinical characteristics of RA and UA patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>RA (n = 64)</th>
<th>UA (n = 31)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of illness (weeks- mean)</td>
<td>65.8</td>
<td>56.4</td>
<td>0.433</td>
</tr>
<tr>
<td>No of joints involved</td>
<td>10.2</td>
<td>8.4</td>
<td>0.011</td>
</tr>
</tbody>
</table>

7.6.4 Number of joints involved

The range of joints was 2-20 joints with a mean number of joints involved among the 95 patients studied being 9.6±3.2SD. The number of joints was significantly different between the RA and the UA patients (P=0.011). The average number of joints involved among the RA patients was higher (10.2 joints) than the UA patients (8.4 joints).

Figure 10: Distribution of joints involved and prevalence of Anti-ccp
8.0 DISCUSSION

8.1 BASELINE CHARACTERISTICS

Ninety five (95) patients were studied and hence the minimum sample size required for the study was met.

From the results, it can be seen that the majority of the study population were female (91.6%) (M:F=1:10). This female preponderance is keeping with other studies done previously. (52,53,54).

The mean age of patients classified as RA as per the ACR criteria was 44.7 years as compared with that of UA which was 41.2 years but this was not statistically significant between the two groups. This figure is closely similar to one found by Oyoo (53) and Bagg et al (55) who found this age to be 44.5 and 43 years respectively. Owino (52) found a mean age of 41.3 years. The age characteristics found in these studies is much lower compared to those recorded in the United Kingdom study by Wiles et al (56) who found a peak age of 45-74 years for females and 65-74 years for males. Similarly younger age groups in black patients (36.6 years) compared to 44.2 years of their Caucasian counterparts was recorded by Mody (57) and Adebajo and Reid in Nigeria (58) and could therefore probably be due to a racial difference. From our study we can deduce that inflammatory arthritis afflicts the relatively young and productive members of our society hence the need to control this condition early enough so as to prevent morbidity and mortality.

8.2 ACR CRITERIA

Sixty four patients (67.4%) satisfied the ACR criteria and were thus classified as RA. These results are similar with those found at baseline in an early arthritis clinic by Harrison BJ et al (59). Among 323 patients studied, 67% satisfied the ACR criteria for RA. Higher number of patients may have satisfied the ACR criteria due to recall bias of the study subjects. This is because questions regarding the ACR criteria may be misinterpreted by the patient and hence explain the high number of patients who satisfied the ACR criteria. Furthermore the sensitivity and specificity of ACR is quite low for the diagnosis of early RA.
8.3 ANTI-CCP

The overall prevalence of Anti-ccp was 47.4% compared to 36.8% for RF. Thus the population with inflammatory arthritis who were Anti-ccp positive was significantly higher than that of RF (p=0.05) and hence the confirmation from this study that Anti-ccp is usually picked early in the disease process as compared to RF which is usually picked late in the disease process. Nielen and coworkers (67) did a study on stored blood samples of blood donors who later developed RA and found that the presence of Anti-ccp preceded the development of RA much earlier in the disease process than RF by a difference of about 4 years.

Vittecoq and coworkers (65) in a similar study found the prevalence of Anti-ccp to be 41% and that of RF to be 28% at presentation to the clinician and these results are comparable to those of our study. They concluded that Anti-ccp detected more positive subjects compared to RF.

The above prevalence of 47.4% was relatively higher compared to 33% that was found by D.M Lee et al (60) in Harvard Medical school. This might have been the case because these investigators looked at the prevalence of Anti-ccp in a study that included patients with SLE, osteoarthritis, spondylitis, JRA, psoriatic arthritis, fibromyalgia that were excluded from our study hence lowering their prevalence.

The greater majority of the patients classified as RA as per the ACR criteria had their blood test positive for Anti-ccp (62.5%) as compared to UA (16.1%) which was statistically significant (p=0.000). Forty (88.9%) of the patients who tested positive for Anti-ccp were classified as RA. D.M Lee et al (60) found almost a similar figure of 83% in these subset of patients. Hence Anti-ccp is significantly correlated with the ACR criteria in classifying patients into RA and UA.

S.K. Sharif et al (66) in Tehran Iran carried out a comparative study of Anti-ccp and RF for the diagnosis of RA among a group of patients with inflammatory arthritis and found a prevalence of 61.7% and 85% for Anti-ccp and RF respectively in patients who were classified as RA by ACR. In patients classified as UA, they found a prevalence of Anti-ccp of 10.9% compared to 16.1% found in our study.
The Anti-ccp results compare well with those found in our study but that of RF is way above what was found in our study. This may be due to the selection criteria used in the Tehran study where patients already diagnosed as RA were compared with those patients with UA.

8.4 RHEUMATOID FACTOR
RF was positive in 50% of the patients classified as RA as compared to 9.7% classified as UA (p=0.000). The prevalence of RF in this cohort of patients classified as RA is lower than earlier studies (63) that had a prevalence of 70-80%. Owino et al (52) found a prevalence of RF of 78.9%. This prevalence might have been lower given the fact that most of the patients recruited into our study had a shorter duration of disease (62.8 wks) than the study that was done by Owino et al (64.97 months). This may also have been due to the fact that the researchers in these studies may not have excluded patients who had comorbidities that can cause RF to be positive. Such patients were excluded from our study. Furthermore, RF tends to become positive as the disease progresses.

This also correlated positively with the ACR criteria but less significantly than Anti-ccp correlation. This further confirms that Anti-ccp picks more patients with RA than does RF. There is therefore the need to adopt Anti-ccp as part of the ACR criteria as it can pick many patients with RA than RF.

8.5 SENSITIVITY AND SPECIFICITY OF RF AND ANTI-CCP
The calculated specificity of Anti-ccp of 83.9% was lower than that of RF in the study subjects but sensitivity higher than that of RF. This was not expected as most studies suggest otherwise. This may be explained by the fact that by using ACR criteria as the gold standard may have falsely increased the sensitivity and specificity of RF. This is because RF is part of the ACR criteria for the diagnosis of RA.
Furthermore, due to recall bias of the study subjects for the various components of the ACR criteria, we may have had patients falsely satisfying the ACR criteria and hence lowering the specificity of the Anti-ccp.

Anti-ccp is a fairly new test and its performance in this part of the world is not known. Hence further longitudinal studies are needed to find the specificity and sensitivity of our population.

8.6 RF, ANTI-CCP AND CLINICAL CHARACTERISTICS

8.6.1 JOINT COUNT

The joints involved in the two groups were statistically significant (10.2 in RA as compared to 8.4 in UA p=0.011). Since the majority of the patients who had RA as per the ACR criteria had positive Anti-ccp, we can deduce that joint count positively correlated with Anti-ccp which as we know is associated with more erosive and severe forms of RA. Anti-ccp has been shown to be an independent predictor of radiological damage and progression (62).
8.6.2 THE RF NEGATIVE PATIENTS WHO HAD POSITIVE ANTI-CCP

In the total study population, 60 patients were RF negative, 20% of whom had positive Anti-ccp. This produces similar results obtained in other studies that found a prevalence of 20-43% (65, 66). D.M Lee et al (60) found 34% of patients who were RF negative testing positive for Anti-ccp. These values suggest important diagnostic utility where previously serology had been unhelpful. Hence from this prevalence of Anti-ccp in seronegative individuals, using Anti-ccp antibody would appear to select seronegative RA patients and so may have important implications for patient management.

Of these patients who were RF negative, 32 satisfied the ACR criteria for RA. This again shows that RF was highly seronegative in patients who satisfied the ACR criteria. This in clinical practice may cause diagnostic and management difficulties.

Of the 32 who satisfied the ACR criteria and were RF negative, 10 were Anti-ccp positive. This also strongly suggests that a greater number of patients with early arthritis will test positive for Anti-ccp earlier than they do for RF.

The number of patients who were negative for both Anti-ccp and RF was 46 bringing the negative concordance rate for the two tests at 48.4%. The positive concordance rate was 33 (34.7%). The number of patients who had positive concordance rate and satisfied ACR was 30. 16 patients were either Anti-ccp positive or RF negative and vice versa.
8.6.3 THE UA PATIENTS WITH POSITIVE ANTI-CCP

Five patients classified as UA tested positive for Anti-ccp which was 5.26% of the total population studied. These group of patients had higher titres for Anti-ccp than the average for the whole study population hence a conclusion can be drawn that despite being negative for ACR, these patients can still be presumed to have RA. Furthermore, three out of five of these patients had their sera test positive for RF. Their mean joint count and the age never differed significantly from the total population.

Their mean Anti-ccp titres (127.64iu) were away above the average for the whole study group (59.8). This information is important as it tells us that Anti-ccp can be used to diagnose patients who have RA despite the fact that they have not satisfied the ACR criteria for RA. These five patients diagnosis of RA may have been missed because of their non-specific signs and symptoms. These are the patients who benefit most from Anti-ccp when the diagnosis of RA is in doubt and hence its clinical utility.

8.6.4 FAMILY HISTORY OF RA

The family history of RA was not statistically significant between the two groups. This may be attributed to the fact that the patients might have had recall bias and hence accurate determination of the true family history is difficult.
9.0 STUDY LIMITATIONS

1. This was a hospital based study and hence generalisability of study results may not apply to the general population.

2. Due to the study design, patients who had RF positive and had a low ESR were not further analysed for Anti-ccp and this could have added more information.

3. Due to the study design follow up of patients was not possible especially the patients who never satisfied the ACR criteria.

4. Components of the ACR criteria may have been misinterpreted by the patients due to recall bias.

10. CONCLUSIONS

There is a high prevalence of Anti-ccp in patients with inflammatory arthritis and the prevalence was higher in those who satisfied ACR criteria than those who did not. The prevalence of RF in this same group of patients was lower than that of Anti-ccp. The prevalence of RF was lower than that of Anti-ccp in those who satisfied the ACR criteria.

A high percentage of patients with inflammatory arthritis satisfied the ACR criteria at 67.4%. Anti-ccp was found to be more sensitive than RF. Many patients (20%) who were RF negative were Anti-ccp positive.

11. RECOMMENDATIONS

From this study it is recommended that RF and Anti-ccp antibodies be used to complement each other in patients with seronegative arthritis. Further evaluation of clinical utility of Anti-ccp for RA diagnosis to include control group of patients with other connective tissue diseases. Where the probability of RA is low in patients who do not satisfy the ACR criteria and the diagnosis is in doubt, Anti-ccp should be done to exclude diagnosis of early RA. That a follow up study be done to evaluate the course of the illness in patients with both positive and negative Anti-ccp.
13.REFERENCES


29. Hoet RM, van Venrooij WJ. The antiperinuclear factor (APF) and antikeratin antibodies (AKA) in rheumatoid arthritis. 1992;299—318.


THE APPENDICES

THE ACR CRITERIA

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

2. Arthritis of three or more joint areas
   At least three joint areas simultaneously have had soft tissue swelling or fluid [not bony overgrowth alone] observed by a physician. The fourteen possible areas are right or left PIP, MCP, wrist, elbow, knee, ankle, and MTP joints.

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

4. Symmetric arthritis
   Simultaneous involvement of the same joint areas [as defined above in 2] on both sides of the body [bilateral involvement of PIPs, MCPs, or MTPs is acceptable without absolute symmetry].

5. Rheumatoid nodules
   Subcutaneous nodules, over bony prominences, or extensor surfaces, or juxta-articular regions, observed by a physician.

6. Serum rheumatoid factor
   Demonstration of abnormal amounts of serum RF by any method for which the result has been positive in <5% of normal subjects.

7. Radiographic changes
   Radiographic changes typical of RA on postero-anterior hand and wrist radiographs, which must include erosions or equivocal bony decalcification localized in or most marked adjacent to the involved joints [osteoarthritis alone do not qualify]. For classification purposes, a patient shall be said to have RA if she / he has satisfied at least four of these seven criteria. Criteria 1 through 4 must have been present for at least six weeks. Patients with two clinical diagnoses are not excluded. Designation as classic, definite, or probable RA is not to be made.
<table>
<thead>
<tr>
<th>Activity</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gait</strong></td>
<td>Symmetry and smoothness of movement</td>
</tr>
<tr>
<td></td>
<td>Normal stride length</td>
</tr>
<tr>
<td></td>
<td>Ability to turn normally</td>
</tr>
<tr>
<td><strong>Spine (standing)</strong></td>
<td></td>
</tr>
<tr>
<td>Inspection from behind</td>
<td>Scoliosis</td>
</tr>
<tr>
<td></td>
<td>Symmetrical muscle bulk</td>
</tr>
<tr>
<td></td>
<td>Level iliac crests</td>
</tr>
<tr>
<td></td>
<td>No popliteal swelling</td>
</tr>
<tr>
<td></td>
<td>Normal hindfoot alignment</td>
</tr>
<tr>
<td><strong>Trigger point tenderness</strong></td>
<td>Pressure over mid-supraspinatus</td>
</tr>
<tr>
<td>Inspection from the side</td>
<td>Kyphosis</td>
</tr>
<tr>
<td></td>
<td>Normal flexion (‘touch your toes’)</td>
</tr>
<tr>
<td>Inspection from in front</td>
<td>Normal cervical lateral flexion (‘touch your ear on your shoulder’)</td>
</tr>
<tr>
<td>Arms (sitting on couch)</td>
<td></td>
</tr>
<tr>
<td>Body Part</td>
<td>Examination</td>
</tr>
<tr>
<td>-----------</td>
<td>-------------</td>
</tr>
<tr>
<td>Hands</td>
<td>Wrist/finger swelling/deformity</td>
</tr>
<tr>
<td></td>
<td>Squeeze across 2nd to 5th metacarpals</td>
</tr>
<tr>
<td></td>
<td>(tenderness indicates synovitis of metacarpophalangeal joints)</td>
</tr>
<tr>
<td></td>
<td>Turn hands over (inspect for muscle wasting, assess normal pronation/supination of forearm)</td>
</tr>
<tr>
<td>Grip strength</td>
<td>Power grip ('make a tight fist')</td>
</tr>
<tr>
<td></td>
<td>Precision grip ('put fingers on thumb')</td>
</tr>
<tr>
<td>Elbows</td>
<td>Full extension ('arms out straight')</td>
</tr>
<tr>
<td>Shoulders</td>
<td>Abduction and external rotation of the shoulders ('hands behind your head')</td>
</tr>
<tr>
<td>Legs (lying on couch)</td>
<td></td>
</tr>
<tr>
<td>Knees</td>
<td>Knee swelling/deformity</td>
</tr>
<tr>
<td></td>
<td>Quadriceps muscle bulk</td>
</tr>
<tr>
<td></td>
<td>Check for knee effusion</td>
</tr>
<tr>
<td></td>
<td>Crepitus during passive knee flexion</td>
</tr>
<tr>
<td>Hips</td>
<td>Check internal rotation of hips</td>
</tr>
<tr>
<td>Feet</td>
<td>Squeeze across metatarsals (tenderness indicates synovitis of metatarsophalangeal joints)</td>
</tr>
<tr>
<td></td>
<td>Check for callouses</td>
</tr>
</tbody>
</table>
STUDY PROFORMA

Serial number............

Demographic data
Age in years .......
Sex (male□, female□)
Marital status
(single□, married□, separated□, divorced□, widowed□)
Education status
(none□, primary□, secondary□, tertiary□)
Place of residence
Rural □ periurban □ urban □

History

How long have you had signs and symptoms of arthritis?
2wks □ 6wks □ 12wks □ 1yr □

Have you been prescribed medication for your illness?
Yes □ no □

If yes to the above question, which ones?
NSAIDS □ DMARDs □ Don’t know □

Do you have family member(s) who have been diagnosed to have RA?
Yes □ No □

If the answer to question is yes, how many?
1 □ 2 □ 3 □ 4 □

Do you have morning stiffness in and around joints?
Yes □ No □

If yes to the above question, how long does it last?.
Do you have pain in the joints?
Yes □ No □
If so do they involve both sides of the body on similar joints?
Yes □ No □
How many joint areas are involved simultaneously?
None □ one □ two □ three □ >3 □
Do you have swelling in the hand joints?
Yes □ No □
Do you have swellings over bony prominences?
Yes □ No □
Have you had a test done on you in a bid to confirm your diagnosis?
Yes □ no □
If yes to the above question, which test is it?
don’t know □ RF □ Anti-CCP □ CRP □
What were the test results for Anti-CCP?
Weak positive □ moderate positive □ strong positive □ negative □
What were the results for RF?
Positive □ negative □
Do you have any other comorbid conditions?
Yes □ No □
If yes to the above question, which conditions?
DM □ Heart disease □ HPT □ MI □ ARF □ CRF □
Physical examination
Number of joints that are tender..................
Symmetrical involvement..........
Number of deformed joints..........
Presence of rheumatoid nodules.............
EXPLANATION AND CONSENT FORM

I, Dr. Amos Ombaso Ayunga, a postgraduate student in Internal Medicine at the University Of Nairobi. I am carrying out a study on patients with Inflammatory arthritis to see whether a certain test is useful at the patients first visit to the clinician. RA is a chronic condition that affects joints and other organ systems and may lead to significant disability if not detected early and treated. Anti-CCP is this new test. It has been found to diagnose RA with almost 100% certainty and hence the ability to distinguish it from other rheumatic diseases.

I will be comparing the performance of this fairly new test with that of the commonly used test called the RF. In addition, I will be comparing these two tests with the classification criteria that is used to classify RA.

The study forms part of the requirements for me to be able to obtain my Master of Medicine degree, but the results of the study are also intended to lead to recommendations which, if implemented, would lead to improved management and quality of life of patients with RA.

Once you agree to participate, you will answer questions of personal nature as laid in the study proforma, physical examination and getting about 5mls of blood. All the information you give and the examination and investigation results will be handled with absolute confidentiality. You will feel a little pain as is normal with standard phlebotomy and the amount of blood drawn will not affect your health.

Your participation in this study is absolutely voluntary and you will not be denied treatment or be in any way penalized for declining to participate.

Your participation in the study bears no cost to you but the laboratory results will be used for your individual benefit.

I ___________________________of______________________________hereby consent/decline to participate in this study, the nature and purpose of which have been fully explained to me.

Signed...........................................................................date..............................................................

Witness..........................................................................date..............................................................