ANTI-CYCLIC CITRULLINATED PEPTIDE AS AN EARLY AND ACCURATE LABORATORY MARKER FOR THE DIAGNOSIS OF RHEUMATOID ARTHRITIS (RA) AND THE PREVALENCE OF HLA-B27 AMONG ANKYLOSING SPONDYLITIS PATIENTS IN GHANA

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by

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DECLARATION

I hereby declare that this submission is my own work towards the PhD and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

Rheumatoid Arthritis (RA) and Ankylosing Spondylitis (AS) are two of the most common autoimmune diseases. This study aimed to obtain the best biomarker for early and accurate diagnosis of RA, determine the prevalence of HLA-B27 among AS patients in Ghana, and examine if there is dyslipidaemia and oxidative stress among them. A total of one hundred and sixty-seven (167) subjects were recruited for this cross sectional study from October 2006 to June 2008. They were made up of one hundred and two (102) RA and sixty-five (65) AS subjects. Both groups were recruited from the orthopaedic departments of Komfo Anokye Teaching Hospital (KATH), Korle-Bu Teaching Hospital (KBTH) and from subjects visiting MEDILAB centres nationwide with laboratory requests to investigate rheumatoid conditions. Those who were confirmed with either RA or AS and consented were recruited. Fifty (50) healthy blood donors with similar age and sex distribution as the subjects were recruited as control group. After ethical approval and informed consent had been obtained, information on socio-demographic characteristics and medical history were obtained from standardized questionnaires which was administered to them and through their medical records. Rheumatoid Factor (RF)-latex and its isotypes (RF-IgA and RF-IgM) were assayed and compared with anti-CCP as specific markers for early diagnosis of RA in Ghana. HLA-B27 was assayed to determine its prevalence among AS subjects. Lipid profile, Tumour Necrosis Factor-α (TNF-α) and Erythrocyte Sedimentation Rate (ESR) were estimated to determine the presence of dyslipidaemia and inflammation among AS and RA subjects. To evaluate the presence of oxidative stress, MDA and vitamin C were also determined among RA and AS subjects. Out of 102 RA subjects, Sensitivity was highest for RF-IgM test (58.45%) followed by anti-CCP antibody (54.34%), RF-latex (52.48%) and RF-IgA tests (28.51%). Specificity of 96.67% was obtained for anti-CCP, 69.52% for RF-IgM, 63.12% for RF-latex and 51.75% for RF-IgA. RA and AS subjects had a higher mean level of total cholesterol (TC) 5.88 ± 0.06 mmol/l. The difference between the RA and AS subjects' cholesterol level and that of the control was significant ($p<0.001$) with a significant ($r^2= 0.0619$, $p= 0.002$) positive correlation between serum triglycerides levels of AS and RA patients and that of the control. RA and AS subjects exhibited a slightly higher mean level of triglycerides 0.88 ± 0.16 mmol/l as compared to 0.56±0.05 mmol/l of controls. The difference between the subjects triglycerides levels and that of the control was significant ($p<0.001$). The mean HDL level for the patients was significantly lower ($p<0.001$) as compared to that of the control. The mean LDL level indicates a significantly higher ($p<0.001$) value as compared to that of the controls with a significant ($r^2= 0.0619$, $p= 0.002$) positive correlation between serum triglycerides, total cholesterol, HDL and LDL of RA subjects. A significant ($p<0.001$) atherogenic ratios i.e. TC/HDL-C and LDL-C/HDL-C were observed among the subjects and controls. Out of 65 AS subjects examined there were

four (4) HLA-B27 positives representing 4.6%, three (3) of the HLA-B27 positives were males, the mean Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) score was 44.7/100. Forty-eight (48) AS patients had sacroilitis in their x-ray reports. None had a family history of AS or any extra-articular manifestations. TNF-α level among RA and AS patients were 11.87 ± 0.30 pg/ml and 13.11 ± 0.50 pg/ml respectively as compared to normal control of 5.70 ± 0.48 pg/ml whiles the mean ESR for AS was 34.64 ± 1.87 mm/hr and that of RA was 24.67 ± 1.87 mm/hr as compared to 9.23 ± 0.91 mm/hr of controls. The mean MDA level for AS subjects was 0.75 ± 0.03 mmol/l and that of RA subjects was 1.57± 0.06µmol/l as compared to normal controls of 0.58 ± 0.02mmol/l. MDA levels had a significant correlation with the serum levels of vitamin C. Vitamin C was 0.85 ± 0.03mmol/l for AS subjects and 0.95 ± 0.02mmol/l for RA subjects as compared to 1.12± 0.01µmol/l of control. This study has demonstrated that anti-CCP has the highest specificity and that the combination of anti-CCP and RF-IgM assay are highly specific and moderately sensitive for diagnosing RA, making this combination of autoantibodies a powerful tool in the serologic assessment of RA in Ghana. The presence of anti-CCP at disease onset means that they have a high positive predictive value of 100, a negative predictive value of 22.6 with 4.98 and 0.68 as positive and negative likelihood ratios respectively, predict the development of erosive joint lesions and the detection of these antibodies can therefore be used in clinical practice to help plan a therapeutic strategy. Findings from this study further showed that HLA-B27 is present in some AS patients. The BASDAI scores and other clinical features clearly denote the presence of the disease with moderate disease activity. Dyslipidaemia is present in early RA and AS subjects, and that proinflammation and inflammation markers plays a pivotal role in the development of atherosclerosis as evidenced in the increase in TNF-α, ESR and a more atherogenic lipid profile, thereby increasing cardiovascular risk. Increased oxidative stress is evidenced by raised MDA and decreased vitamin C and that lipid peroxidation is a giant distracter in RA and AS.

ACKNOWLEDGEMENT

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ABBREVIATIONS

AAU Acute Anterior Uvetis

ACR American College of Rheumatology

AFA Antifilaggrin Antibodies

AKA Antikeratine Antibodies

ANCA Antineutrophil Cytoplasmic Antibodies

ANTI-CII Antibody Response to Collagen Type II

APF Antiperinuclear Factor

ARA American Rheumatism Association

AS Ankylosing Spondylitis

BASDAI Bath Ankylosing Spondylitis Disease Activity Index

Bcl-2 B cell lymphoma 2

Bip Heavy Chain Binding Protein

CCP Cyclic Citrullinated Peptide

CD Cluster of Differentiation

CRP C - reactive protein

CVD Cardiovascular Disease

DC Dendritic cell

DMARDs Disease Modifying Antirheumatic Drugs

ELISA Enzyme-Linked Immunosorbent Assay

ERA Early Rheumatoid Arthritis

ERAD Endoplasmic Reticulum-Associated Degradation

ESR Erythrocyte Sedimentation Rate

FLS Fibroblast-Like Synoviocytes

GPI Glucose-6-Phosphate Isomerase

HDL-C High Density Lipoprotein Cholesterol

HLA Human Leukocyte Antigen

hnRNP-A2 Heterogenous nuclear ribonucleoprotein A2

HSP Heat Shock Protein

IFN Interferon

Ig Immunoglobulin

IL Interleukin

KATH Komfo Anokye Teaching Hospital

KBTH Korle-Bu teaching hospital

LDL-C Low-Density Lipoprotein Cholesterol

LPO Lipid Peroxides

MAP Mitogen-Activated Protein

MCP Metacarpophalangeal

MCTD Mixed Connective Tissue Disease

MDA Malondialdehyde

MHC Major Histocompatibility Complex

MMP Matrix Metalloproteinase

MRI Magnetic Resonance Imaging

MTP Metatarsophalangeal

NFkβ Nuclear Factor Kappaβ

NK Natural Killer

NSAIDs Nonsteroidal Anti-Inflammatory Drugs

OA Osteoarthritis

OPGL Osteoprotegrin ligand

PAD Peptidylarginine Deiminase

PIP Proximal Interphalangeal Joints

PUFA Poly Unsataurated Fatty Acid

RA Rheumatoid arthritis

RANKL Receptor Activator of Nuclear Factor Kappa β Ligand

RF Rheumatoid Factor

ROC Receiver Operating Characteristics

ROS Reactive Oxygen Species

SERPINS Serine Proteinase Inhibitors

SLE Systemic Lupus Erythematosus

TC Total Cholesterol

TcR T cell Receptor

TGF Transforming Growth Factor

TGFB Transforming Growth Factor

Th T Helper

TIMPS Tissue Inhibitors of Metalloproteinases

TLR Toll-Like Receptor

TNF Tumour Necrosis Factor

UPR Unfolded Protein Response

Chapter 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

Ghana's disease profile is characterized by high levels of communicable and a rising number of non-communicable diseases. Rheumatoid conditions accounts for 1.9% of the top ten causes of morbidity and mortality (PPME-GHS, 2005). Rheumatoid Arthritis (RA) and Ankylosing Spondylitis (AS) are two of the most common autoimmune diseases affecting 0.5–1.5% of US population (Helmick *et al.*, 2008). The direct and indirect costs are estimated to be over US $ 120 billion.

RA is a chronic inflammatory multisystem autoimmune disorder of undetermined aetiology involving primarily the synovial membranes and articular structures of multiple joints (Feldmann *et al.*, 1996). AS is a chronic progressive inflammatory arthritis, affecting primarily the spine and sacroiliac joints, causing eventual fusion of the spine (Sengupta and Stone, 2007). RA and AS severely affect the identity and sense of confidence of patients. Research examining the impact of RA and AS on patients indicates that they are associated with stress and depression and that these psychological factors can have negative consequences for patients' families and other members of their social networks (Yelin *et al.*, 1980). Getting early and accurate diagnosis of RA and AS is more relevant for therapeutic decision – making than predicting whether an arthritis syndrome will ever satisfy a set of classification criteria. Early and aggressive intervention with new and effective drugs can alter the course of the disease, reverse morbidity and increase life expectancy (de Vries-Bouwstra *et al.*, 2005). Most autoantibodies in autoimmune diseases are not disease specific. Recent studies have however, demonstrated that

anti-cyclic citrullinated peptide (anti-CCP) antibodies can detect early RA (Kroot *et al.*, 2000; Schellekens *et al.*, 2000).

The most important genetic predisposing condition for the development of AS is the Human Leukocytes Antigen, B27 (HLA-B27). This antigen is said to be uncommon in Africans compared with Europeans. Previous survey of B27 positive African Americans found the relative risk of AS to be lower than in B27 positive Caucasians, supporting the theory that some ethnic groups may be genetically protected from spondyloarthritis (Brown *et al.*, 1997). In some African populations like Gambia and Senegal, HLA-B27 prevalence is between 3–6% of the general population. However, some other genetic markers may be involved in the causation of AS in the HLA-B27 negative population (Hill *et al.*, 1991). Studies concerning clinical presentation and presence of extra-articular manifestations such as anterior uveitis compared with the situation in Western Europe to the disease in Africa has not been well documented, most affected individuals do not have a family history of AS, and the patients are older at onset of the disease in Africa.

Mortality in RA and AS patients is mainly associated with cardiovascular disease (CVD) which is affected by dyslipidaemia (Wallberg-Jonsson *et al.*, 1997; Bjornadal *et al.*, 2002). It is also known that dyslipidaemia increases the incidence of cardiovascular disease in the general population (Goodson and Solomon, 2006). There have been a few reports on lipid levels and their association with disease activity prior to the fulfillments of all the America College of Rheumatology (ACR) and Modified New York criteria. There have been studies reporting either increased or decreased or similar levels for total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) in comparison to control subjects after the administration of drugs (Asanuma *et al.*, 1999). Studies concerning increased erythrocytes sedimentation rate (ESR) in

cardiovascular events have already been documented (del Rincon *et al.*, 2001), but those regarding ESR and tumour necrosis factor (TNF) as inflammatory and proinflammatory components of cardiovascular events has not been evaluated.

Oxidative stress has been suggested to relate to the pathogenesis of RA and AS (Sies, 1997). However, the data on AS and RA patients related to oxidative stress are inconsistent. Many investigators have suggested that RA and AS patients are more prone to lipid peroxidation (Ozkan *et al.*, 2007). Levels of malondialdehyde (MDA), vitamin C and vitamin E have been reported to be higher or normal in AS and RA patients depending on disease activity (Ozgocmen *et al.*, 2004).

1.2 PROBLEM STATEMENT

A major problem to overcome in diagnostic research is the lack of an independent gold standard tests for early diagnosis of RA and AS. In early cases of RA and AS, clinical symptoms are milder and non-specific and patients will not fulfill all the ACR and MNY criteria. It is imperative to predict the clinical outcome of RA and AS patients for therapeutic decision –making than satisfying a set of classification criteria, therefore, the detection of disease specific autoantibody is of great diagnostic and therapeutic importance. The tendency to develop AS is genetically inherited and strongly associated with HLA-B27 gene. The prevalence of HLA-B27 among causacian patients with AS is about 90%. In most West-African countries, HLA-B27 prevalence among AS patients is found between 3-6%. It is therefore important to determine the association between HLA-B27 and the Ghanaian AS patients, determine the prevalence of HLA-B27 and other clinical features among Ghanaians with AS. Mortality in RA and AS patients is known to be associated with CVD. However, the data on AS and RA patients related to dyslipidaemia, inflammation and oxidative stress has not been evaluated.

1.3 RATIONALE OF STUDY

The lack of appropriate health care facilities and the scarcity of rheumatologists make early diagnosis of AS and RA difficult. In Ghana, diagnosis is made when the disease conditions are far advanced with their corresponding disabilities and morbidity. There is lack of suitable biomarkers to help in early diagnosis and monitoring of disease activity. Moreover, there is no readily available data on prevalence of RA and AS making early awareness and diagnosis difficult in Ghana. The HLA-B27 gene is found with highest prevalence in patients with AS (>90%). However, definitive diagnosis of AS and its subsequent association with HLA-B27 in Ghana has not been evaluated. In order to keep abreast with new trends and to aid in early diagnosis, extensive research is needed on RA and AS. There are evidence of interrelationships between dyslipidaemia and inflammation, showing a deterioration of the lipid profile during increased disease activity. Cardiovascular risk in rheumatic diseases needs to be investigated against the background of mounting evidence revealing the pivotal role of inflammation in the development of cardiovascular disease (CVD). The chronic inflammatory nature of rheumatic diseases, such as rheumatoid arthritis (RA) and ankylosing spondylitis (AS), raises the question whether the increase in inflammatory burden causes a higher prevalence of CVD. It is imperative therefore to evaluate TNF-α and ESR as cardiovascular risk factors among RA and AS patients. Oxidative stress has been implicated in many diseases, such as atherosclerosis, RA and AS. There is therefore the need to investigate the relationship among oxidants, antioxidants and pathogenesis of RA and AS.

1.4 HYPOTHESIS

1. Anti-CCP is a better diagnostic marker for RA than RF and its isotypes.
2. HLA-B27 positivity in AS patients is rare in Ghana.
3. AS patients in Ghana have moderate BASDAI score.
4. The onset of Extra –articular manifestations of AS in Ghana, occur late.
5. There is dyslipidaemia and inflammation among AS and RA patients
6. There is oxidative stress among AS and RA patients

1.5 MAIN OBJECTIVE

The main objective of this research is to compare biomarkers for early and accurate diagnosis of RA, determine the prevalence of HLA-B27 among AS patients in Ghana and examine if there is dyslipidaemia and oxidative stress among them.

1.6 SPECIFIC OBJECTIVES

1. To compare the usage of Rheumatoid Factor (RF)-latex and its isotypes (RF-IgA and RF-IgM) with anti-CCP as specific markers for early diagnosis of RA in Ghana.
2. To use anti-CCP antibodies to distinguish rheumatoid arthritis from other autoimmune diseases.
3. To determine the prevalence of HLA-B27 among AS patients.
4. To determine disease activity among AS patients using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) scores.
5. To determine the risk of developing cardiovascular disease among AS and RA patients using their lipid profiles, TNF-alpha and ESR.
6. To determine whether oxidative stress is associated with RA and AS using MDA and vitamin C.

Chapter 2

LITERATURE REVIEW

2.1 ARTHRITIS

Arthritis is the inflammation of one or more joints; a joint is where two bones meet to allow movement of body parts. Inflammation of the joints can be caused by gout, rheumatiod arthritis, ankylosing spondylitis and osteoarthritis. The inflammation in RA and AS causes swelling, pain, stiffness, and redness in the joints (Feldmann *et al.*, 1996).

The World Health Organization (WHO) considers rheumatism or rheumatoid disease to be an umbrella term for all diseases of the musculoskeletal and locomotor systems, which are associated with pain and loss of movement. This definition has also been adopted by the International League of Associations for Rheumatology (ILAR), the European League Against Rheumatism (EULAR) (Combe *et al.*, 2007) and the American College of Rheumatology (ACR) (Arnett *et al.*, 1988).

2.2 RHEUMATOID ARTHRITIS OVERVIEW

2.2.1 History and Definition

The name rheumatoid arthritis is derived from the Greek *rheumatos* meaning "flowing", the suffix-*oid* meaning "in the shape of", *arthr* meaning "joint" and the suffix *-itis*, a "condition involving inflammation." RA was first described as *goutte asthénique primitive* by Landré-Beauvais in 1800 (Landre-Beauvais, 2001). In 1859, Garrod named the disease rheumatoid arthritis (Storey, 2001).

Table 2:1 ACR Classification Criteria

1.Morning stiffness	Morning stiffness in and around the joint, lasting at least 1 hour before maximal improvement
2.Arthritis of three or more joints areas	At least 3 joint areas simultaneously have had soft tissue swelling or fluid
3.Arthritis of hand joints	At least 1 area swollen in wrist, Metacarpophalangeal (MCP) or proximal interphalangeal joints (PIP) joint
4.Symmetric arthritis	Simultaneous involvement of the same joint areas on both sides of the body involving PIP, MCP, and MTP.
5.Rheumatoid nodules	Subcutaneous nodules over bony prominence, extensor surfaces or juxta-articular regions, observed by a physician
6.Rheumatoid factor	Detected by method positive in less than 5% of normal controls.
7. Radiographic changes	Radiographic changes typical on posteroanterior hand and wrist radiograph with erosions

Patients fulfilling at least 4 of these 7 criteria are classified as having RA. Criteria 1 to 4 must have been present for at least 6 weeks, (Arnett et al., 1988)

2.2.2 Epidemiology of RA

Rheumatoid arthritis is a worldwide disease affecting adults with a peak incidence between the fourth and sixth decade. About two-thirds of patients with RA are women (Sokka *et al.*, 2009). The prevalence in Sweden and parts of the western world has been reported to be between 0.5 and 1% (Kvien *et al.*, 1997; Simonsson *et al.*, 1999; Carmona *et al.*, 2002; Silman and Pearson, 2002). The incidence is higher in native American-Indian populations, *e.g.* about 5 % in the Pima Indians (Jacobsson and Pillemer, 1994) and 7 % in the Chippewa Indians

(Harvey *et al.*, 1981) and lower in rural African populations (Brighton *et al.*, 1988; Silman *et al.*, 1993), China and Japan (Lau *et al.*, 1993; Shichikawa *et al.*, 1999).

2.3 DISEASE COURSE, OUTCOME AND MORTALITY

RA is a progressive disease with joint destruction, subsequent disability and socioeconomic consequences for the individual patients (Scott *et al.*, 2005). There is considerable variation between patients in the disease course, the pattern of joints involved, the presence of autoantibodies and/or presence of articular manifestation (Huizinga *et al.*, 2005). Health-economical calculations have shown that cost, both direct and indirect is associated with increased disability (Kobelt *et al.*, 2002). Mortality in RA has been mainly attributed to cardiovascular disease (Wallberg-Jonsson *et al.*, 1997), and particularly in patients with extra-articular disease (Turesson *et al.*, 2002). Other comorbidities are infections (Doran *et al.*, 2002) and lymphomas (Ekstrom *et al.*, 2003).

2.4 IMPACT OF GENDER AND SEX HORMONES

More women than men are affected by RA (Symmons *et al.*, 1994). This implicates a plausible role for sex hormones in susceptibility and pathogenesis. In women peak incidence is observed in the peri-menopausal and postpartum periods (Goemaere *et al.*, 1990). Furthermore, pregnancy has a protective effect (Silman *et al.*, 1992), which may be explained by the fact that pregnancy induces a shift from T-helper type 1 reaction typical for RA towards type 2 reaction (Mishan-Eisenberg *et al.*, 2004). The use of oral contraceptives also has a protective effect, whereas the use of hormone replacement therapy does not (Doran *et al.*, 2004). However, hormone replacement therapy has a beneficial effect in post-menopausal patients with established RA (D'Elia *et al.*, 2003).

2.5 CLINICAL PRESENTATION OF RA

In patients with RA, chronic inflammation leads to the destruction of the cartilage, bone and ligaments causing deformity of the joints. Damage to the joints can occur early in the disease and be progressive (Feldmann *et al.*, 1996). RA is characterized by inflammation of the synovial membrane of diarthrodial joints. Early indications of RA are swelling and pain of the proximal interphalangeal and metacarpophalangeal joints. Later, the larger joints become affected, especially those of the knee, elbow and ankle (Smolen and J.S., 1996).

Large numbers of activated leukocytes infiltrate the synovial membrane, causing hyperplasia and inflammation, which in most cases lead to progressive destruction of cartilage and bone. Since RA is a systemic autoimmune disease, other parts of the body may become affected at a later stage. The symptom that distinguishes RA from other forms of arthritis is inflammation and swelling of soft-tissue of many joints at the same time (Smolen, 2007).

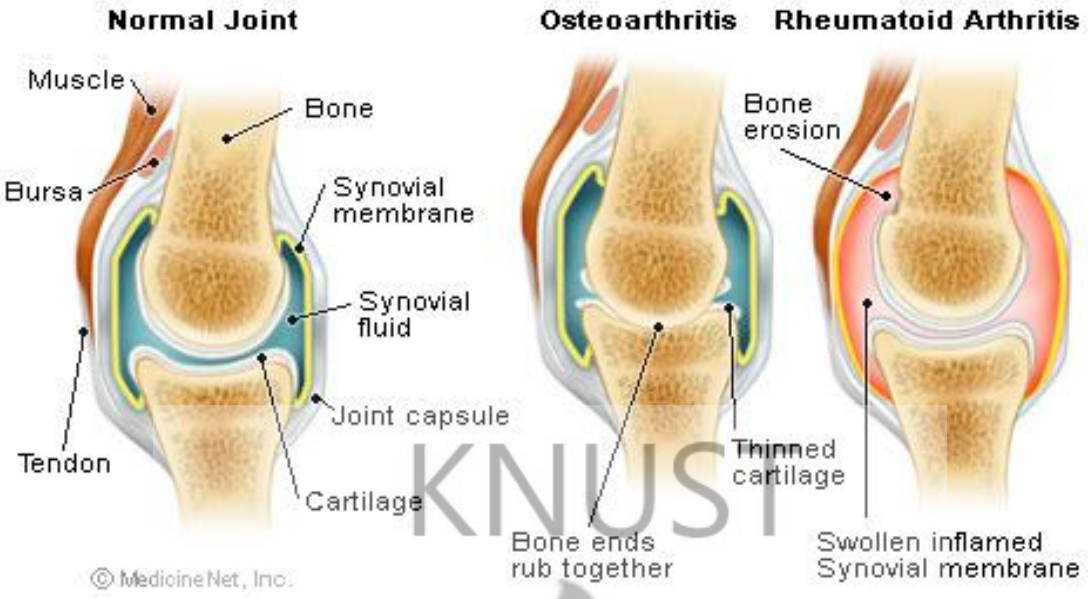

Figure 2-1Schematic views of a normal joint, osteoarthritis and rheumatoid arthritis.

(Shiel *et al.*, 2010)

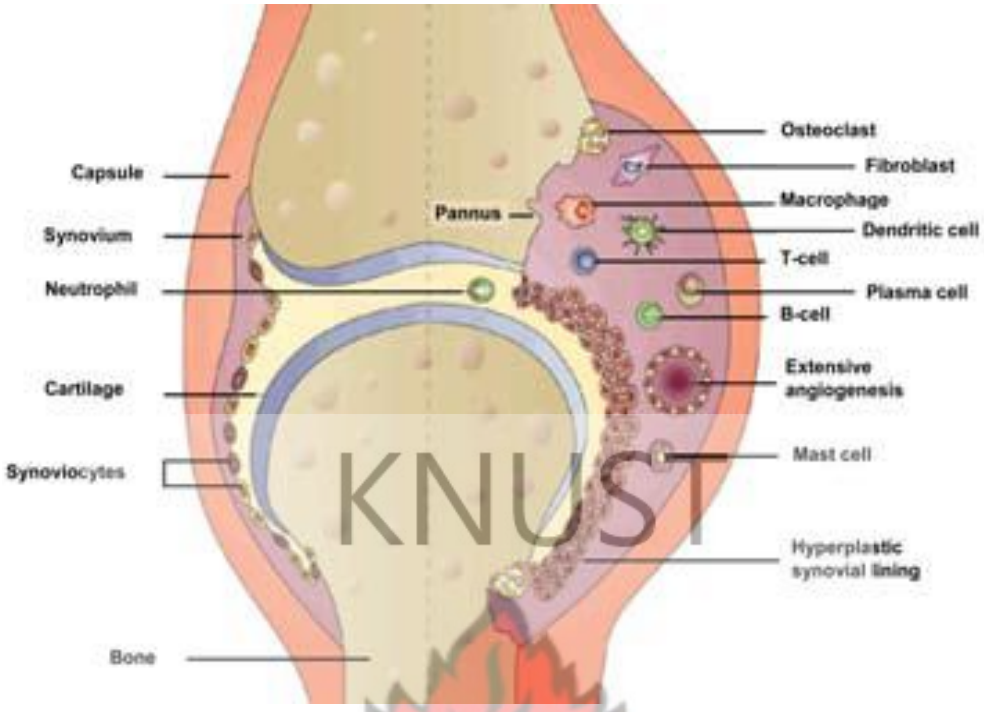

Figure 2-2 Schematic views of a normal (left) and arthritic (right) joints.
Source: Smolen et al., (2007).

2.6 PATHOGENESIS OF RHEUMATOID ARTHRITIS

Genetic studies have demonstrated that a genetic predisposition resides in the HLA-DR locus (Gabriel *et al.*, 2003). There is also evidence that environmental factors, such as infectious agents, oral contraceptives and smoking may play a role (Stites *et al.*, 1994). Ninety percent (90%) of patients with RA have the cluster of markers known as the HLA-DR4/DR1 cluster, whereas only 40% of controls do. Thus, in theory, RA requires susceptibility to the disease through genetic endowment with specific markers and an infectious event that triggers an autoimmune response. The infectious agent that normally triggers the inflammatory response is viral infections. Once triggered, the immune response causes inflammation of the synovium.

2.7 SYNOVIA

2.7.1 Normal synovium

The synovium covers all non-cartilage intra-articular surfaces. The normal synovial lining layer consists of only 1-3 loosely organised, avascular cell layers, and is not supported by any basement membrane. Two cell types in the intima are identified by electron microscopy: macrophage-like synoviocytes and fibroblast-like synoviocytes (FLS) (Barland *et al.*, 1962). Macrophages express major histocompatibility complex (MHC) class II, cluster of differentiation (CD) 163, CD68 and FcR IIIa (Bhatia *et al.*, 1998). In normal synovia they are a minority, whereas in RA synovia up to 80% may be macrophages. FLS do not express MHC class II or CD68 and have no phagocytic ability. FLS produce synovial fluid produced from filtered plasma. In normal joints the synovial fluid just lubricates the cartilage parts of the joint. Lower down, in the synovial sublining, there are only a few cells and scattered blood vessels. The functions of synovial tissue in healthy individuals are maintenance of an intact tissue surface, lubrication and nutrition of the cartilage.

2.7.2 RA Synovium

In RA, the synovial tissue is characterised by a prominent inflammation, where both *FLS* and synovial macrophages contribute (Tak and Bresnihan, 2000). The synovial hyperplasia may have several causes (Mor *et al.*, 2005). Several growth factors driving fibroblast proliferation are over-expressed in the synovia and FLS have mutations in proliferation-regulating proteins, among them the tumour suppressor gene p53 and these mutations are frequent at sites with cartilage damage (Seemayer *et al.*, 2003). Furthermore, an impaired apoptosis with more long-lived cells has been suggested as RA FLS express high levels of B cell lymphoma 2 (Bcl-2), consistent with an anti-apoptotic phenotype (Perlman *et al.*,

2000). IL-15, produced from FLS and synovial macrophages, supports further

increased Bcl-2 expression and cell survival (Kurowska *et al.*, 2002). FLS respond to, and produce, a wide range of inflammatory mediators, such as proinflammatory cytokines and pro-angiogenic factors. Signal transduction pathways, like nuclear factor kappa β (NFKB) and mitogen-activated protein (MAP) kinase-related pathways, are activated in FLS (Mor *et al.*, 2005). A direct contact between IL-15-producing RA FLS and T cells promotes T cell activation (Miranda-Carus *et al.*, 2004).

The proliferating synovial tissue subsequently forms a pannus that invades the cartilage and bone. FLS contribute significantly to cartilage degradation through the production of enzymes such as matrix metalloproteases (MMPs). TNF, IL-1a and growth factors induce MMP expression in FLS (MacNaul *et al.*, 1990; Shingu *et al.*, 1993). Bone destruction is mediated mainly by osteoclasts, derived from macrophage precursor cells influenced by osteoprotegrin ligand (OPGL), produced by FLS and receptor activator of nuclear factor kappa β (NFK B) ligand (RANKL) (Mor *et al.*, 2005). FLS stimulated with TNF may also directly invade bone (Pap *et al.*, 2003).

Macrophages are also abundant in the inflamed thick synovial tissue in RA. They express MHC class II and produce several proinflammatory cytokines, such as IL-1 (Miyasaka *et al.*, 1988; Ulfgren *et al.*, 2000), IL-6 (Okamoto *et al.*, 1997), TNF (Firestein *et al.*, 1990; Chu *et al.*, 1991; Ulfgren *et al.*, 2000), IL-15 (McInnes *et al.*, 1996) and IL-18. They also produce some proteolytic enzymes, but their matrix-degrading ability is modest in comparison with FLS (Gracie *et al.*, 1999). Dendritic cells (*DCs)* are present in the synovial tissue arranged with lymphocytes (van Dinther-Janssen *et al.*, 1990), immature DCs are detected in the lining, and mature DCs exclusively in lymphocyte infiltrates (Page *et al.*, 2002). DCs with the capacity

to produce a great amount of proinflammatory cytokines are also present in the synovial tissue (Cavanagh *et al.*, 2005) as well as Toll-like receptors (TLRs) (Roelofs *et al.*, 2005).

T cells are present in the sublining of the synovial tissue in RA. T cells express the surface marker CD3 and either CD4 or CD8. The majority in the synovial tissue are CD4+. A few B cells are present in the synovial membrane in RA. The formation of antibodies to immune complex are common in RA and immune complexes with anti-citrulline specificity are detected in synovia in murine models (Lundberg *et al.*, 2005). In the synovial tissue, neutrophils are rare, but they are demonstrated in the cartilage-pannus junction and are abundant in the synovial fluid (Pillinger and Abramson, 1995), where the survival has been suggested to be prolonged due to an impaired ability to undergo apoptosis (Edwards *et al.*, 2004). Neutrophils are recruited to the synovial fluid by chemoattractants (C5a, IL-8) and immune complexes. Neutrophils contribute to cartilage destruction through the release of lysozomal enzymes and oxygen radicals (Liu and Pope, 2004).

2.8 PATHOPHYSIOLOGY OF RA

The cause of RA is unknown. Several possible associations have been described; notable among them are genetic predisposition, autoimmune response, psychological stress, hormone interaction and viral infection (hepatitis B, parvovirus and rubella). Various inflammatory disorders of remote organ systems are present and contribute to the presenting problem (Ruddy, 1989).

RA is a diffuse systemic disease involving many areas of the body. The presenting complaint may be remote from a joint or may involve inflammatory symptoms at a joint. Early and intermediate molecular mediators of inflammation include tumour

necrosis factor alpha (TNF-α), interleukins IL-1, IL-6, IL-8 and IL-15, transforming growth factor beta, fibroblast growth factor and platelet-derived growth factor. Once the inflammatory reaction is established, the synovium thickens, the cartilage and the underlying bone begin to disintegrate and evidence of joint destruction accrues. The inflammation associated with rheumatoid arthritis primarily attacks the linings of the joints; cells in the synovial membrane divide and grow producing inflammation pain and stiffness in the joint of hand and feet (Khan *et al.*, 2009).

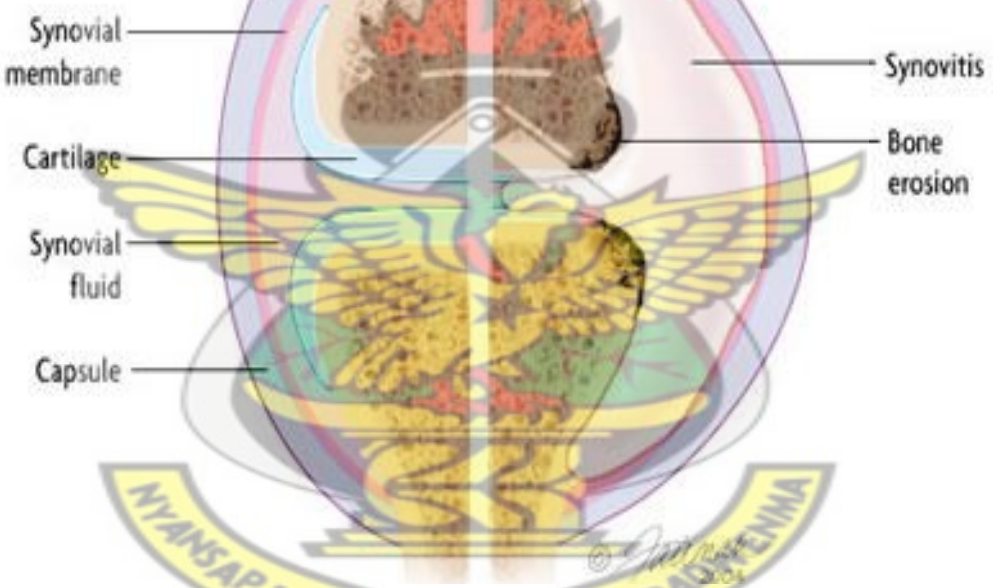

Figure 2-3 Schematic views of a normal synovial (left) and RA synovium (right) (Smolen, 1996)

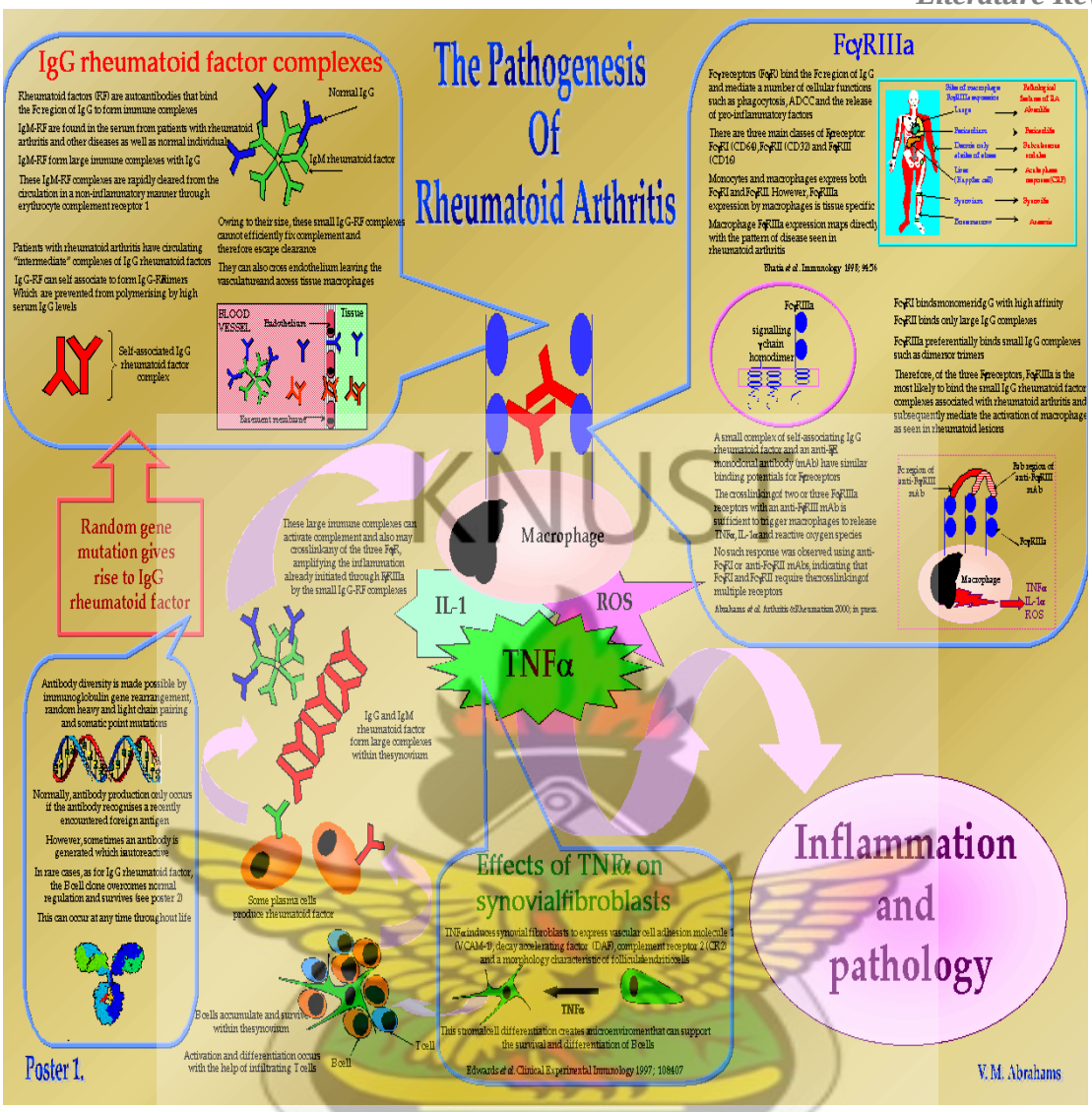

Figure 2-4 Schematic view of RA pathogenesis.

(Edwards *et al.*, 1997)

2.9 AUTOIMMUNITY

Autoimmunity is a loss of self tolerance. Tolerance or the unresponsiveness of the immune response to antigens occurs both peripherally and centrally (Stites *et al.*, 1994). There are two main types of autoimmune diseases: organ specific and systemic. There are many mechanisms that are responsible for self-tolerance and

when these mechanisms break down, auto immunity can result. The mechanism responsible for breaking self tolerance is not well understood. Autoimmune diseases are more likely to affect people with a particular allotype, exposure to sequestrated antigens to immune systems and molecular mimicry (Stites *et al.*, 1994). These factors lead to an immune response against the cross-reactive epitomes giving rise to an autoimmune disease (Silman and Pearson, 2002). Central tolerance occurs in immature lymphocytes in the thymus and bone marrow. Immature lymphocytes which can recognize self see these antigens in the thymus and bone marrow and are deleted or inactivated. Peripheral tolerance occurs in mature lymphocytes that see self – antigen in peripheral tissues. It provides for maintenance of self-tolerance for clones that escape destruction in the thymus or bone marrow which is important for tolerance to tissue-specific self-antigens not found in the generative organs (Stites *et al.*, 1994). Common targets of organ specific autoimmune responses include thyroid, adrenals, stomach, and pancreas. The non-organ specific reactions include rheumatic diseases that involve primarily the skin, joints, kidney and muscle (Naparstek and Plotz, 1993).

2.10 INFLAMMTION AND INFLAMMTORY RESPONSE

Inflammation resulting from any form of tissue injury causes an increase in the concentration of a number of liver-derived plasma proteins, which appear to have important functions in the inflammatory process (Whicher *et al.*, 1984). The acute phase response is accompanied by several other systemic responses. The measurement of acute phase proteins in plasma thus provides a clinically valuable indication of the presence of inflammation and its extent (Whicher *et al.*, 1984).

The most commonly used measurements of the acute phase response are ESR, plasma viscosity, CRP, orosomucoid, haptoglobin and alpha 1-antitrypsin (Whicher *et al.*, 1984). The most potent inducers of hepatic synthesis of the acute phase proteins are the cytokines interleukin 1, interleukin 6 and tumour necrosis factor, which interact mutually in a complicated way (Wollheim and Eberhardt, 1992). The various acute phase proteins respond differently to different combinations of cytokines, which may explain the occurrence of different patterns of acute phase response. Inflammation is basically a protective mechanism. The leakage of water and protein into the injured area brings humoral factors, including antibodies into the locale and may serve to dilute soluble toxic substances and wash them away. The adherence and migration of leukocytes brings them to the local site to deal with infectious agents. There are also instances in which no causative toxic substance or infectious agent can be found to account for the inflammation. This is the case in rheumatoid arthritis and rheumatic fever. Such diseases may be examples in which an uncontrolled or misdirected inflammatory response with an autoimmune component is turned against the host.

2.11 TNF-ALPHA

Tumour necrosis factor-alpha (TNF-A) is a pleiotropic inflammatory cytokine. It was first isolated by Carswell *et al.*, (1975). The cytokine possesses both growth stimulating properties and growth inhibitory processes, and it appears to have self regulatory properties as well. TNF-A induces neutrophil proliferation during inflammation, but it also induces neutrophil apoptosis upon binding to the TNF-R55 receptor (Murray *et al.*, 1997). The cytokine is produced by several types of cells, but especially by macrophages. Tracey *et al.*, (1990) suggest two beneficial functions of TNF-A which have led to its continued expression. First, the low

levels of the cytokine aid in maintaining homeostasis by regulating the body's circadian rhythm. Furthermore, low levels of TNF-A promote the remodeling or replacement of injured and senescent tissue by stimulating fibroblast growth. Additional beneficial functions of TNF-A include its role in the immune response to bacterial, certain fungal, viral and parasitic invasions as well as its role in the necrosis of specific tumors. Lastly it acts as a key mediary in the local inflammatory immune response. TNF-A is an acute phase protein which initiates a cascade of cytokines and increases vascular permeability, thereby recruiting macrophage and neutrophils to a site of infection. TNF-A secreted by the macrophage causes blood clotting which serves to contain the infection (Janeway *et al.*, 1999). The pathological activities of TNF-A cause necrosis of some types of tumors; it also promotes the growth of other types of tumor cells. High levels of TNF-A correlate with increased risk of mortality (Rink and Kirchner, 1996). TNF-A participates in both inflammatory disorders of inflammatory and non inflammatory origin (Strieter *et al.*, 1993).

2.11.1 Structure/Binding sites

TNF-A, can either bind directly to TNFR-55 and TNFR-75 receptors through cell-to-cell contact or undergo cleavage and bind in its soluble form. TNF-A shares only 36% amino acid sequence homology with TNF-B, also called lymphotoxin (LT) (Meager and Anthony., 1991).

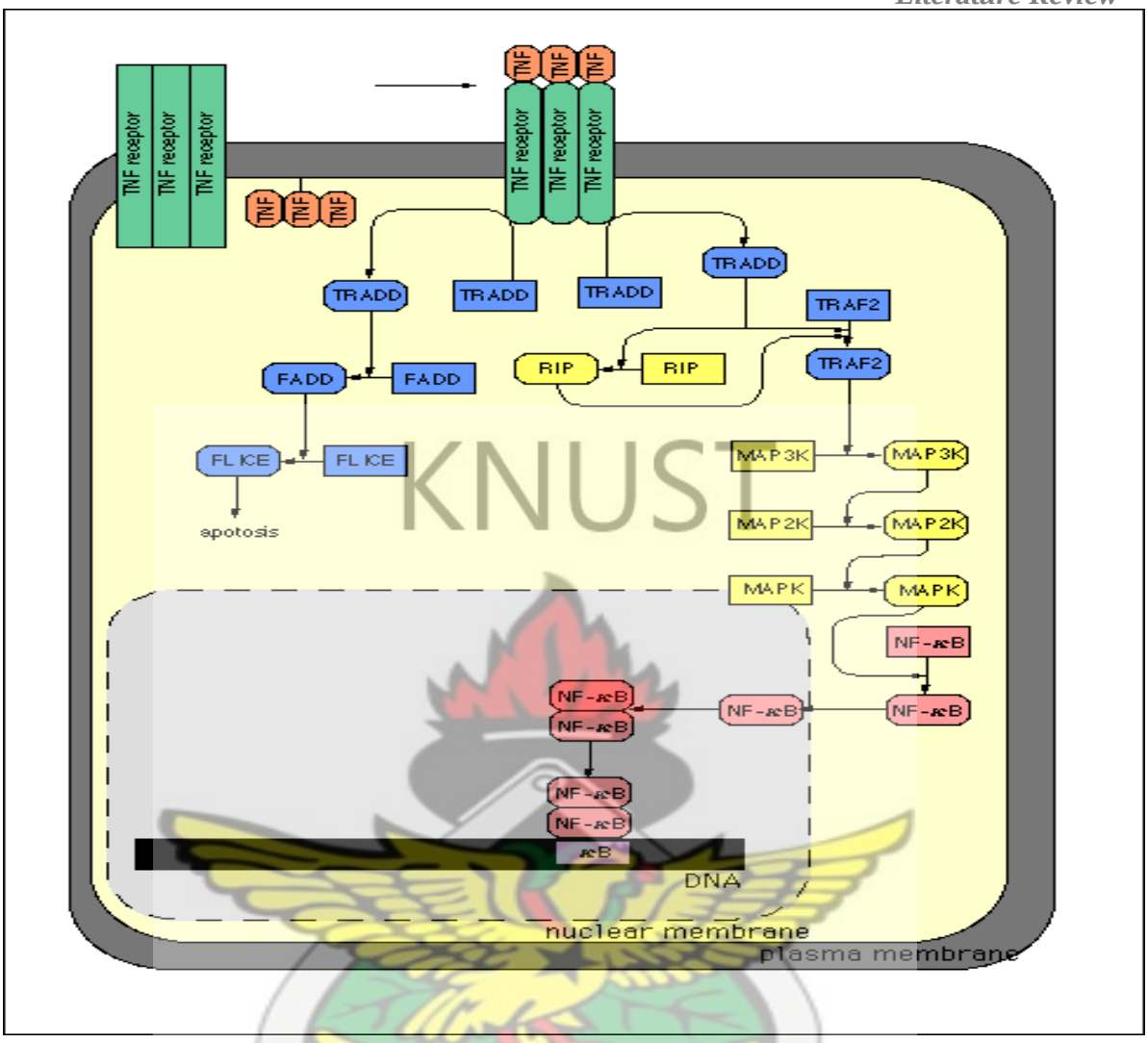

Figure 2-5 Signal transduction pathway initiated by TNF-alpha binding to its receptor, TNFR initiate clustering and signal transduction.

(Higashi *et al.*, 1998)

2.12 AUTOANTIBODIES IN RHEUMATOID DISEASES

The detection of autoreactive antibodies against intracellular autoantigens is essential for establishing diagnosis, prognosis as well as monitoring the disease course of autoimmune rheumatic diseases. Therefore, the methods for autoantibody detection should be reliable, reproducible, validated and easy-to-perform in the every day clinical practice (van Boekel *et al.*, 2002).

Systemic rheumatic diseases are among the most complex disorders because their clinical presentation and constellation of findings are in part reflected by the wide spectrum of autoantibodies found in the sera of patients suffering from RA (Smolen and Steiner, 1998).

Autoantibodies are a common and characteristic feature of rheumatic autoimmune diseases. Although the majority of autoantibodies do not seem to play a major pathogenetic role in these disorders, some of them have proven extremely useful as diagnostic tools and indicators of disease activity. A number of autoantibodies are associated with RA, most of them are not disease specific making it extremely difficult to distinguish it from other joints and bone diseases. The lack of a specific marker antibody is particularly true for RA (Egeland and Munthe, 1983). This has stimulated a search for novel antibodies and their respective target molecules that could be useful for the diagnosis of RA. In addition, identification of these targets might further enlighten our understanding of the pathogenesis of this disorder. Among the autoantibodies described in recent years, several are promising candidates as diagnostic indicators for RA and have become part of the diagnostic repertoire including antikeratin, anticitrullinated peptides, anti-RA33, anti-Sa, and anti-p68 autoantibodies and have been shown to have >90% specificity for RA (van Zeben *et al.*, 1992).

2.12.1 Rheumatoid factor

Rheumatoid factor is circulating antibody acting against multiple antigenic determinants on the Fc fragment of the IgG molecule. The conventional agglutination and immunoturbidimetric techniques measure predominantly IgM class of RF. However, radioimmunoassay or enzyme-linked immunosorbent assay (ELISA) allow measurement of RF belonging to all the major immunoglobulin classes (IgM, IgG and IgA) (Harris, 1997). RF -IgM is the main isotypes identified

by clinically available diagnostic assays for RF detection (Westedt *et al.*, 1985). The discovery of RF (Waaler, 1940; Rose *et al.*, 1949) made it possible to distinguish seropositive arthritis. Among immunological markers, RF is the most studied and has been associated with a more severe radiological outcome in early RA (Combe, 2001) and the titre of RF at baseline has also been reported to correlate with radiological damage after 3 years of follow-up (Paimela *et al.*, 1995). In studies of various isotypes of RF, some studies have reported IgA-RF to be the best predictor of radiological damage (Scott *et al.*, 1984; Teitsson *et al.*, 1984), whilst others found no significant prediction of erosive disease with IgA-RF (Eberhardt *et al.*, 1990).

The presence of RF is included among the original and revised criteria for the classification of RA as accepted by the American College of Rheumatology (ACR) (Arnett *et al.*, 1988). However, the proportion of patients with RA positive for RF has ranged from 30% to more than 90% in various studies (Wolfe *et al.*, 1991). Different laboratory methods such as agglutination, radioimmunoassay, or enzyme linked immunosorbent assay (ELISA) have been used, but these methods do not have equivalent sensitivity, specificity, and reproducibility.

Most routine laboratory tests in current use (haemagglutination, latex agglutination, nephelometry, and turbidimetry assays) are based on the ability of RFs to agglutinate sheep red blood cells, latex, or similar particles (polystyrene, bentonite, acryl) coated with IgG. They detect mainly the IgM isotype of RFs (IgM RF), which are more efficient in agglutination reactions owing to their polyvalency, but the exact contributions of IgM, IgG, and IgA RFs are not known (Roberts-Thomson *et al.*, 1982).

The most consistent serological finding in patients with RA is an increase in the concentration of RF- IgM in blood and synovial fluid. RF- IgM has been reported to

occur in approximately 70-80% of patients with confirmed RA. The concentration of RF tends to be highest when the disease peaks and tends to decrease during prolonged remission. RF-IgM is found in 1 to 4% of the general population. RF is present in 75% of adult RA patients with the highest incidence of RF occurring in persons over 65 years of age (Egeland and Munthe, 1983). Increased titers may accompany a variety of acute immune responses, particularly viral infections and a number of other diseases (infectious mononucleosis, tuberculosis, leprosy, various parasitic diseases, liver disease, sarcoidosis, and systemic lupus erythematosus). Despite this, RF is widely used as a diagnostic marker for RA.

2.12.2 Anti-RA 33 antibodies

The 33KDa antigen RA33 was first described by Hassfeld and coworkers when it was recognized in 36% of sera from RA patients on immunoblots from soluble nuclear extracts of HeLa cells (Hassfeld *et al.*, 1989). Only 1% of the normal sera recognized the antigen, and thus it has been suggested that RA33 is a marker for early arthritis. Anti-RA33 antibodies are directed to the heterogeneous nuclear ribonucleoprotein A2 (hnRNP-A2), a nuclear protein that is involved in mRNA splicing and transport (Hassfeld *et al.*, 1993). The antibodies occur in approximately one-third of RA patients but can also be detected in 20–30% of patients with SLE and in up to 40% of patients with the rare overlap syndrome mixed connective tissue disease (MCTD). The sensitivity of RA patients for anti-A2/anti-RA33 autoantibodies is therefore low (~40%). Nevertheless, in a representative cohort of patients with various rheumatic diseases including autoimmune and non-autoimmune arthrities, the specificity of anti-RA33 antibodies for RA was approximately 90% (Steiner *et al.*, 1992). However, if a diagnosis of SLE and MCTD (or MCTD alone) is excluded or if there is an absence of autoantibodies associated with SLE (such as anti-DNA, anti-Sm, and anti-U1 RNP antibodies), the specificity

of anti-RA33 antibodies for RA can be as high as 96% (Hassfeld *et al.*, 1993). It has been suggested that RA33 is a marker for early arthritis. However, the autoantibodies to RA33/A2 protein are not exclusively found in RA patients. They are also present in SLE and mixed connective tissue disease sera (Skriner *et al.*, 1997). Importantly, other arthritides such as osteoarthritis, reactive arthritis, and psoriatic arthropathy are usually anti-A2/anti-RA33 negative (Isenberg *et al.*, 1994).

2.12.3 Sa proteins

Anti-Sa antibodies are directed to a 50 kDa protein of unknown structure and function that has been isolated from human tissues (spleen, placenta, rheumatoid synovium). Anti-Sa autoantibodies are detected in approximately 40% of patients with established RA but less often in patients with early disease (Despres *et al.*, 1994). The reported specificity of anti-Sa antibodies for RA range between 92 and 98%, which compares favorably with marker antibodies for other autoimmune diseases. As suggested by Hayem *et al.*, (1999) who found the incidence of these antibodies to be significantly increased in RA patients with severe destructive disease, determination of anti-Sa antibodies may be of prognostic benefit. In a recent prospective study in patients with recent onset synovitis, anti-Sa had the highest specificity and prognostic value of all autoantibodies investigated (Saulot *et al.*, 2000). Moreover, these antigens can be detected at an early stage of the disease, although this is with a rather low sensitivity (26%). Sa is possibly a citrullinated form of the intermediate filament protein vimentin (Menard *et al.*, 2000).

2.12.4 Heavy chain binding proteins (p68)

Autoantibodies to a ubiquitously expressed 68 kDa glycoprotein were described by Blass *et al.*, (1995). The target of anti-p68 antibodies was recently identified as the chaperone, or stress protein immunoglobulin heavy-chain binding protein

(BiP). It is also known as glucose-regulated protein of 78 kDa (grp78), a member of the 70 kDa heat-shock protein family, and is localized in the endoplasmic reticulum. Anti-BiP autoantibodies are found in the sera of more than 60% of RA patients (Specker *et al.*, 1997; Blass *et al.*, 2001). The specificity of anti-BiP antibodies for RA has been reported as 96%, making these antibodies promising candidates for the diagnosis of RA. Similar to hnRNP-A2/RA33 and fibrin, BiP has been shown to be highly expressed in synovial tissue. Because it seems to form a target for auto reactive T cells of RA patients, BiP may be one of the antigens driving the pathologic autoimmune process in RA. Autoantibodies to the heavy chain binding protein (BiP) occur in about 64% of RA patients and appear to be highly specific for the disease (Corrigall *et al.*, 2001), although this awaits confirmation from clinical studies. The BiP protein can be detected in the cytoplasm and the endoplasmic reticulum of cultured cells, whereas under stress, it re-localizes to the nucleus. Combined with a change in glycosylation, this non-physiological localization of BiP may become antigenic during RA development (Blass *et al.*, 2001).

2.12.5 Anticalpastatin

Calpains are calcium-ion-dependent neutral cysteine proteinases. Two forms of calpains exist: μ-calpains (calpain I, which needs micromolar concentrations of Ca^{2+} for activity) and m-calpains (which require millimolar amounts of Ca^{2+} for activity). Substrates of these enzymes are highly diverse and include cytoskeletal proteins, nuclear proteins, cytokines and extracellular matrix proteins including proteoglycans (Menard and el-Amine, 1996). Elevated levels of extracellular calpain in the inflamed synovium have been reported, which suggests that calpains might be secreted by synovial cells and could play a role in cartilage degradation in RA (Yamamoto *et al.*, 1992; Szomor *et al.*, 1995).

Calpastatin is the natural inhibitor of calpains. Using immunoblots of recombinant protein, autoantibodies directed to calpastatin can be found in about 45% of the RA sera, but also sera from SLE, myositis and systemic sclerosis contain antibodies directed to calpastatin (Mimori *et al.*, 1986). A recent study using recombinant fusion proteins with truncated forms of calpastatin showed that anticalpastatin antibodies can also be found in sera from healthy individuals, even in comparable numbers, relative to RA patients (Lackner *et al.*, 1998). Autoantibodies directed to calpastatin could increase calpain activity, leading to enhanced cartilage damage, and could therefore contribute to the severity of the disease.

2.12.6 Glucose-6-phosphate isomerase (GPI)

Recently described as a novel auto antigen in RA, antibodies to GPI have been detected in one study in 64% of RA patients, but not in controls. In humans, anti-GPI antibodies appear to be produced locally, as titers were higher in RA synovial fluid than in RA sera. It is also worth noting that the same auto antigen is targeted in a mouse model of RA (Schaller *et al.*, 2001).

2.12.7 Anti-perinuclear factor (APF)

In 1964 a highly specific RA antibody system directed to a protein component present in the keratohyaline granules in the cytoplasm of differentiating buccal mucosa cells was described. The antigen was referred to as perinuclear factor, and the antibody activity became known as antiperinuclear factor (APF) (van Jaarsveld *et al.*, 1999). APF antibodies have relatively high sensitivity with a strong specificity (73–99%). The APF test never became popular because of several practical inconveniences (Nienhuis and Mandema, 1964; Hoet and van Venrooij, 1992).

2.12.8Anti-Filaggrin (AFA) and Anti-Keratin Antibodies (AKA)

A related group of RA-specific autoantibodies, the so-called antikeratine antibodies (AKA), was first described in 1979 (Young *et al.*, 1979). These antibodies stain keratin-like structures in the cornified layer of oesophagus cryostat sections but do not recognize cytokeratins. AKA can be detected by indirect immunofluorescence in 36–59% of RA sera with a specificity of 88–99% (Sebbag *et al.*, 1995). It has been demonstrated that the citrulline moiety is essential for the autoantigenicity of filaggrin and that the citrullinated filaggrine is the antigen that is detected in the APF and AKA tests (Vincent *et al.*, 1999). When combined with AKA testing, a sensitivity of 64% was achieved, with no loss of AFA's specificity (99%); however, the sensitivity seems to be dependent on the filaggrin purification method (Youinou *et al.*, 1990).

2.12.9 Anti-cyclic citrullinated peptide (Anti-CCP)

One way of bypassing the difficulty of obtaining a filaggrin antigen that is not only sufficiently pure but also contains a reproducible citrulline content is to use citrullinated peptides themselves as the antigen. Recent clinical studies indicate that this anti-CCP test is extremely specific (98%) and sensitive (68-75%) (Schellekens *et al.*, 2000). The ability to diagnose early RA and detect RA in early synovitis patients, as well as differentiate RA from other connective tissue diseases such as SLE, further adds to the value of anti-CCP as bio-marker for RA (Vasishta, 2002; Pinheiro *et al.*, 2003; Tampoia *et al.*, 2005).

Native citrulline-containing peptides are only produced by enzymatic conversion of peptidylarginine to citrulline, because citrulline is a non-coded amino acid in vivo. The enzymes involved in this conversion are peptidylarginine deiminases (PADIs). Five PADIs isoenzymes have been detected in humans, and two of them; PADIs2 and PADIs4 have been detected in human RA synovial tissues (Vossenaar *et al.*, 2003). Previously, PADIs4 gene was associated with RA (Jansen *et al.*, 2002).

These facts strongly suggest that citrullination of self proteins and production of autoantibodies against those citrullinated proteins play pathologic roles in RA. Consequently, identification of the substrates of PADIs is important for the investigation of autoimmunity in RA. All PADIs are dependent on calcium ions for activity.

Figure 2-6 Deimination of Arginine

(Molberg and Sollid, 2006).

In 1998, Schellekens and colleagues reported that autoantibodies reactive with linear synthetic peptides containing the unusual amino acid citrulline were present in 76% of RA sera with specificity for RA of 96%. The antibodies in patients with RA that recognised the citrulline containing epitopes were predominantly of the IgG class and of relatively high affinity (Schellekens *et al.,* 2000). Subsequenly, Schellekens and colleagues reported that an ELISA test based on cyclic citrullinated peptide (CCP) showed superior performance characteristics to one based on the linear version in the detection of antibodies to RA (Schellekens *et al.,* 2000). Most citrullinated proteins and peptides are recognised by autoantibodies in RA sera although with differing sensitivities and specificities. These findings suggest an

important role for citrullinated antigens in the diagnosis of RA (van Boekel *et al.*, 2002; Hueber *et al.*, 2003).

2.12.10 Other RA associated autoantibodies

RA patients produce autoantibodies directed to many autoantigens, but most of them are not specific for RA. Among these nonspecific antibodies are those directed to a wide variety of cartilage proteins such as collagen and fibronectin, but also antiphospholipid antibodies and anti-neutrophil cytoplasmic antibodies (ANCA) can often be found (Mulder *et al.*, 1993). ANCA can be subdivided into two groups depending on their localization: cytoplasmic ANCA, and perinuclear ANCA (Charles and Maini, 1993). ANCA can be found in up to one-third of the RA patients, but can also be detected in other autoimmune conditions and various infectious diseases (Kallenberg *et al.*, 1992; Schnabel *et al.*, 1996).

Anticollagen antibodies are present only in about 30% of HLA-DR4-positive RA patients (Ronnelid *et al.*, 1994). Although anti-Collagen type II (anti-CII) autoantibodies can be found in serum of only a small proportion of the RA patients, these antibodies and anti-CII-producing B cells are present in the joints of the majority of these patients (Tarkowski *et al.*, 1989). Anticollagen autoantibodies show a low specificity and can also be found in other autoimmune disorders (e.g. SLE, 20% occurrence). Anti-CII- IgG titers in serum and synovial fluid appear to directly correlate with levels of acute-phase reactants, and of cytokines such as tumour necrosis factor-α and interleukin-6 (Kim *et al.*, 2000). Autoantibodies to fibronectin are found in about 14% of RA patients and show a low specificity 34% (Atta *et al.*, 1995).

Autoantibodies directed to the nonhistone chromosomal proteins HMG1 and HMG2 can be detected in about 25–40% of the RA patients, but similar frequencies

have also been reported for SLE, Sjögren's syndrome and scleroderma patients (Uesugi *et al.*, 1998).

2.13 TREATMENT OF RHEUMATOID ARTHRITIS

Modern therapy for RA is based on knowledge of the severity of the natural history of the disease. The history of RA includes a long period from the 1950s through to the mid-1980s in which RA was regarded in the majority of patients as a disease with a good prognosis based on epidemiological data (Kelley *et al.*, 1985) This traditional teaching was that RA could be controlled in most patients with bed rest (Short and Bauer, 1948), aspirin, and later with alternative nonsteroidal anti-inflammatory drugs (NSAIDs). However, it was recognized during the mid-1980s from clinical cohorts that short-term drug efficacy was not translated into long-term effectiveness, as most patients experienced severe functional declines (Pincus *et al.*, 1984), radiographic progression (Scott *et al.*, 1984), work disability, and premature mortality (Yelin *et al.*, 1980).

These reports led to calls for early and aggressive use of disease modifying anti-rheumatic drugs (DMARDs), including aggressive strategies to prevent future damage and functional loss (Wilske and Healey, 1989; Fries, 1990; Emery and Salmon, 1995). Gold sodium thiomalate was among the first drugs to be shown to be disease-modifying over the long term (Luukkainen *et al.*, 1977).

2.13.1 General principles of drug therapy for RA patients

Several general principles characterize the contemporary approach to patients with RA. Identification of RA in the early stages is both important and difficult. Criteria for RA have been developed since 1907 (Allbutt and Rolleston, 1907). However, even the American College of Rheumatology ACR 1987 revised criteria do not differentiate patients with early RA from other types of recent onset

inflammatory polyarthritides (Harrison *et al.*, 1998; Saraux *et al.*, 2001). The risks of side effects of traditional RA drugs are substantially greater than side effects of contemporary DMARDs (Pincus and Callahan, 1993). Early treatment may prevent the development of RA (Van Dongen *et al.*, 2007) whereas even a short delay of therapy of four months reduces the likelihood of achieving remission (Mottonen *et al.*, 2002). There was therefore the need for an early and aggressive use of disease modifying anti-rheumatic drugs (DMARDs), including aggressive strategies to prevent future damage and functional loss (Emery and Salmon, 1995).

2.13.2 Biological agents

Five biological agents, including three which interfere with the actions of tumour necrosis factor alpha (TNF-*a*)—etanercept, infliximab, and adalimumab, one with T-cell actions—abatacept, and one with B-cell actions—rituximab, are approved for use to treat RA in the US and other countries (Sokka *et al.*, 2005). These agents represent a major advance for the armamentarium of antirheumatic drugs for patients who have poor or incomplete responses to methotrexate monotherapy or a combination with other DMARDs. It is important to recognize such incomplete responses within 3–6 months of treatment, to prevent long-term damage in the 20–30% of patients who appear to require biological agents to control inflammatory activity. According to guidelines in many countries, biological agents should be considered if patients do not respond to traditional DMARDs including methotrexate during the first few months (Miyasaka and Koike, 2007).

Treatment with DMARDs only after erosions i.e., joint damage, has been replaced by early, aggressive intervention. Judgment of efficacy as significant differences from placebo has been replaced by tight control of inflammation. Intamuscular gold and penicillamine have been replaced by methotrexate, as monotherapy or used in combination with sulfasalazine and/or hydroxychloroquine, as well as

targeted therapies with biological agents. Patient outcomes appear much improved at this time compared to earlier periods.

2.14 ANKYLOSING SPONDYLITIS (AS)

2.14.1 History

It was not until the late nineteenth century when the neurophysiologist Vladimir Bekhterev of Russia in 1893,Adolph Strümpell of Germany in 1897, and Pierre Marie of France in 1898 gave adequate descriptions which permitted an accurate diagnosis of AS. For this reason, Ankylosing spondylitis (**AS**) is also known as Bechterew's disease; Bechterew syndrome; Marie Strümpell disease; Marie Struempell disease and Spondyloarthritis. AS is a chronic, painful, progressive inflammatory arthritis primarily affecting spine and sacroiliac joints, causing eventual fusion of the spine; it is a member of the group of the autoimmune spondyloarthropathies with a probable genetic predisposition (Brewerton *et al.*, 1973). Complete fusion results in a complete rigidity of the spine, a condition known as bamboo spine. AS is a seronegative spondyloarthropathy (SpA) that mainly affects the vertebrae and the peripheral joints. The genetic marker HLA-B27 was found to play a key role in the occurrence of this disease (Schlosstein *et al.*, 1973). The association of HLA-B27 with AS was first described in 1973, and is among the strongest described for a HLA locus (Brown *et al.*, 1998). The tendency to develop AS is believed to be genetically inherited, and the majority (nearly 90%) of patients with AS are born with the HLA-B27 gene. Blood tests have been developed to detect the HLA-B27 gene marker, and have furthered our understanding of the relationship between HLA-B27 and ankylosing spondylitis. The HLA-B27 gene appears only to increase the tendency of developing ankylosing spondylitis, while some additional factor(s), perhaps environmental, are necessary for the disease to appear or become expressed

2.14.2 Clinical presentation of AS

The clinical manifestations of AS are believed to result from a combination of an immunogenetic predisposition and a triggering biomechanical, inflammatory, or infectious event that leads to the disease. The precise sequence of the immune activation and consequent histopathological characteristics has remained undefined (Jimenez-Balderas and Mintz, 1993). The role of an antecedent infection in the onset of AS has not been clearly established, although significant evidence of an immunoreactivity against Klebsiella in this patient population may represent a primary pathogenic step. Furthermore, a biomechanical triggering event can initiate either exposure to previously immune-sequestered autoantigens or provide a route for bacterial seeding to initiate inflammation. The disease may result in erosion at the joint between the spine and the hip bone (the sacroiliac joint), and the formation of bony bridges between vertebrae in the spine, fusing those bones. In addition, bones in the chest may fuse. Each individual tends to have his or her own unique pattern of presentation and activity of the illness. The initial inflammation may be a result of an activation of the body's immune system by a bacterial infection or a combination of infectious microbes. Once activated, the body's immune system becomes unable to turn itself off, even though the initial bacterial infection may have long subsided. Chronic tissue inflammation resulting from the continued activation of the body's own immune system in the absence of active infection is the hallmark of an inflammatory autoimmune disease (Reveille *et al.*, 2001).

The symptoms of AS are related to inflammation of the spine, joints, and other organs. Fatigue is a common symptom associated with active inflammation. Inflammation of the spine causes pain and stiffness in the low back, upper buttock area, neck, and the remainder of the spine. The onset of pain and stiffness is

usually gradual and progressively worsens over months. Occasionally, the onset is rapid and intense. The symptoms of pain and stiffness are often worse in the morning or after prolonged periods of inactivity. The pain and stiffness are often eased by motion, heat and a warm shower in the morning. AS often affects patients in adolescence, the onset of low back pain is sometimes incorrectly attributed to athletic injuries in younger patients (Kim *et al.*, 2005).

Patients who have chronic, severe inflammation of the spine can develop a complete bony fusion of the spine (ankylosis). Once fused, the pain in the spine disappears, but the patient has a complete loss of spine mobility. These fused spines are particularly brittle and vulnerable to breakage (fracture) when involved in trauma, such as motor vehicle accidents. A sudden onset of pain and mobility in the spinal area of these patients can indicate bone breakage (fracture). The lower neck (cervical spine) is the most common area for such fractures.Chronic spondylitis and ankylosis cause forward curvature of the upper torso (thoracic spine), limiting breathing capacity. Spondylitis can also affect areas where ribs attach to the upper spine, further limiting lung capacity. AS can cause inflammation and scarring of the lungs, causing coughing and shortness of breath, especially with exercise and infections. Therefore, breathing difficulty can be a serious complication of AS (van der Linden *et al.*, 1983).

Patients with AS can also have arthritis in joints other than the spine. Patients may notice pain, stiffness, heat, swelling, warmth, and or redness in joints such as the hips, knees, and ankles. Occasionally, the small joints of the toes can become inflamed, or "sausage" shaped. Inflammation can occur in the cartilage around the breast bone (costochondritis) as well as in the tendons where the muscles attach to the bone (tendinitis) and ligament attachments to bone. Some patients with this disease develop achilles tendinitis, causing pain and stiffness in the back of the heel, especially when pushing off with the foot while walking up stairs.

Inflammation of the tissues of the bottom of the foot, plantar fasciitis, occurs more frequently in persons with AS (McGonagle and Emery, 2000).

Other areas of the body affected by AS include the eyes, heart and kidneys. Patients with AS can develop inflammation of the iris called iritis. Iritis is characterized by redness and pain in the eye, especially when looking at bright lights. Recurrent attacks of iritis can affect either eye. In addition to the iris, the ciliary body and choroid of the eye can become inflamed and this is referred to as uveitis. Iritis and uveitis can be serious complications of AS that can damage the eye and impair vision (Gran and Skomsvoll, 1997).

A rare complication of AS involves scarring of the heart's electrical system, causing an abnormally slow heart rate. A heart pacemaker may be necessary in these patients to maintain adequate heart rate and output. The part of the aorta closest to the heart can become inflamed, resulting in leakage of the aortic valve. These patients can develop shortness of breath, dizziness and heart failure. Advanced spondylitis can lead to deposits of protein material called amyloid into the kidneys and result in kidney failure. Progressive kidney disease can lead to chronic fatigue and nausea and can require removal of accumulated blood poisons by a filtering machine (Hamersma *et al.*, 2001).

2.14.3 Methods for diagnosing Ankylosing Spondylitis

Diagnosis of AS depends on the typical clinical presentation with supportive evidence from imaging studies and laboratory tests showing an elevated erythrocytes sedimentation rate (ESR), C-reactive protein (CRP) and low haemoglobin with a positive HLA-B27 assay and fulfillment of Modified New York criteria (van der Linden *et al.*, 1984). The bath ankylosing spondylitis disease activity index (BASDAI) scores is also used for accessing disease activity. Modified New York criteria for the diagnosis of AS consist of;

- Clinical Criteria
  - Low back pain, > 3 months, improved by exercise, not relieved by rest
  - Limitation of lumbar spine motion, sagittal and frontal planes
  - Limitation of chest expansion relative to normal values for age and sex
- Radiologic Criteria
  - Sacroilitis grade ≥ 2 bilaterally or grade 3 – 4 unilaterally
- Grading
  - Definite AS if radiologic criterion present plus at least one clinical criteria
  - Probable AS if: Three clinical criteria with radiologic criterion but no signs or symptoms satisfy to clinical criteria

2.14.4 Epidemiology of AS

Prevalence of AS is approximately 1% worldwide and the direct and indirect costs are estimated to be over 120 billion dollars, the occurrence rate ranges from 0.5% to greater than 5% depending on ethnic variation. AS is three times less common in American blacks than in whites (Mijiyawa *et al.*, 2000). It is rare in African blacks of unmixed ancestry. A histocompatibility antigen HLA-B27, which does not exist in African blacks of unmixed ancestry, and is present in 8% of white and 2% to 4% of the American black population, is strongly associated with AS and Reiter's disease. HLA-B27 is present in more than 80% of white patients with AS or Reiter's disease but in less than 60% of American black patients. Other genetic and environmental factors may be of major importance in the genesis of these diseases in American blacks (Gran and Husby, 2003).

The HLA-B27 gene appears only to increase the tendency of developing AS, while some additional factor(s), perhaps environmental, are necessary for the disease to

become expressed. For example, while 7% of the United States population has the HLA-B27 gene, only 1% of the population actually has AS. Even among HLA-B27 positive individuals, the risk of developing AS appears to be further related to heredity. In HLA-B27 positive individuals who have relatives with the disease, their risk of developing ankylosing spondylitis is 12%. The disease can occur at any age but tends to peak in the fourth and fifth decades of life. The demographic, lifestyle and environmental factors interact to present high levels of morbidity and mortality in a particular country (Gran and Husby, 2003).

2.15 ENVIRONMENTAL TRIGGERS

Although various other spondyloarthropathies have been linked to infectious origins, the role of microbial pathogenesis in the development of AS has remained undefined (Rashid and Ebringer, 2007). Animal studies have shown that the genetically susceptible HLA-B27-transgenic rats only exhibit the disease phenotype on exposure to a pathogen, but less definitive evidence exists among human patients, and no organism has been identified in synovial fluid and sacroiliac joints (Braun *et al.*, 1997; Martinez *et al.*, 2004).

Circumstantial evidence of microbial involvement has been borne out in demographic studies suggesting higher rates of enteric infection among patients with AS and undifferentiated spondyloarthropathy who have active disease. There are specific bacterial agents with an established relationship to HLA-B27, and these include Campylobacter, Chlamydia, Shigella, Salmonella, Yersinia and Klebsiella. Of specific interest is Klebsiella pneumoniae infection as a triggering or perpetuating factor in the pathogenesis of AS through the mechanism of molecular mimicry (Ebringer and Wilson, 2000; Leirisalo-Repo *et al.*, 2003).

2.16 PATHOLOGICAL FEATURES OF AS

Inflammation in AS occurs primarily at the sacroiliac joints, but can involve entheses, vertebral bodies adjacent to the intervertebral discs and peripheral joint of the synovium. The extraarticular pathological features include involvement of the ophthalmic, cardiovascular, pulmonary, gastrointestinal, and renal systems. In AS, the apophyseal, costovertebral, and sacroiliac joints of the axial skeleton are most typically affected and may ultimately become ankylosed. The enthesitis that is typical of AS occurs at fibrocartilaginous sites, with the enthesis originating at the cartilage and attaching at the epiphyses or apophyses. Affected entheseal fibrocartilage structures are generally exposed to significant shear stress and microtrauma, and it is suspected that this pathomechanism is necessary to expose tissue antigens to circulating lymphocytes. The accompanying osteitis is not haphazard, but rather follows the lines of mechanical stress in the bone. In genetically susceptible individuals, such exposed antigens can elicit autoinflammatory and autoimmune reactions. The more extensive oligoclonal expansion of CD8+ T cells that occurs in patients with AS compared with healthy HLA-B27+ individuals further supports the antigen-specific pathological mechanism of this disease (Benjamin and McGonagle, 2001).

The precise sequence of the immune activation and consequent histopathological characteristics has remained undefined. Strong associations with the presence of HLA-B27 could result from various molecular abnormalities ranging from the presentation of novel arthritogenic peptides to an abnormal autoimmune stimulation to an anomalous microbial tolerance. A better comprehension of the sequence of molecular events that actualize AS will facilitate the development of more specific and targeted therapies (Benjamin *et al.*, 2007).

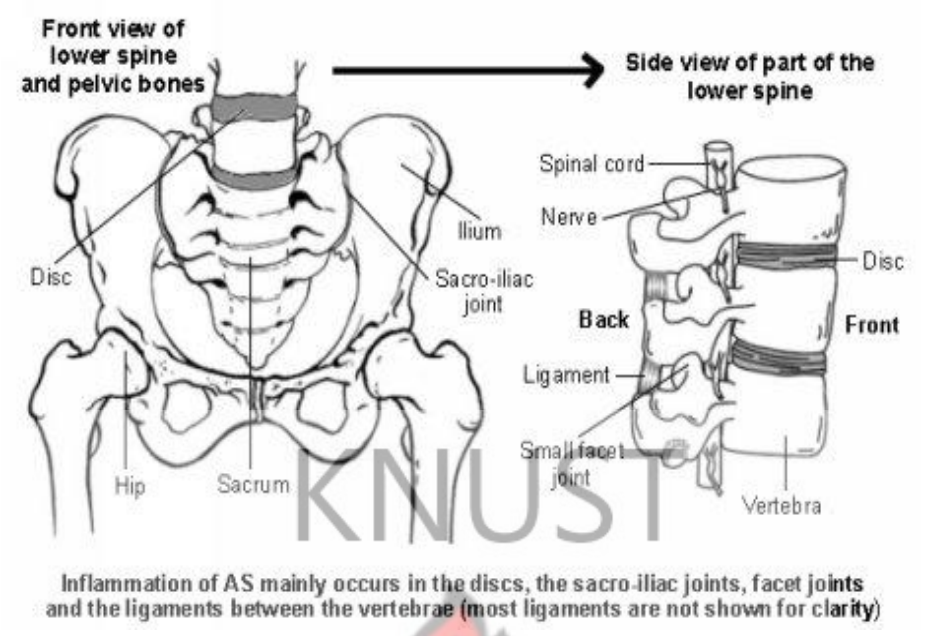

Figure 2-7 Sacro – iliac joints in AS

2.17 HUMAN LEUKOCYTE ANTIGEN-B27 (HLA-B27)

HLA-B27 is a MHC Class I molecule that is encoded on chromosome 6. Although it is ubiquitous among cell types, its expression is higher on antigen-presenting cells. After translation and tertiary folding, this protein binds to β2-microglobulin and is loaded with an oligopeptide (Brewerton *et al.*, 1973). These peptides are normally derived from self-proteins, but antigenic peptides may be displayed when intracellular microbes infect the cell. The trimolecular complex travels through the golgi apparatus to the cell surface where the antigenic peptide is presented to CD8+ lymphocytes or NK cells. There is a strong genetic association between HLA-B27 and AS, with the protein isoform being found in more than 90% of afflicted patients; however, fewer than 5% of HLA-B27 positive individuals will develop AS (Schlosstein *et al.*, 1973).

There are at least 25 allele subtypes of *HLA-B27* that encode 23 different gene products. The most common subtype (B*2705) is thought to be the parent molecule

from which the other types have evolved and is the most closely associated with the risk of AS. Other subtypes that confer disease susceptibility include B*2701, B*2702, B*2704, and B*2707, whereas the B*2706 and B*2709 alleles that are common in South-East Asia and Sardinia do not have an association with AS (Ball and Khan, 2001).

Another explanation for disease susceptibility in certain HLA-B27 positive individuals may be the expression levels in antigen-presenting cells. HLA-B27 has been found to be more highly expressed in peripheral blood mononuclear cells in HLA-B27 positive patients with AS. Furthermore, expression levels were found to be higher in AS patients with B*2705 than in healthy individuals with B*2709 and B*2705 (van der Linden *et al.*, 1983; Braun *et al.*, 1998).

2.18 THE ROLE OF HLA-B27 IN AS

The predominant genetic association with AS is the MHC group of molecules, specifically HLA-B27. There are significant associations of AS with HLA-B27, and the estimated overall contribution to AS susceptibility is estimated to be between 20 and 40%. An understanding of these genes and their protein products provides insight into the underlying disease pathogenesis and may permit the development of novel avenues of treatment.

Several theories have been promoted with regard to the molecular pathogenetic role of HLA-B27 in AS. These include the presentation of arthritogenic peptides, aberrant folding of surface heavy chains, HLA-B27 misfolding, and enhanced intracellular microbial survival (Reveille, 2006).

2.19 PRESENTATION OF ARTHRITOGENIC PEPTIDE

Human leukocyte antigen-B27 may have the ability to bind unique antigenic peptides leading to the CD8+ cytotoxic T-cell responses to these self or bacterial

sequences. The ensuing cytolytic response leads to tissue injury and diffuse inflammation. Unlike most other MHC Class I molecules, there is a restriction for peptides with arginine at the P2 position to bind to HLA-B27. Single amino acid changes from aspartate in the B*2705 allele to histidine in the B*2709 allele leads to a loss of the association with AS. In general, subtypes not associated with AS have a restriction for nonpolar C-terminal residues such as aliphatic chains and phenylalanine, whereas disease-associated molecular subtypes are able to bind peptides with a C-terminal tyrosine (Fiorillo *et al.*, 1998).

2.20 EVIDENCE OF OTHER GENETIC ASSOCIATIONS IN AS

There is significant evidence to suggest that other genetic factors act to determine which of these patients have a heightened susceptibility to developing the disease and family studies consistently show that HLA-B27 positve patients who have a first-degree relative with AS have rates of disease development 6-16 times greater than those without such a family history (Calin *et al.*, 1983).

2.21 TREATMENT

The general goals of treatment for patients with AS are: (1) *symptomatic relief*—to eliminate, or reduce to the minimum level possible, symptoms such as pain and stiffness; (2) *restore function*—to return the patient to the best possible functional capacity; (3) *prevent joint damage*—for those patients with hip or shoulder and/or peripheral joint involvement, to prevent joint destruction or bony ankylosis; (4) *prevent spinal fusion*—to prevent progressive bony erosions and ankylosis of the spine to maintain spinal mobility, and prevent development of spinal deformities; (5) *minimise extraspinal and extra-articular manifestations*—to reduce the impact of AS associated disorders such as uveitis and aortic valve insufficiency; and (6) *prevent*

complications of spinal disease—to prevent spinal fractures and flexion contractures, especially of the cervical spine (Sengupta and Stone, 2007).

2.21.1 Anti-inflammatory painkillers

Anti-inflammatories are used to ease a flare-up of symptoms. They reduce inflammation and ease pain. However, they do not alter the course of the disease. An important part of these drugs is to ease pain so that regular exercises can be done without much discomfort. There are several different brands of anti-inflammatory painkillers. For example, ibuprofen, diclofenac and naproxen. Side-effects sometimes occur with these drugs.

2.21.2 TNF antagonists

TNF is released by some cells involved in inflammation. Drugs that counter TNF (TNF antagonists) have been shown to reduce inflammation and ease symptoms in some people with AS. TNF antagonists include etanercept and infliximab. They are given by injection and require special monitoring as some people develop serious side-effects. Occasionally, other drugs are sometimes used. A steroid injected directly into a badly inflamed joint is sometimes used to ease symptoms. Some drugs that are used to reduce joint damage in rheumatoid arthritis are sometimes tried, for example, sulphasalazine and methotrexate. They do not work as well as in rheumatoid arthritis, but may be considered when non-spine joints are affected (Sidiropoulos *et al.*, 2008).

2.21.3 Exercise and posture

Exercise is the most important treatment of AS. Good posture and a regular exercise help to keep a full range of spinal movement and to prevent spine from stiffening up. Regular exercises also limit the extent of any spinal deformity that may develop, ease back pain and maintain the full range of movement of the spine.

2.22 PROGNOSIS FOR PEOPLE WITH ANKYLOSING SPONDYLITIS

Despite the fact that there is no cure for AS, the outlook is quite good. After an initial period of inflammation, the disease settles down to a low level of activity. Flare-ups of symptoms occur from time to time, often mild or moderate. In most cases, regular exercise and medication keep symptoms away. The lower spine tends to become stiffer and less flexible over the years. Uveitis (eye inflammation) may lead to blindness if not treated. People with established AS have an increased risk of fracturing the spine if they are involved in a high impact accident such as a car crash (Zochling *et al*., 2006).

2.23 OXIDATIVE STRESS

In normal healthy state, a balance is maintained between free radicals and antioxidants; when this equilibrium breaks down, oxidative stress arises within due to increase free radical or reduced antioxidant system. Free radicals act on cell membranes of different organelles of cells and cause cell injury and death by oxidative reactions; they also cause lipid peroxidation. The poly unsaturated fatty acids (PUFA) of cell membrane are more susceptible to this injury. Free radicals increase the permeability of cells, leading to calcium influx and altered pH of the cell (Jang and Pezzuto, 1998).

Oxidative stress plays a significant role in the pathogenesis of AS and RA. During inflammatory processes as in RA and AS, more free radicals are produced which lead to increase lipid peroxidation and oxidative stress (Darlington and Stone, 2001). Increased oxidative stress and a low antioxidant status in these patients have been reported (Blake *et al*., 1981; Tak and Bresnihan, 2000). It is observed that plasma levels of vitamin C and retinol inversely correlate with variables related

to disease activity, thus proposing a dietary antioxidant interventions in these patients (Verstraeten and Dequeker, 1986; Comstock *et al*., 1997).

Malondialdehyde (MDA) is the end-product of lipid peroxidation, a process where reactive oxygen species degrade polyunsaturated lipids causing toxic stress in cells and form an advanced glycation endproducts. The production of this aldehyde is used as a biomarker to measure the level of oxidative stress.

2.24 DYSLIPIDAEMIA

Increased premature mortality in RA and AS patients' results from accelerated atherosclerosis. There is an accumulating evidence of intriguing interactions between dyslipidaemia, inflammation and atherosclerosis (Goodson and Solomon, 2006). There is a worsening of the lipid profile during increased disease activity leading to an atherogenic lipid profile which is characterised by a reduced level of high-density lipoprotein cholesterol (HDLc) and increased levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDLc) and triglycerides (TG). An important prognostic indicator for future cardiovascular disease (CVD) is the atherogenic index, which is the ratio of total cholesterol to HDL-c. Tumour necrosis factor-alpha (TNF-α) is a pivotal cytokine in chronic inflammation and affects lipid metabolism, insulin resistance and endothelial function. It also promotes the inflammatory response and has been implicated in the pathophysiological processes of RA and AS (Kotler, 2000). Plasma concentrations of TNF-α are directly associated with the degree of early atherosclerosis and the impairment of vascular functions in rheumatoid conditions (Skoog *et al*., 2002).

Chapter 3

MATERIALS AND METHODS

3.1 STUDY DESIGN

This was a cross sectional study that was carried out from October, 2006 to June 2008. Standardized questionnaires were administered to patients diagnosed with either RA or AS and those who consented were recruited. The accuracy of the self-reported information on clinical history and socio-demographic information collected from the patients were assessed through record reviews of hospital database with a 100% rate of accuracy.

3.2 STUDY SITE

The Komfo Anokye Teaching Hospital (KATH), situated in the Ashanti Region, Kumasi, is one of the leading tertiary hospitals and a major referral centre in the country. KATH serves the Brong Ahafo, Northern, Upper East and Upper West regions of Ghana and also serves as the teaching Hospital for the Kwame Nkrumah University of Science and Technology. It orthopeadic department is one of the biggest tertiary care centre in the country and more than 45% of the total cases seen in the four regions are processed through this department with its catchment population estimated to be about 10 million people. The Korle Bu Teaching Hospital (KBTH) is the premier health care facility in Ghana. It is the only tertiary hospital in the southern part of Ghana and it is also a teaching hospital affiliated with the medical school of the University of Ghana. Its orthopeadic department processed most of the cases within southern Ghana. Medilab Diagnostic Centre is also the premier private laboratory in Ghana and has branches

in Accra, Kumasi, Obuasi, Sunyani, Techiman and Takoradi. These centres of excellence were used as study sites and they give a national representation.

3.3 STUDY POPULATION

3.3.1 Subject selection

Subjects for this study were both male and female patients visiting the orthopeadic departments of KATH, KBTH and Medilab centres. Thus the demographics of the patients that were tested in this study were not limited to a specific social group. The patients in this study originated from various social and ethnic groups as well as geographically distinct areas from the vast territory (ies) of the country. After an explanation of the purpose of the study, all the participants were invited to participate. They were informed that the study was confidential and that the information provided by them would not affect their treatment or management of the disease status. A total of one hundred and sixty seven (167) individuals, eighty (80) from KATH, fifty-five (55) from KBTH and thirty two (32) from Medilab joined in the research. Written informed consent was obtained from each participant and the information regarding the protocol and informed consent was presented to them at the appropriate literacy level. The medical records of each consenting participant were reviewed to ascertain whether they had been previously tested for the tests under investigations. The study was conducted in a confidential manner and random unique study-generated numbers were employed to identify the participants. They were divided into two (2) main groups. One group consisted of one hundred and two (102) patients diagnosed as having Rheumatoid Arthritis (RA) using America College of Rheumatology Criteria (ACR), x-ray examination and confirmed by a rheumatologist. The other group consisted of sixty-five (65)

patients diagnosed as having Ankylosing Spondylitis (AS) using modified New York criteria and confirmed by a rheumatologist.

3.3.2 Exclusion criteria

- Patients whose diagnosis could not be confirmed.
- Patients with other forms of arthritis apart from autoimmune induced arthritis.
- Confirmed RA and AS patients with Diabetes Mellitus and Hypertension.
- Juveniles with either RA or AS were also not taken into account.

3.3.3 Control selection

Fifty (50) blood donors with similar age and sex distribution as the subjects were recruited as control group. They comprised of 50 healthy blood donors from KATH donor clinic who had not been diagnosed with any form of arthritis or chronic joint pain. The exclusion criteria for the control cases were the same as for the subjects.

3.4 STANDARDIZED QUESTIONNAIRES ADMINISTRATION

All the consenting participants completed a structured questionnaire assessing socio-demographic characteristics, clinical and extra articular manifestations, family history, presence of other autoimmune conditions, drug history and risk profile for rheumatoid arthritis and ankylosing spondylitis. Physical examination was done to evaluate the general health condition for each participant. The America College of Rheumatology (ACR) classification criteria, which embody the risk profile for rheumatoid arthritis subjects was included. Furthermore, the Modified New York criteria and the Bath Ankylosing Spondylitis Disease Activity

Index (BASDAI) which access the risk profile and disease activity for anklyosing spondylitis subjects were also included in the questionnaire.

3.5 DETERMINATION OF DISEASE ACTIVITY USING BASDAI SCORE

The Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) was included into the questionnaire to assess disease activity. It is known that the BASDAI is superior in terms of symptoms considered and their weighting. The BASDAI is user friendly, highly reliable, reflects the entire spectrum of the disease, and is sensitive to clinical changes. It is also superior to the Newcastle Enthesis Index (Garrett *et al.*, 1994). Calin *et al.*, (1999) have further assessed the validity of the BASDAI and confirmed it. BASDAI consists of 10cm visual analog scales used to answer 6 questions pertaining to the 5 major symptoms of AS:

- Fatigue
- Spinal pain
- Joint pain / swelling
- Areas of localized tenderness
- Morning stiffness/duration.

To give each symptom equal weighting, the mean of the two scores relating to morning stiffness was taken. The resulting 0 to 50 score was divided by 5 to give a final 0 – 10 BASDAI score. Score from all questions were calculated using a ruler. The mean measurement (score) of questions 5 and 6 was added to the scores from questions 1 to 4. This total was then divided by 5 to give the average. This gave the BASDAI score. The higher the BASDAI score, the more severe the subject disability due to AS disease activity.

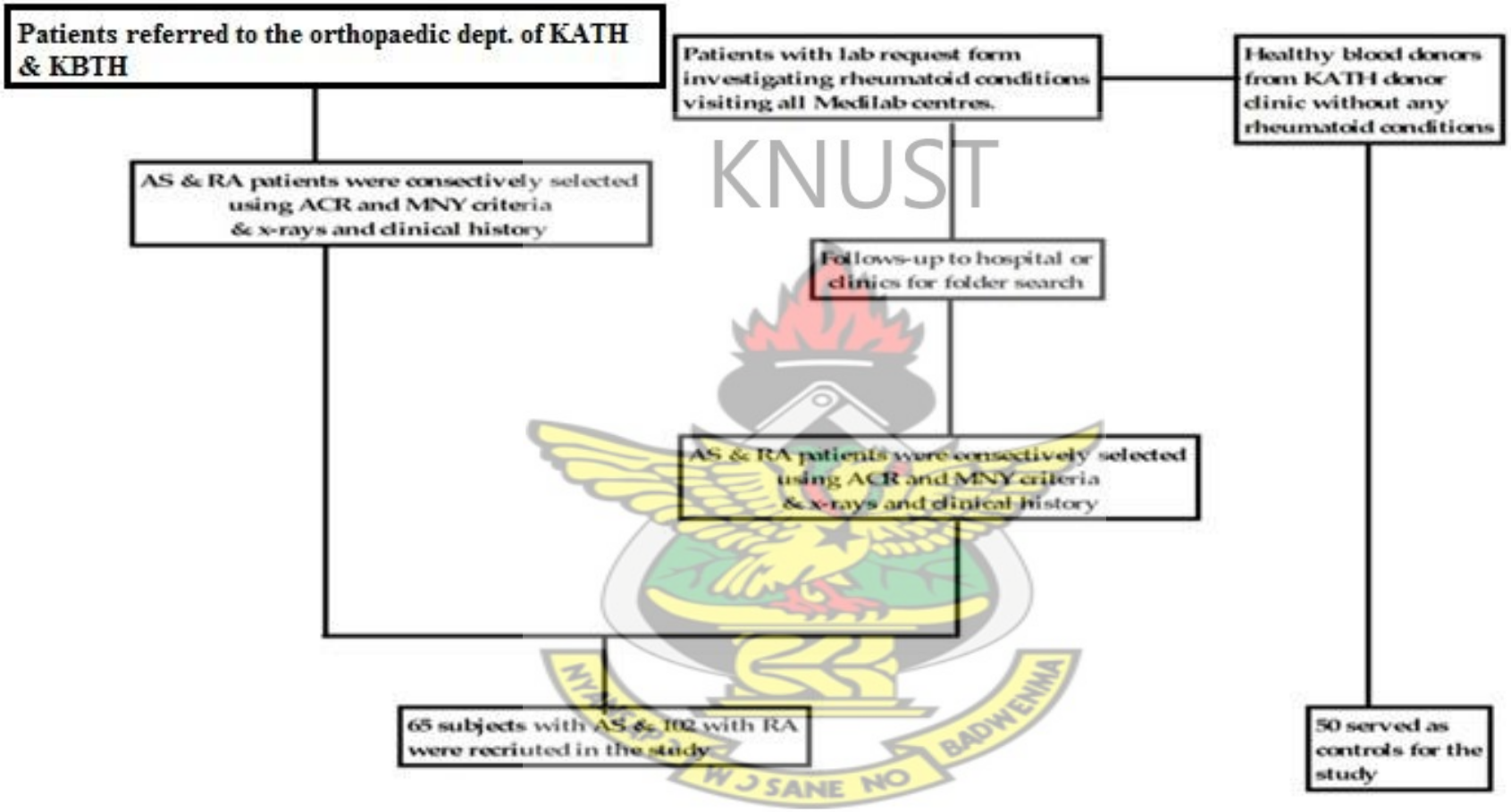

Figure 3-1 Flow diagram for the recruitment of the study subjects

3.6 BLOOD SAMPLE COLLECTION

Blood samples were collected from the ante-cubital vein from all subjects and controls. A rubber tourniquet was applied for less than one minute and the site to be punctured disinfected with 70% methylated spirit. Six (6) mls of blood sample was collected from each respondent and 3mls of it was dispensed into a vacutainer® containing two drops of the anticoagulant, ethylenediaminetetraacetic acid (EDTA). This sample was then used for the haematological analysis and HLA-B27 within three hours of collection. The rest of the blood sample was dispensed into a vacutainer®, allowed to clot and centrifuged at 3000 revolution per minute (rpm) for 10 minutes to obtain the serum which was stored at -20^{0}C until it was used for the biochemical and serological analysis.

3.7 METHODOLOGY

3.8 HAEMATOLOGICAL ASSAY

Haematological parameters assayed were haemoglobin concentration (Hb) and Erythrocyte Sedimentation Rate (ESR).

3.8.1 Haemoglobin Estimation (Cyanmethaemoglobin method)

Drabkin's reagent was used for the quantitative, colorimetric determination of haemoglobin concentration in whole blood. The procedure is based on the oxidation of haemoglobin and its derivatives (except sulfhemoglobin) to methemoglobin in the presence of alkaline potassium ferricyanide. Methemoglobin reacts with potassium cyanide to form cyanmethemoglobin, which has maximum absorption at 540 nm. The colour intensity measured at 540 nm is proportional to the total haemoglobin concentration (Drabkin and Austin, 1935). The EDTA blood specimen collected were vortexed to obtain homogenous samples, 20µl of blood was pipetted into 5mls of Drabkins reagent, vortexed and

allowed to stand for at least 15 minutes. The mixture was transferred into cuvette of pre-warm spectrophotometer, (spectrumlab™ 5A, USA) and absorbance read at 540nm against reagent blank. The Hb value was read from a previously calibrated graph in grams per deciliter.

3.8.2 Erythrocyte Sedimentation Rate estimation (ESR)

Using vortexed sample of the EDTA blood collected, the ESR was determined using the Westerngren tube method, one part of disodium citrate to four parts of whole blood (Expert Panel on Blood Rheology of the International Council for Standardization for Haematology, 1993). A clean dry standard Westergren tube was filled with the blood and adjusted to the zero mark. It was then placed in a vertical position on a bench. After exactly one hour, the distance from the bottom of the surface meniscus to the top of the column of sedimenting red cells was recorded in mm/hr as the ESR.

3.9 HLA-B27 ESTIMATION

HLA-B27 IMS-sandwich ELISA has high sensitivity and specificity, easy operation and clear interpretation. It combines immuno-magnetic separation (IMS) and ELISA techniques for the rapid detection of HLA-B27 antigen in whole blood samples. The HLA-B27 IMS ELISA kit was purchased from Taiwan Advance Bio-Pharmaceuticals, Taiwan. Tests were performed according to the manufacture's instructions.

In a 96-microtiter plate, 50µl of whole blood was incubated with 50µl of monoclonal anti-B27-coated magnetic beads (MB) and horseradish peroxidase (HRP)-labelled anti-CD45 monoclonal antibody (McAb). The anti-HLA-B27 McAb (ABC-m3) on the magnetic bead was allowed to react for 10 minutes with the B27

antigen, and at the same time, the HRP-labelled anti-CD45 McAb reacted with the CD45 leucocytes common antigen. During the reaction, MB attached B27-positive cells, which were also labeled with HRP-CD45 McAb, were isolated from peripheral blood mononuclear cells by adherence to the magnetic plate under the tray. HLA-B27 negative cells did not bind to the MB and were washed away. After washing and removing the supernatant four times, each well was filled with tetramethylbenzamidine (TMB) substrate and left for 10 minutes at room temperature. After adding the stopping solution, the absorbance (A) of each well was measured using an ELISA plate reader (DNM 9602 New Tech Corp.) at a wavelength of 550nm.

3.10 BIOCHEMICAL ASSAY

Biochemical parameters assayed included total cholesterol, triglycerides, high density lipoprotein, low density lipoprotein, vitamin C and Malondialdehyde.

3.10.1 Total cholesterol

Total cholesterol was assayed using a kit from Human Diagnostic™ (Wiesbaden, Germany) which employs an enzymatic colorimetric method with lipid clearing factor. The cholesterol is determined after enzymatic hydrolysis and oxidation (Allain *et al.*, 1974). The indicator quinoneimine is formed from hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidase (Tamaoku *et al.*, 1982).

$$\textbf{Cholesterol Esters} \xrightarrow{CE} \textbf{Cholesterol} + \textbf{Fatty Acids}$$

$$\textbf{Cholesterol} + O_2 \xrightarrow{CO} \textbf{Cholest} - 4 - en - 3 - one + H_2O_2$$

$$4 - AA + ADPS + H_2O_2 \xrightarrow{POD} \textbf{Quinoneimine (red dye)} + 4H_2O$$

Briefly, the reagents, standards and samples were brought to room temperature and 1 ml of enzyme reagent pipetted into test tubes labelled blank, samples and standard. 10ul of samples and standard was added into respective test tubes, mixed thoroughly and incubated for 5 minutes at 37^0C or 10 minutes at room temperature. The absorbance of the standard and the samples were read at 550 nm against the reagent blank. The total cholesterol concentration of the sample was calculated as follows:

$$\frac{Absorbance\ of\ Test}{Absorbance\ of\ Standard} x\ Conc.\ of\ Standard = Conc.\ total\ cholesterol$$

3.10.2 Triglycerides

Triglycerides were assayed using a kit from Human Diagnostic® (Wiesbaden, Germany) which employs an enzymatic colorimetric method with lipid clearing factor. The present method uses a modified Trinder (Trinder, 1969; Barham and Trinder, 1972) colour reaction to produce a fast, linear, endpoint reaction (Fossati and Prencipe, 1982; McGowan *et al.*, 1983). Triglycerides in the sample are hydrolyzed by lipase to glycerol and fatty acids. The glycerol is then phosphorylated by ATP to glycerol-3-phosphate (G -3-P) and ADP in a reaction catalyzed by glycerol kinase. G-3-P is then converted to dihydroxyacetone phosphate (DAP) and hydrogen peroxide by glycerophosphate oxidase (GPO). The hydrogen peroxide then reacts with 4-aminoantipyrine (4-AAP) and 3, 5-dichloro-2-hydroxybenzen (3, 5-DHBS) in a reaction catalyzed by peroxidase to yield a red coloured quinoneimine dye. The intensity of the colour produced is directly proportional to the concentration of triglycerides in the sample.

$$Triglycerides + H_2O \xrightarrow{Lipase} Glycerol + Fatty\ Acids$$

$$Glycerol + ATP \xrightarrow{Glycerolkinase} G-3-P + ADP$$

$$G-3-P + O_2 \xrightarrow{glycerolphosphate\ oxidase} DAP + H_2O_2$$

$$H_2O_2 + 4AAP + 3,5-DHBS \xrightarrow{peroxidase} Quinoneimine\ (red\ dye) + 2H_2O$$

Briefly, the reagents, standards and samples were brought to room temperature. One (1) ml of monoreagent was pipetted into test tubes labelled blank, samples and standard before 10 ul of samples and standard were pipetted into their respective test tubes, mixed thoroughly and incubated for 5 minutes at 37^0C or 10 minutes at room temperature. The absorbance of the standard and the samples were read at 550 nm against the reagent blank. The triglycerides concentration of the sample was calculated as follows:

$$\frac{Absorbance\ of\ Test}{Absorbance\ of\ Standard} x\ Conc.of\ Standard = Conc.of\ triglycerides$$

3.10.3 HDL and LDL cholesterols

High density lipoprotein (HDL) Cholesterol was assayed using a kit from Human™ Diagnostic (Wiesbaden, Germany) which employs a precipitant and a standard. The chylomicrons, very low density lipoproteins (VLDL) and low density lipoproteins (LDL) are precipitated by the addition of phosphotungstic acid and magnesium chloride (Warnick *et al.*, 1985; Stein and Myers, 1995). After centrifugation the supernatant fluid contains the HDL fraction, which is assayed

for HDL Cholesterol with the HUMAN™ cholesterol liquicolor test kit. The reagents, standards and samples were brought to room temperature and 1 ml of precipitant aliquoted into test tubes labelled samples. Subsequently 500 ul of samples were pipetted into respective test tubes, mixed thoroughly and incubated for 10 minutes at room temperature. The mixture was then centrifuged for at least 2 minutes at 10000 g, or for 10 minutes at 4000 g. After the centrifugation, the clear supernatant was separated from the precipitate within one hour. 1 ml of enzyme reagent (HUMAN™ cholesterol liquicolour reagent) was pipetted into test tubes labeled blank, samples and standard. 100 ul of samples and HDL standard were then added to their respective test tubes, mixed thoroughly and incubated for 5 minutes at 37^0C or 10 minutes at room temperature. The absorbance of the standard and the samples were read at 550 nm against the reagent blank. The HDL cholesterol concentration of the sample was calculated as follows:

$$\frac{Absorbance\ of\ Test}{Absorbance\ of\ Standard} x\ Conc.of\ Standard = Conc.of\ HDL - C$$

The LDL-Cholesterol concentration (LDL-C) was calculated from the total cholesterol concentration (TC), HDL-Cholesterol concentration (HDL-C) and the triglycerides concentration (TG) according to Friedewald's equation (Friedewald *et al.*, 1972).

$$LDL = TC - HDL - \frac{TG}{5} \quad mmol/l$$

3.11 OXIDATIVE STRESS MARKERS

3.11.1 Ascorbic acid

Ascorbic acid (vitamin C) was assayed using the micro techniques of clinical chemistry developed by Samuel Natelson in 1961 (Natelson, 1961). Ascorbic acid in plasma is oxidized by Cu^{2+} to form dehydroascorbic acid, which reacts with acidic 2, 4- dinitrophenylhydrazine to form a red dihydrazone, which is measured at 520nm. Ascorbic acid should be analyzed immediately or not later than 3 hour if the specimen is refrigerated. 0.4 ml of serum was added rapidly to 1.6 ml of 10% trichloroacetic acid, mixed thoroughly and allowed to stand at room temperature for five minutes. The mixture was centrifuged at 2000 rpm for five minutes. One milliliter (1ml) of the supernatant was pipetted into the test tube for the test and 0.4 ml of dinitriphenyl hydrazine reagent was then pipetted into all the three test tubes labelled; sample, standard, and blank. One (1) ml of sample and standard were then added to their respective test tubes. 1 ml of trichloroacetic acid was also added to the test tube labelled blank. The test tubes were stoppered and incubated at 37^0C for three hours. The mixture was then chilled in ice bath after which 1.6 ml of cold 65% H_2SO_4 was added and mixed thoroughly. The mixture was allowed to stand for 30 minutes at room temperature, and the absorbance of the standard and test read against blank at 520nm in a spectrophotometer (spectrumlab™ 5A, USA). The concentration of ascorbic acid was calculated as follows:

$$\frac{Absorbance\ of\ Test}{Absorbance\ of\ Standard} x\ Conc.\ of\ Standard = Conc.\ of\ Vitamin\ C$$

3.11.2 Malondialdehyde (MDA) determination

The method used for this assay was based on that described by Kamal (Kamal *et al.*, 1989). MDA levels were determined by the MDA-Thiobarbituric acid (TBA) test which is the colorimetric reaction of MDA and TBA in acid solution. TBA reacted with MDA, a secondary product from lipid peroxidation, which generated an adduct of a red colour and was measured spectrophotometrically. 0.5 ml of serum was added to 2.5 ml of 20% trichloroacetic acid (TCA) after which 1 ml of 0.67% TBA was added to the mixture and incubated at 100°C for 30 minutes. After cooling, the sample was extracted with 4 ml n-butanol and centrifuged at 3000 rpm for 10 min. The absorbance of the supernatant was read at 535 nm and the results were expressed as μmol/l, using the extinction coefficient of 1.56 x 10^5l/mmol cm.

3.12 SEROLOGICAL ASSAY

Serological parameters assayed included;

- Rheumatoid Factor Latex agglutination (RF-Latex)
- Rheumatoid Factor Immunoglobulin A (RF-IgA)
- Rheumatoid Factor Immunoglobulin M (RF-IgM)
- Anti-cyclic citrullinated peptide (Anti –CCP)
- Tumour necrosis factor alpha (TNF-α)

3.12.1 Rheumatoid Factor Latex Agglutination

Latex agglutination slide test for the qualitative and semi quantitative test of Rheumatoid Factor (RF) in human serum was purchased from Smartest diagnostic, (USA). The Latex RF direct test is based upon the immunological reaction between the rheumatoid factor (RF) in the serum and the corresponding human antibody

IgG coated onto polystyrene latex particles. When the serum containing RF is mixed with the RF latex, a resulting agglutination will be observed.

Reagents and respondent samples were brought to room temperature prior to use, one drop (25 µl) of the non-diluted patient sample was put on the slide. Also one drop of the positive control and negative control was put on separate cells of the test slide. One drop of each sample was gently mixed with the contents of the latex reagent. The mixture was stirred with the mixer provided, covering the whole surface of each cell. Gently, the slides were rotated for 2 minutes and observed under light, whether there was absence or presence of agglutination. A negative and a positive control was used with each serial samples.

3.12.2 RF-IgA Estimation

RF-IgA was assayed using AUTOSTAT™II (Hycor Biomedical Ltd, Edinburgh, UK) enzyme linked immunosorbent assay (ELISA) kit specific for RF-IgA. The Autostat™II assay for detection of autoantibodies is a solid phase immunosorbent assay in which the analyte is indicated by a colour reaction of an enzyme and substrate. The Autostat™II wells are coated with purified antigen. On adding diluted serum to the wells the antibodies present bind to the antigen. After incubating at room temperature and washing away unbound material, horseradish peroxidase conjugated anti-IgA monoclonal antibody was added, which binds to the immobilised antibodies.

Following further incubation and washing, tetra-methyl benzidine substrate (TMB) was added to each well. The presence of the antigen-antibody-conjugate complex turns the substrate to a dark blue colour. Addition of the stop solution turns the colour to yellow. The colour intensity was proportional to the amount of autoantibodies present in the original serum sample. All reagents were brought to

room temperature (18-25°C). All serum samples and assay controls were diluted 1/100 in sample diluent by adding 5µl to 495µl sample diluent. Standards were not diluted. 100µl of the standards, diluted control or diluted patient sample were pipetted into the wells. To achieve blanking on the plate reader a 'no serum' control of 100µl of sample diluent was added to the first two wells. This acted as the zero point for the curve. The wells were incubated at room temperature (18-25°C) for 30 minutes.

The wells were washed three times with diluted wash buffer. This was done manually with a multichannel pipette. The wells were emptied and tapped dry on paper towel. 100µl of ready-to-use conjugate was added to each well. The wells were incubated at room temperature (18-25°C) for 15 minutes and washing repeated as above. 100µl of ready-to-use TMB substrate was added to each well. The wells were incubated at room temperature (18-25°C) for 15 minutes. 50µl of stop solution was added to each well and gently tapped to ensure uniform colour distribution and read within 15 minutes. The absorbance of the well contents was read at 450nm on a plate reader (DNM 9602 New Tech Corp). The blank was subtracted from the optical densities of the standard, controls and patient samples.

3.12.3 RF-IgM Estimation

The Diagnostic Automation, Inc. (DAI, Chicago, Ilinois, USA) Rheumatoid Factor (RF) Enzyme-Linked Immunosorbent Assay (ELISA) kit was used for the detection of RF-IgM antibodies in human serum to RF antigen and as an aid in the diagnosis of rheumatoid arthritis. The purified antigen is bound to a solid phase microassay well. Patient serum samples to be assayed for antibody are first diluted and are added to each well. If antibody is present in the patient's serum, antigen-antibody complexes are formed. After washing the unbound serum from the well, horseradish peroxidase conjugated anti-human IgM was added to the wells and allowed to incubate. The

conjugate will bind to human antibody which is present. After washing the unbound conjugate from the wells, TMB substrate solution was added and incubated. The enzyme conjugate present will react with the H_2O_2 substrate and tetramethylbenzidine (TMB) chromogen, resulting in blue color development. The addition of 1N H_2SO_4 stops the enzymatic reaction and turns the blue color to yellow. The absorbance of the solution, measured at 450 nm, is directly related to the concentration of IgM antibody bound to the well.

Briefly, all reagents were brought to room temperature (21 - 25° C) before use. All samples and controls were also vortexed before use. For each test serum, calibrator and control to be assayed, a 1:21 serum dilution was prepared. 10 µl of serum sample was added to 200 µl of serum diluent and mixed thoroughly. To individual wells, 100 uL of the appropriate diluted calibrator, controls and patient sera were added. 100 ul of serum diluent was added to reagent blank well. Each well was incubated at room temperature (21-25) ^{oC} for 30 minutes ± 1 minute. Liquid was aspirated from all wells and the washing procedure was repeated twice (for a total of three (3) washes). After the final wash, the plate was blotted on paper toweling to remove all liquid from the wells. 100 ul conjugate was added to each well, including reagent blank well. Avoiding bubbles upon addition as they may yield erroneous results. Each well was incubated at room temperature (21-25) ^{o}C for 30 minutes ± 1 minute. Washing was repeated as above. 100 ul of chromogen/substrate solution (TMB) was added to each well, including reagent blank well, maintaining a constant rate of addition across the plate. Each well was incubated at room temperature (21-25) ^{o}C for 15 minutes ± 2 minutes. Reaction was stopped by addition of 100 ul of stop solution (1N Sulfuric Acid) following the same order of chromogen /substrate (TMB) addition, including the reagent blank well; the plate was tapped gently along the outsides, to mix contents of the wells. The developed colour was read on an ELISA plate reader (DNM

9602 New Tech Corp). The plate was held up to 1 hour after addition of the stop solution before reading at 450nm.

3.12.4 Anti-CCP Estimation

The DIASTAT™ Anti-CCP (Axis Shield, UK) assay kit was used for the detection of the IgG class of autoantibodies specific to cyclic citrullinated peptide (CCP) in human serum. It is intended to aid in the diagnosis of Rheumatoid Arthritis (RA). Autoantibody levels represent one parameter in a diagnostic process, encompassing both clinical and laboratory-based assessments. The wells of the microtitre strips are coated with a highly purified synthetic cyclic peptide containing modified arginine residues. During the first incubation, specific autoantibodies in diluted serum or plasma bind to the antigen-coated surface. The wells are then washed to remove unbound components. In the second incubation, the conjugate, an enzyme-labelled monoclonal antibody to human IgG, binds any surface-bound autoantibodies. After further washing, specific autoantibodies are traced by incubation with the substrate. Addition of stop solution terminates the reaction, resulting in a coloured end-product. The amount of conjugate bound is measured in absorbance units. In the qualitative protocol, the amount of conjugate bound by the sample is compared with that bound by the reference control. In the semi-quantitative protocol, the concentration of anti-CCP can be estimated by interpolation from a dose-response curve based on standards (Sebbag *et al.*, 1995; Schellekens *et al.*, 1998; Girbal-Neuhauser *et al.*, 1999).

Semi-Quantitative protocol**:** standards, positive and negative controls, and samples were assayed, wells were referenced for identification. 100µl of referenced control/standards in duplicate, pre-diluted (1:100) positive and negative controls, and pre-diluted (1:100) patient samples were pipetted into appropriate wells. The wells were incubated for 60 minutes at 18-25°C, strip contents was decanted by

quick inversion over a sink with paper towels. Wells were washed three times with a minimum of 200µl diluted wash buffer, decanted and blotted after each wash step.

100µl of conjugate was added to each well and incubated 30 minutes at 18-25°C, washing was repeated. 100µl of substrate was added to each well and incubated for 30 minutes at 18-25°C without decanting and 100µl stop solution was added to each well in the same order and rate as the substrate, wells was tapped gently to mix and strips read at 550nm using plate reader (DNM 9602 New Tech Corp.).

3.12.5 TNF-α Estimation

Human TNF-α TiterZyme® Enzyme Immunometric Assay (Assay Design, USA) test kit was used. It is a complete test for the quantitative determination of human TNF-α in biological fluids. It uses a monoclonal antibody to human TNF-α immobilized on a microtiter plate to bind the human TNF-α in the standards or sample. After a short incubation the excess sample or standard is washed out and a biotinylated polyclonal antibody to human TNF-α is added. This antibody binds to the human TNF-α captured on the plate. After a short incubation the excess antibody is washed out and streptavidin, conjugated to horseradish peroxidase is added, which then binds to the biotinylated human TNF-α antibody. Excess conjugate is washed out and substrate is added. After a short incubation, the enzyme reaction is stopped and the colour generated is read at 450 nm. The measured optical density is directly proportional to the concentration of human TNF-α in either standards or samples (Klareskog and McDevitt, 1999).

All reagents were brought to room temperature for at least 30 minutes prior to opening. 100 µl of standard diluent was pipetted into the S0 (0 pg/ml standard) wells. 100 µl of standards was put into wells #1 through #7. 100 µl of the samples

were also put into the appropriate wells. The plate was tapped gently to mix the content; the plate was sealed and incubated at 37 °C for 2 hours. The content of the wells were emptied and washed by adding 400 µl of wash solution to every well, washing was repeated 3 more times for a total of 4 washes. After the final wash, the wells were emptied by firmly tapping the plate on a towel to remove any remaining wash buffer. 100 µl of yellow antibody was put into each well, except the blank. The plate was sealed and incubated at 37 °C for 1 hour. The content of the wells were emptied and washed by adding 400 µl of wash solution to every well; washing was repeated 3 more times for a total of 4 washes. After the final wash, wells were aspirated and the plate tapped on a towel to remove any remaining wash buffer. 100 µl of blue conjugate was added to each well, except the blank. The plate was sealed and incubated at 37 °C for 30 minutes, the content of the wells were emptied and washed as above. The washing was repeated and wells emptied as above. 100 µl of substrate solution was put into each well and incubated for 30 minutes at room temperature, 100 µl stop solution 2 was also put into each well. The plate reader (DNM 9602 New Tech Corp.) was blanked against the blank wells and the optical density read at 450 nm, the mean of the optical density of the blank was subtracted from all the readings.

3.13 STATISTICAL ANALYSIS

Statistical comparisons were analyzed using the one way ANOVA followed by Bonferroni's Multiple Comparison test. Continuous variables were expressed as their mean ± SEM, while categorical variables were expressed as proportion. Correlations were evaluated using the Pearson's correlation analysis. The Receiver

Operator Characteristic (ROC) was used to analyze for the sensitivity and specificity of the various RA biomarkers. The sensitivity and specificity for RA subjects and controls were computed for each of the four tests, along with the 95% confidence intervals (CI) and their differences were tested with McNemar's test.

For all statistical comparisons, the level of significance was set at $p<0.05$. All data analysis was carried out using GraphPad Prism for Windows version 5.00 (GraphPad Software, San Diego, CA, USA).

Chapter 4

RESULTS

4.1 DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

There were 167 respondents comprising of 102 patients with RA and 65 with AS. The proportion of adults with RA was higher for women than for men in a ratio of 2.5:1. For AS, the female to male ratio was 1:3. Median age for RA was 49.96 yrs and AS was 46.75 yrs. Seventy-one (43%) of all respondents with chronic joint symptoms reported limitations in activities because of their joint symptoms. Their mean symptom duration was more than 8 months. Prevalence of rheumatoid conditions increased with increasing age of respondents. There was no significant difference in educational level among AS and RA patients as the disease tend to occur among people with different educational background. None had a family history of Ankylosing Spondylitis, psoriasis, or chronic bowel disease.

4.2 HAEMATOLOGICAL INVESTIGATIONS

4.2.1 Haemoglobin

There was a significant difference ($F=11.37$; $df= 2$; $p<0.0001$) in the haemoglobin levels between the control group and the RA and AS subjects. The mean haemoglobin was 11.27 ± 0.15 g/dl for RA and 11.86±0.17 g/dl for AS subjects as compared to 13.99 ± 0.15 g/dl of control group (Figure 4.1). Bonferroni's Multiple Comparison test gave no significant difference ($p>0.051$) between the haemoglobin levels of RA and AS subjects.

4.3 COMPARISON OF RF-LATEX AND ITS ISOTYPES WITH ANTI-CCP

Out of 102 RA patients, 52.90% were positive with RF-IgM, 41.20% with RF-Latex, 27.40% with RF-IgA and 72.50% with anti-CCP (Table 4.1). Using receiver operating characteristic (ROC) curve, sensitivities and specificities were computed under the ROC curve. Sensitivity for RA was highest for RF-IgM test (58.45%) followed by anti-CCP antibody (54.34%), RF-latex (52.48%) and RF-IgA tests (28.51%). Specificity of 96.67% was obtained for anti-CCP, 69.52% for RF-IgM, 63.12% for RF-latex and 51.75% for RF-IgA as shown in table 4.2.

Table 4:1 Demographic and RF markers of subjects with RA

PARAMETERS	VALUES
Mean Age	49 yrs
Mean Symptom duration	> 8 months
Male to Female ratio	1:2.5
RF-IgA positive	27.40%
RF-IgM positive	52.90%
RF-Latex positive	41.20%
Anti-CCP positive	72.50%

Anti-CCP: Anti-Cyclic Citrullinated Peptide, RF-Latex: Rheumatoid Factor, RF-IgA: Rheumatoid Factor immunoglobulin A, RF-IgM: Rheumatoid Factor immunoglobulin M.

Table 4:2Comparison of RF-Latex and its isotypes (RF-IgA and RF-IgM) with anti-CCP

	RF-IgM	RF-IgA	RF-LATEX	ANTI-CCP
AUC	0.612	0.668	0.0611	0.905
SE	0.0507	0.0471	0.0511	0.0487
95% CI	0.54-0.68	0.59-0.73	0.55-0.64	0.85-0.71
Z-STATISTICS	2.215	3.561	3.309	2.987
P-VALUE	0.0267	0.0004	0.0031	0.0028
CUT-OFF	>1.1	>21.4	>20.1	>5.2
SENSITIVITY	58.45	28.51	52.48	54.34
SPECIFICITY	69.52	51.75	63.12	96.67
+LR	5.17	6.52	5.09	4.98
-LR	0.73	0.83	0.54	0.68
+PV	96.3	97.1	95.8	100
-PV	21.4	19.3	20.4	22.6

AUC: Area under the curve, SE: shared epitome, +LR: Positive likelihood ration, -LR: Negative likelihood ratio, +PV: Positive predictive value, -PV: Negative predictive value

Table 4:3 Numbers of ACR criteria and disease duration for positive Anti-CCP

Age Group	(n)	%	No. ACR Criteria	Symptom Duration (months)	Mean Anti-CCP value
21-30	2	1.9	**3**	**< 1**	5.1
31-40	12	11.8	4	1—6	5.45
41-50	34	33.3	5	7—9	5.80
51-60	**36**	**35.3**	**5**	**10—12**	**5.82**
61-70	11	10.8	6	13	6.1
>70	7	6.9	6	>13	7.44

ACR; America college of rheumatology, ANTI-CCP; anti-cyclic citrullinated peptide

Table 4.3 indicates that the anti-CCP was detectable even in subjects who do not satisfy all the ACR criteria; moreover, anti-CCP concentration tends to increase with increasing symptoms duration, age and the number of ACR criteria. The highest prevalence of RA subject is those within 51-60 years. These diagnostic features of anti-CCP enable it to serve as an early and definitive marker for RA.

The sensitivity and specificity among RA subjects were computed for each of the four tests, along with the 95% confidence intervals (CI), the differences were tested with McNemar's test. The ROC for anti-CCP showed excellent discrimination between RA subjects and controls as indicated in table 4.3. The kappa statistic was used to examine whether the tests tended to identify the same patients as positive

or negative were also computed in table 4.4. The combination of anti-CCP and RF-IgM gave an excellent agreement in identifying positive RA subjects.

Table 4:4 ROC with confidence interval for the prediction of RA

RA markers	Sensitivity 95% (CI)	Specificity 95% (CI)
Anti-CCP	0.54(0.50-0.75)	0.96(0.86-1.00)
RF-IgA	0.28(0.30-0.65)	0.51(0.60-0.90)
RF-IgM	0.58(0.50-0.65)	0.69(0.60-0.90)
RF-Latex	0.52(0.48-0.65)	0.63(0.60-0.90)

ROC: Receiver Operating Characteristics, CI: Confidence Interval

Table 4:5 Comparison of agreement in identifying RA patient as positives and controls as negative

Parameters	Agreement among RA patients	Agreement among control subjects
Anti-CCP vrs RF-IgM	0.85(0.45-0.75)	0.05(0.1-0.100)
Anti-CCP vrs RF-IgA	0.30(0.20-0.40)	0.30(0.15-0.75)
Anti-CCP vrs RF-Latex	0.45(0.35-0.45)	0.07(0.19-0.75)

4.4 PREVALENCE OF HLA-B27 AMONG AS SUBJECTS

Tables 4.5 and 4.6 show general and clinical characteristic features for AS subjects. There were sixty-five (65) AS subjects of which three (3) were HLA-B27 positives representing 4.6%. All the three (3) HLA-B27 positives were males and their mean Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) score was 44.7/100. Forty-eight (48) AS subjects had sacrolitis in their x-ray reports; one patient had bilateral sacrolitis with peripheral oligoarthritis. None had a family history of Ankylosing Spondylitis, psoriasis, extra-articular manifestation or chronic bowel disease.

Table 4:6 Demographic and clinical characteristics of AS subjects

PARAMETERS	VALUES
Mean Age	46yrs
Mean Symptom duration	> 8 months
Male to Female ratio	3:1
Hb(gdl^{-1})	11.27
ESR($mmhr^{-1}$)	34.64
HLA-B27 positives	4.61%
Mean BASDAI score	44.7/100
Sacroiliitis (x-ray)	73.80%

HLA-B27: Human Leukocytes Antigen, BASDAI: Bath Ankylosing Spondylitis Disease Activity Index, ESR; Erythrocytes Sedimentation Rate

Table 4:7 Clinical features of Ghanaian patients with Ankylosing Spondylitis*

Patients no.	Axial Involvements	Peripheral Arthritis	Enthesopathy	Extraarticular Manifestation
15	bilateral sacroilitis	Absent	Absent	Absent
48	bilateral sacroilitis	Absent	Absent	Absent
94	bilateral sacroilitis	Oligoarthritis	Absent	Absent
97	bilateral sccroilitis	Absent	Absent	Absent

Patients were three men and one woman, and they fulfilled the New York criteria for Ankylosing Spondylitis. None had a family history of Ankylosing Spondylitis, psoriasis, or chronic bowel disease

4.5 DYSLIPIDAEMIA

4.5.1 Lipid profile

4.5.1.1 Total cholesterol

Using one-way ANOVA, RA and AS subjects exhibited higher mean serum levels of total cholesterol (TC) 5.88 ± 0.06 mmol/l than the controls (4.22 ± 0.17 mmol/l). The difference between the RA and AS subjects cholesterols compared to the control was statistically significant (F=7.69; df=2; p<0.001) (Table 4.7). Pearson's correlation method of analysis revealed a significant (r^2= 0.0619, p= 0.002) correlation between serum triglycerides and the serum total cholesterol level of AS subjects as shown in table 4.8.

4.5.1.2 Triglycerides

Using one-way ANOVA analysis, RA and AS subjects exhibited a slightly higher mean serum level of triglycerides 0.88 ± 0.16 mmol/l as compared to 0.56±0.05 mmol/l of controls. The difference between the subjects triglycerides levels and that of the control was statistically significant (F=9.50; df=2; p<0.001) (Table 4.7).

4.5.1.3 HDL

Table 4.7 shows that the mean HDL levels for AS and RA subjects was significantly lower (F=1.13; df=2; p<0.001) as compared to the control from one-way ANOVA analysis.

4.5.1.4 LDL

The mean LDL level using ANOVA analysis indicates significantly higher (F=4.72; df=2; p=0.001) value as compared to the controls. Pearson's correlation method of analysis revealed a significant (r^2= 0.0619, p= 0.002) positive correlation between

serum triglycerides, total cholesterol, HDL and the serum LDL of RA subjects as shown in tables 4.7 and 4.8.

4.5.1.5 Atherogenic Index and Ratios

A statistically significant ($F=4.23$; $df=2$; $p<0.001$) atherogenic index and ratios i.e. TC/HDL-C and LDL-C/HDL-C were observed among the subjects and controls as shown in table 4.7.

Table 4:8 Comparisons of lipid profile and atherogenic index between subjects and control

PARAMETERS	SUBJECTS	CONTROL	P-VALUE
TC (mmol/l)	5.88±0.06	4.22±0.17	0.001
TG (mmol/l)	0.88±0.02	0.56±0.05	0.001
HDL-C (mmol/l)	1.07±0.01	1.52±0.02	0.001
LDL-C (mmol/l)	3.97±0.04	2.41±0.07	0.001
Atherogenic index (mmol/l)	4.49±0.07	1.77±0.04	0.001
TC:HDL ratio	5.27±0.02	2.77±0.04	0.001
LDL:HDL ratio	3.60±0.05	1.58±.001	0.001
TG:HDL ratio	0.80±0.01	0.36±0.02	0.001

The data are expressed as mean ± SEM, TC: total cholesterol, TG: triglycerides, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein. Atherogenic index = [(total cholesterol – HDL cholesterol)/HDL cholesterol].

Table 4:9 Pearson's correlation coefficients between lipid profile parameters for controls (lower-left hand side) and patients (upper right-hand side)

	AS TC level	**AS TG level**	**AS HDL level**	**AS LDL level**	**RA TC level**	**RA TG level**	**RA HDL level**	**RA LDL level**
AS TC level		0.06	0.16	0.88	-0.02	-0.15	0.05	-0.01
P value		0.61	0.18	0	0.85	0.22	0.67	0.89
AS TG level	0.06		-0.11	-0.28	-0.09	0.14	0.01	-0.16
P value	0.61		0.38	0.02	0.43	0.26	0.94	0.19
AS HDL level	0.16	-0.11		-0.07	-0.16	-0.11	-0.04	-0.12
P value	0.18	0.38		0.55	0.19	0.37	0.72	0.32
AS LDL level	0.88	-0.28	-0.07		0.05	-0.17	0.01	0.09
P value	0.00	0.02	0.55		0.64	0.15	0.88	0.45
RA TC level	-0.02	-0.09	-0.16	0.05		0.45	0.11	0.85
P value	0.85	0.43	0.19	0.64		0	0.15	0
RA TG level	-0.15	0.14	-0.11	-0.17	0.45		0.00	0.06
P value	0.22	0.26	0.37	0.15	0.00		0.929	0.37
RA HDL level	0.05	0.01	-0.04	0.01	0.11	0.00		-0.24
P value	0.67	0.94	0.72	0.88	0.15	0.92		0.00
RA LDL level	-0.01	-0.16	-0.12	0.09	0.85	0.06	-0.24	
P value	0.89	0.19	0.323	0.45	0.00	0.37	0.00	

Correlation is significant at the 0.001 level (2-tailed). TC: total cholesterol, TG: Triglycerides, HDL-C: High Density Lipoprotein Cholesterol, LDL-C: Low Density Lipoprotein Cholesterol.

4.6 PROINFLAMMATORY AND INFLAMMATORY MARKERS

4.6.1 TNF-α

TNF-α level as a pro-inflammatory marker among RA and AS subjects were 11.87 ± 0.30 pg/ml and 13.11 ± 0.50 pg/ml as compared to normal control of 5.70 ± 0.48 pg/ml (figure 4.1). The differences between control group and RA and AS subjects were statistically significant (p<0.001).

4.6.2 ESR

From the one-way ANOVA analysis there was a significant difference (F=43.48; df= 2; p<0.0001) in the ESR values between the control group and the RA and AS subjects. The mean ESR value for AS was 34.64 ± 1.87 mm/hr and that of RA was 24.67 ± 1.87 mm/hr as compared to 9.23 ± 0.91 of controls. Using Bonferroni's Multiple Comparison Test, there was a significant difference (p<0.0001) between RA and AS ESR values. The standard deviation was 24.17 implying that there is an inflammation as shown in figure 4.2.

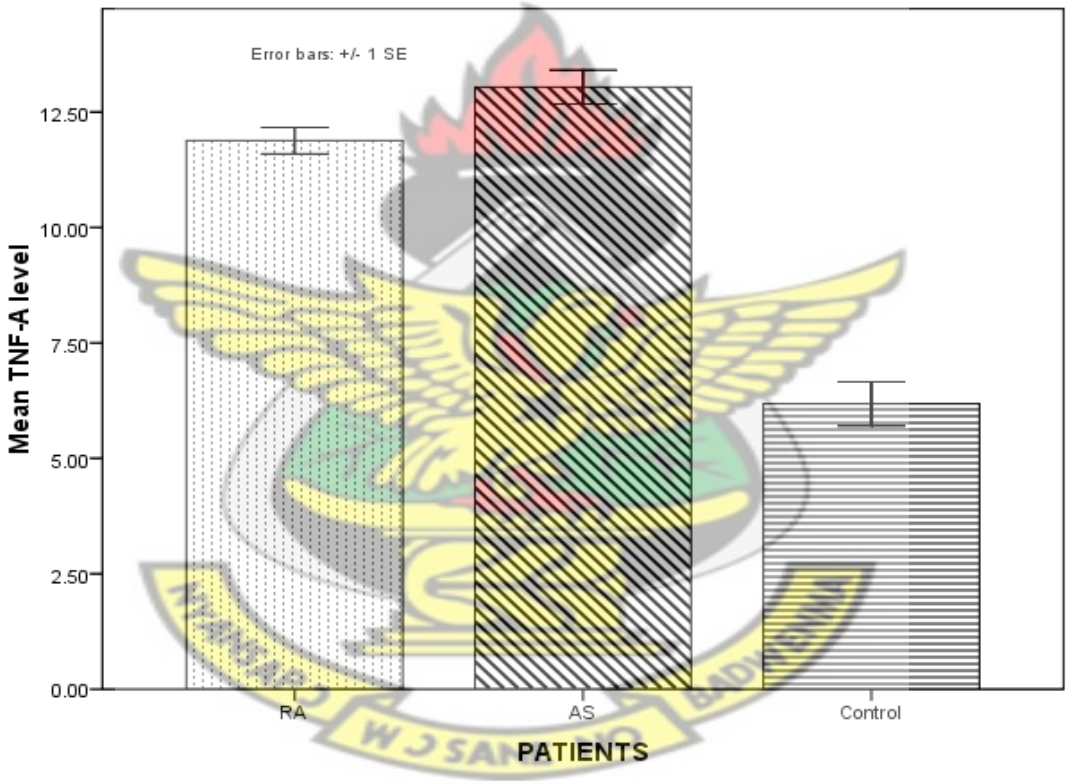

Figure 4-1Comparisons of TNF-α level between subjects and control.

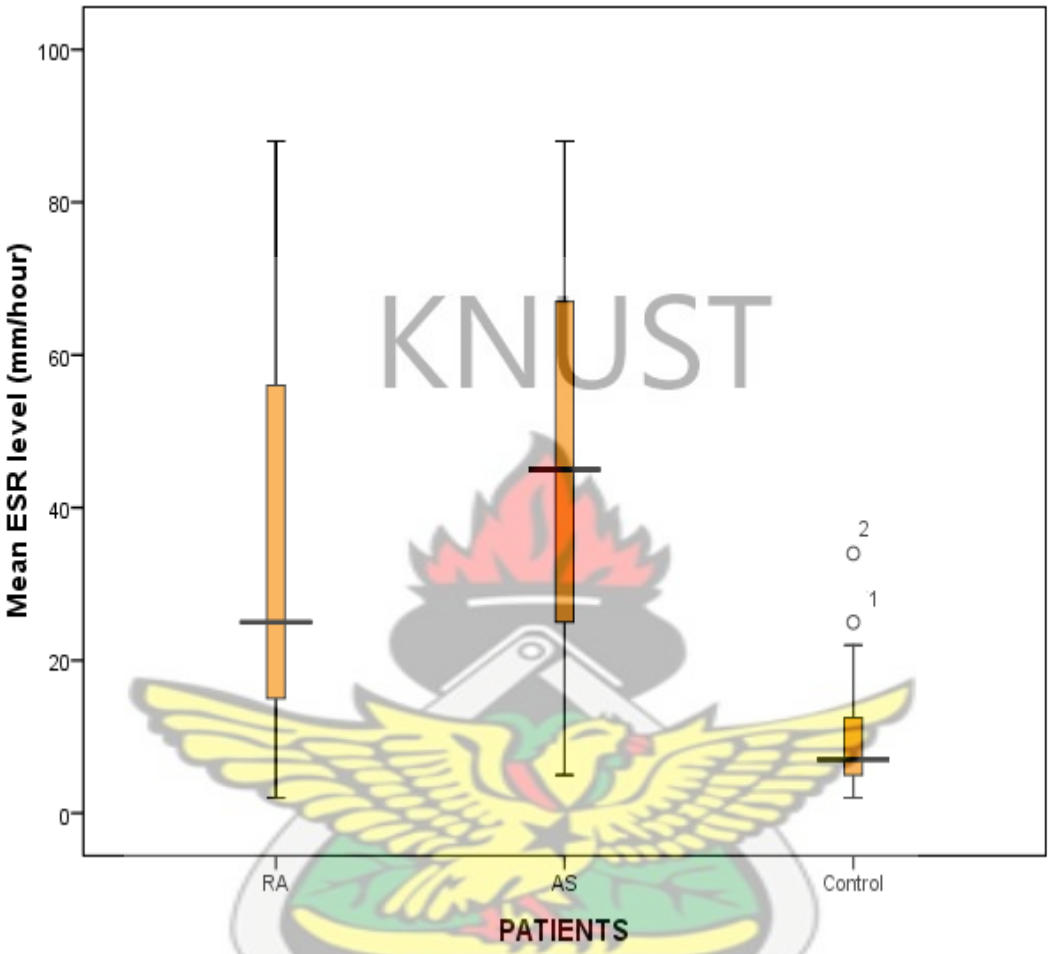

Figure 4-2 Comparisons of ESR levels between subjects and control.

4.7 OXIDATIVE STRESS MARKERS

4.7.1 Vitamin C

From the one-way ANOVA analysis, there was a significant difference (F=4.68; df=2; p<0.0001) between control group and subjects. Vitamin C as an anti-oxidant was low, 0.85 ± 0.03 mmol/l for AS patients and 0.95 ± 0.02 mmol/l for RA patients as compared to 1.12± 0.01 mmol/l of control. Using Bonferroni's Multiple Comparison Test, there was no significant difference (P>0.012) between vitamin C levels of RA and AS subjects (figure 4.3).

4.7.2 MDA

The difference between MDA level for AS subjects (0.75 ± 0.03 mmol/l) and that of RA subjects (1.57± 0.06 mmol/l) was statistically significant (p>0.001) as compared to normal controls of 0.58 ± 0.02 mmol/l (figure 4.4). Using Bonferroni's Multiple Comparison Test, there was a significant difference (P>0.001) between MDA levels of RA and AS subjects (figure 4.6). MDA levels also had a significant correlation with the serum levels of vitamin C (table 4.9).

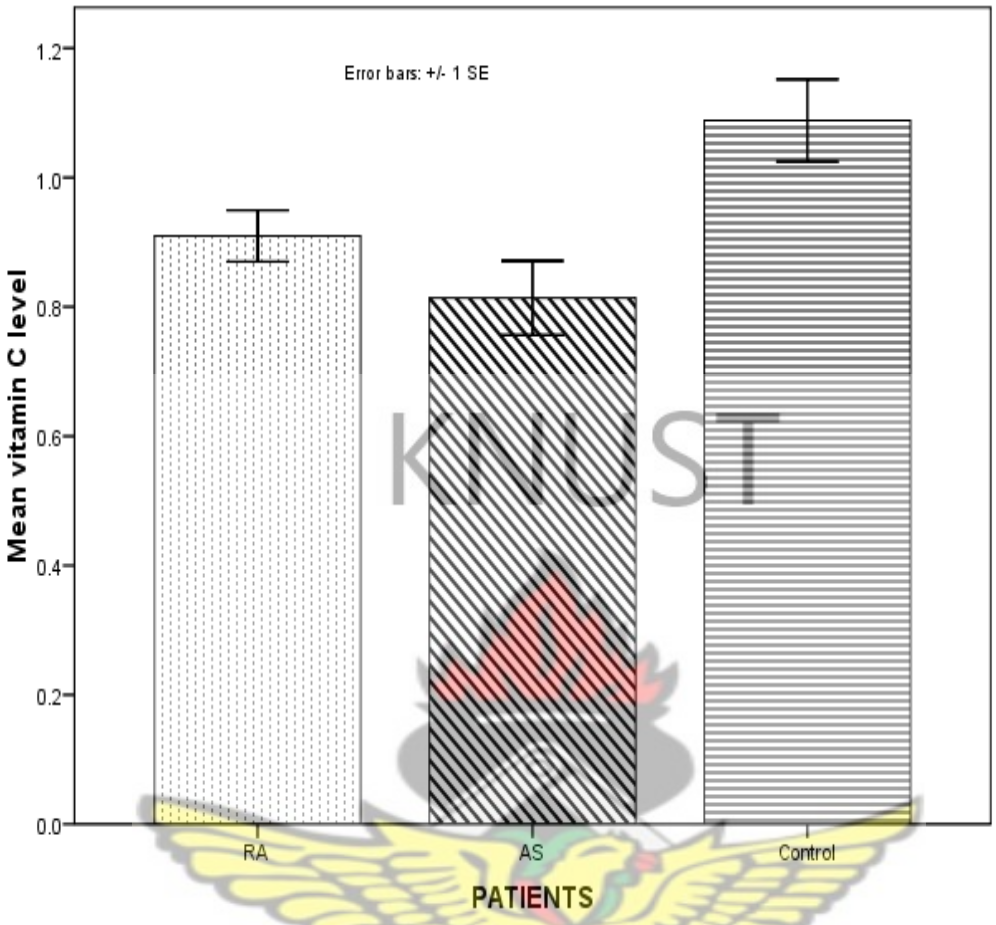

Figure 4-3 Comparisons of vitamin C level between subjects and control

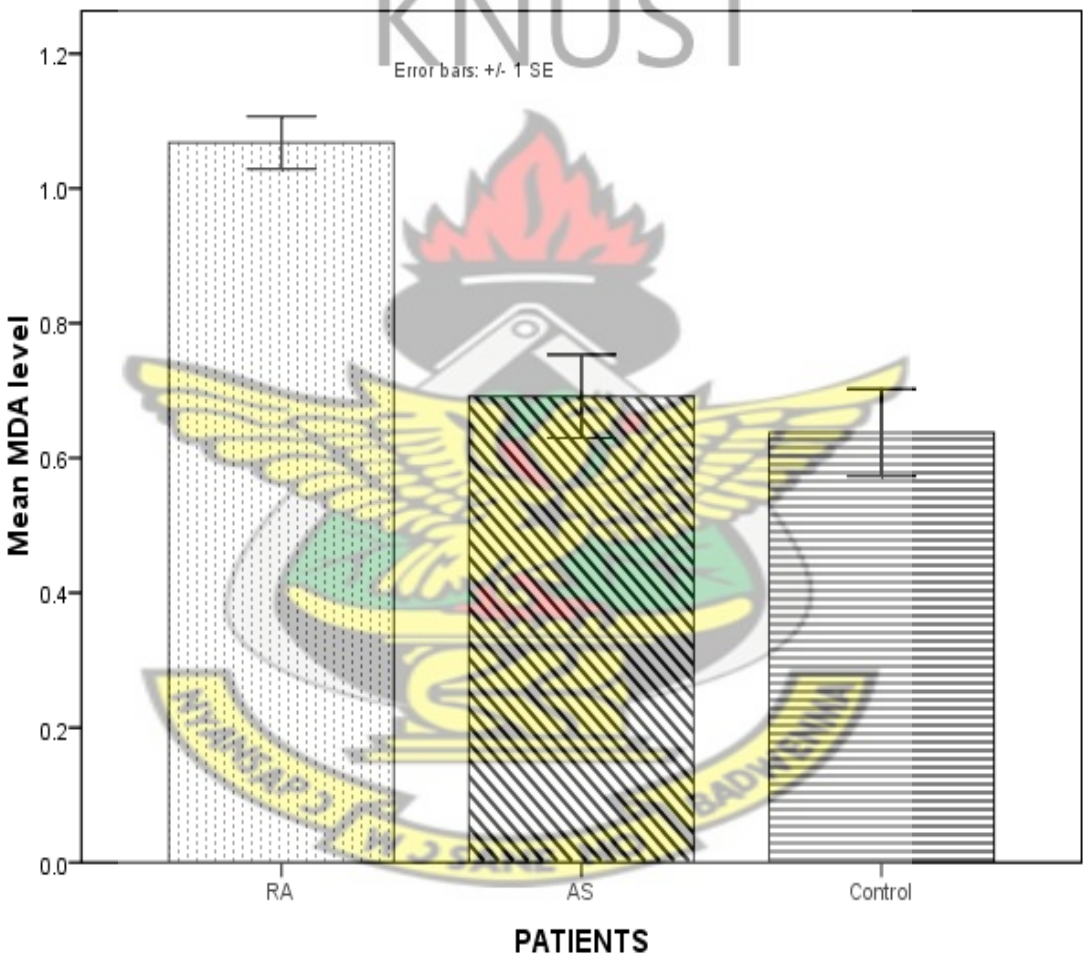

Figure 4-4 Comparisons of MDA level between subjects and control

Table 4:10 Pearson's correlation coefficients between oxidative stress parameters for controls (lower-left hand side) and patients (upper right-hand side)

	AS Vitamin C level	**AS MDA level**	**RA Vitamin C level**	**RA MDA level**
AS Vitamin C level		-0.24	0.20	-0.25
P Value		0.04	0.09	0.03
AS MDA level	-0.24		-0.21	0.18
P Value	0.04		0.08	0.13
RA Vitamin C level	0.20	-0.21		-0.05
P Value	0.09	0.08		0.52
RA MDA level	-0.25	0.18	-0.05	
P Value	0.03	0.13	0.52	

**Correlation is significant at the 0.05 level (2-tailed), MDA: malondialdehyde*

4.8 THE EFFECT OF LIPID PROFILE AND OXIDATIVE STRESS ON DISEASE ACTIVITY

Disease activity was measured among AS subjects using the BASDAI scores. Using the Pearson's correlation method of analysis, there was a significant correlation (r^2= 0.019, *p*= 0.001) between total cholesterol, LDL-c and BASDAI scores. There were negative correlations between triglycerides, HDL and the BASDAI scores, but were not statistically significant (table 4.10). Vitamin C exhibited a negatively significant correlation ((r^2= 0.025, *p*= 0.000) with the BASDAI scores as shown in table 4.11.

Table 4:11 Pearson's correlation coefficients between lipid profile parameters for AS subjects and BASDAI score.

	BASDAI SCORE	TRIG	HDL cholesterol	LDL cholesterol	Total cholesterol
BASDAI SCORE		-0.05	0.06	0.17	0.13
P value		0.59	0.50	0.01	0.01
TRIG	-0.05		-0.08	-0.09	0.29
P value	0.59		0.38	0.36	0.00
HDL cholesterol	0.06	-0.08		-0.15	0.14
P value	0.50	0.38		0.13	0.13
LDL cholesterol	0.17	-0.09	-0.15		0.85
P value	0.01	0.36	0.13		0.00
Total cholesterol	0.13	0.29	0.14	0.85	
P value	0.01	0.00	0.13	0.00	

*Correlation is significant at the 0.05 level (2-tailed), **. Correlation is significant at the 0.01 level (2-tailed).*

Table 4:12 Pearson's correlation coefficients between oxidative stress parameters for AS subjects and BASDAI score.

	BASDAI SCORE	VITAMIN C	MDA
BASDAI SCORE		-0.27	0.12
P value		0.00	0.20
Vitamin C	-0.27		-0.10
P value	0.00		0.31
MDA	0.12	-0.10	
P value	0.20	0.31	

Correlation is significant at the 0.05 level (2-tailed),

Chapter 5

DISCUSSION

5.1 SOCIO-DEMOGRAPHY

From this study the proportion of adults reporting with RA was higher for women than for men. These differences between genders existed in the prevalence, age at onset, and level of production of autoantibodies. Furthermore, women reported more symptoms and other health-related conditions than men. These findings are similar to those of Sokka *et al.*, (2009), who also reported higher proportion of RA among women. This implicates a plausible role for sex hormones in susceptibility and pathogenesis. In women peak incidence is observed in the peri-menopausal and postpartum periods. Furthermore, pregnancy has a protective effect (Silman *et al.*, 1992), which may be explained by the fact that pregnancy induces a shift from T-helper type 1 reaction typical for RA towards type 2 reaction. The use of oral contraceptives is also known to have a protective effect, whereas the use of hormone replacement therapy does not.

Moreover, these differences are attributed to the fact that women have less strength than men, which has a major effect in the functional status of patients with RA as it does in the healthy population. Moreover, given the fact that women are the "weaker vessels" concerning musculoskeletal size and strength and their baseline values are lower than men's, the same burden of a musculoskeletal disease appear to be more harmful to women than to men.

On the other hand, more men than women reported with AS in the ratio of 3:1. In general, it takes longer time for women to be diagnosed with spondylitis than it does for men. Some women have a mild form of the disease that may not be as easily detected as it is in men. Again the disease may progress more slowly in women. Moreover, because AS is a chronic condition the incidence is fairly low.

Many rheumatologists also believe that the number of women with AS is underdiagnosed as women tend to manifest symptoms in their lower spinal area (lumbar, sacrum, hips, and pelvis) instead of their shoulders/upper chest/neck (where the first symptoms appear in male patients). Spondylitis can affect different parts of the body (neck, peripheral joints) in women than in men (low back, spine), yet the diagnostic criteria are based on men's symptoms. Some women with spondylitis develop symptoms that resemble fibromyalgia or early rheumatoid arthritis. The average age of onset does not differ significantly between the sexes, but spinal fusion (ankylosis) may progress more slowly in women than men. Women tend to be worse off than men when it comes to pain and the need for drug therapy. The slower and relatively incomplete progression of spinal fusion in women can mean that it takes longer for pain to decrease as a result of spinal fusion. These differences agreed with the prevalence of HLA-B27. However, men reported more symptoms and poor BASDAI scores, including scores for pain, depression, and other comorbidity than women. Mijiyawa *et al*., (1996) also reported similar proportion in a study which reviewed 26 patients from three African studies, of which 24 were males suggesting that the male to female ratio in Africans (3:1) is similar to those in whites where males by far outnumbered females.

5.2 HAEMOGLOBIN

Haemoglobin levels among AS and RA patients were low as compared to the control. The low Hb observed in this study is similar to that of Wilson *et al*., (2004) where anaemia was found to be a common complication among subjects with rheumatoid conditions, affecting about 60 percent of AS and RA patients. He

attributed this to low serum iron concentrations in conjunction with adequate iron stores. The anaemia in patients with rheumatoid conditions is due to the presence of inflammatory cytokines which leads to reduced production of erythropoietin and red blood cell counts. Moreover, in response to inflammatory cytokines, increasing IL-6 causes the liver to produce high amount of Hepcidin which stops ferroportin from been released from iron stores. The immune complex formed also directly blunt erythropoeisis by decreasing the ability of the bone marrow to respond to erythropoietin. Iron utilization is impaired, with decreased serum iron and transferrin concentrations and an increased synthesis of ferritin (Goodnough and Marcus, 1997). The subsequent effect is increased lactoferrin which binds and anaemia (Tanaka *et al.*, 1999).

5.3 COMPARISONS OF RHEUMATOID ARTHRITIS MARKERS

In many early cases of RA, clinical symptoms are milder and nonspecific and patients will not fulfill all ACR classification criteria for RA. Therefore, the detection of a disease-specific autoantibody is of greater diagnostic and therapeutic importance. In this study anti-CCP antibodies were detected in 52% of patients with less than eight months duration (Table 4.1). This is comparable to that of Nell *et al.*, (2004) in which anti-CCP antibodies were detected in roughly 50-60% of patients with early RA at baseline (usually less than 6 months of symptoms).

The specificity obtained for the anti-CCP test (96.6%) in this study is similar to those obtained by Schellenkens *et al.*, (2000) (97.8%), Goldbach-Mansky *et al.*, (2000) (91.8%) and Bizzaro *et al.*, (2001) (95.4%). The specificity of the anti-CCP antibody test (96.6%) was significantly higher ($p<0.001$) than that for RF-IgM (69.5%), RF-Latex (63.12%) and RF-IgA (51.75%) isotypes.

The sensitivity for anti-CCP (54.34%) however, did not significantly (> 0.02) differ from that of RF-IgM (58%) and RF-Latex (52%). The sensitivity of RF-IgA was 28.1. Although there is some consensus from various studies in the literature about specificity, there are variations in diagnostic sensitivity ranging from 40-75% (Bas *et al.*, 2003).These variations can be due to different cut-off values determined from ROC curve. The sensitivities obtained for RF-Latex, RF-IgM and RF-IgA were similar to that of anti-CCP antibody, hence the better diagnostic accuracy of anti-CCP antibody was mainly due to its higher specificity. The anti-CCP antibody test has moderate sensitivity and excellent specificity, RF-IgA has poor sensitivity and moderate specificity. RF-IgM and RF-Latex have moderate sensitivities and good specificities, thus, while negative results for RF-IgA and RF-IgM do not rule out the diagnosis of RA, positive tests for anti-CCP antibodies and RF-IgM practically establish diagnosis. The combination of anti-CCP antibody and RF-IgM positivity improved specificity over RF-latex positivity alone. In the present study, anti-CCP was compared not only with RF-IgM but also with RF-Latex and RF-IgA in a population which fulfilled four or more of ACR criteria as well as those with less than four symptoms who therefore do not fulfill ACR criteria.

Nevertheless, Lindqvist *et al.*, (2005) demonstrated that anti-CCP and RF-IgA predict the development of rheumatoid arthritis in pre-disease serum samples. In this study, the mean anti-CCP in the patients (5.9pg/ml) was significantly higher ($p<0.0001$) than that of the control (2.3pg/ml). The specificity of anti-CCP extends to patients with early disease in whom a timely diagnosis is most needed, for example, those with symptoms of duration from 1-6 months have a mean anti-CCP of 5.45 pg ml^{-1} thus anti-CCP antibodies also identify subsets of patients who are likely to have substantial ongoing disease activity, accrue more damage and

will benefit from early aggressive treatment. The low sensitivity of the test (40-75%) in most published cohort (Girbal-Neuhauser *et al.*, 1999; Bas *et al.*, 2003) indicates that a negative anti-CCP antibody does not exclude the disease, but its high specificity means that a positive result increases the probability that the patient will have RA. This study also demonstrated the additional prognostic value of anti-CCP antibodies in patients with severe joint destruction and active disease compared with the different RF isotypes; for example anti-CCP was detected in up to 24.4% of RF-IgM negative sera and 30.5% of combined RF-IgM and RF-IgA negative sera of suspected rheumatoid patients who satisfied four or more of ACR criteria, this is similar to that obtained by Vallbracht *et al.*, (2004), who reported that, as a screening method for rheumatoid arthritis, the RF-IgM and the anti-CCP assays are superior to other RF isotypes.

In view of the high specificity of these cyclic citrullinated antibodies, the test is particularly useful in differential diagnosis between RA and other arthritis that is clinically similar to RA and may be positive for rheumatoid factor (RF), such as hepatitis C virus (HCV)-associated cryoglobulinemia undifferentiated polyarthritis or Sjo¨gren syndrome. In some cases these antibodies may be detected many years before the onset of the first symptoms.

The presence of anti-CCP antibodies has already been proposed for inclusion among the classification and diagnostic criteria for RA (Wiik *et al.*, 2004). As a screening method for rheumatoid arthritis, the RF-IgM and the anti-CCP assays are superior to other RF isotypes. This set of diagnostic and prognostic markers would allow the clinician to choose a more powerful disease modifying antirheumatic drug early in the course of disease, even when clinical judgment might not yet indicate the need for such drugs.

5.4 PROINFLAMMATORY AND INFLAMMATORY MARKERS

There are accumulating evidence of intriguing relationships between inflammation, dyslipidaemia and atherosclerosis showing a worsening of the lipid profile during increased disease activity. Erythrocyte Sedimentation Rate (ESR) and tumour necrosis factor (TNF) which measures the level of inflammation and proinflammatory activities were respectively determined among Rheumatoid Arthritis and Ankylosing Spondylitis patients. ESR and TNF were significantly higher ($p<0.001$) among RA and AS patients. ESR values between 2-87 mm/hr and 8-88 mm/hr were obtained for males and females respectively, the women in this study had a higher ESR scores, and this could be explained in part by the female hormone oestrogen, which effect tends to raise the ESR. A mean ESR of 34.63 mm/hr and a standard deviation of 24.17 were obtained implying that there is inflammation, the mean ESR of controls being 9.23 ± 0.91 mmol/l.

When evaluating the relative efficacy of different inflammatory markers in an effort to detect the effects of treatment in RA, Bull *et al.*, (1989) found ESR to be the best single marker. Even though ESR has repeatedly been found a poorer indicator of disease activity than C-reactive protein (CRP) in RA (Blackburn, 1994), van der Heijde *et al.*, (1992) achieved a 75% success rate in predicting the absence or presence of radiological damage by means of the baseline ESR value and HLA-DR4 status over a follow-up of two years. Because ESR is sensitive to immunoglobulins, it thus measure general severity better, even though it is a poorer measure of inflammation. The combination of ESR and CRP however, is

thought to yield useful information that is often not apparent when only a single test is used (Wolfe and Michaud, 1994).

Change of proteins within red blood cells cause them to bind to one another making them denser than normal red blood cells. ESR is a widely used laboratory measure of disease activity in clinical medicine; it indirectly reflects the potentially increased concentrations of serum proteins, particularly asymmetric molecules, such as fibrinogen and other acute phase proteins and immunoglobulins. ESR is also affected by factors independent of inflammation, such as erythrocyte morphology (Wollheim and Eberhardt, 1992).

In this study, a significantly ($p<0.001$) increased levels of TNF-α were observed among RA and AS subjects as compared to normal control. This finding is similar to that of Maury *et al*., (2003) who reported that in healthy people, TNF values are maintained at normal levels by a variety of anti-inflammatory cytokines, but people with auto-immune diseases such as RA and AS have increased TNF in their bodies. This excess TNF-alpha leads to inflammation in the sacroiliac joints of such patients. Furthermore, Gorman *et al*., (2002) and Weaver (2004) showed that anti-TNF drugs reduce the fatigue, pain, swelling, improve mobility and prevent further damage among RA and AS patients. This anti-tumour-necrosis-factor (anti-TNF) therapy is highly effective in ankylosing spondylitis and rheumatoid arthritis. The major anti-TNF-alpha drugs currently available include infliximab and etanercept.

Increase in ESR and TNF- α also serves to mediate inflammation and atherosclerotic vascular disease. TNF- α in particular, serves as a mediator of systemic and vascular wall inflammation, both in rheumatoid conditions and in atherosclerotic vascular disease. Plasma concentrations of TNF-α are directly

associated with the degree of early carotid atherosclerosis and the impairment of vascular functions in rheumatoid conditions (Klareskog and McDevitt, 1999; Skoog *et al.*, 2002).

5.5 PREVALENCE OF HLA-B27 AMONG AS SUBJECTS

Human Leukocyte Antigen B-27 (HLA-B27) is an inherited class I surface gene marker encoded by the B locus in the major histocompatibility complex (MHC) on chromosome 6 and presents antigens to T-cells. HLA-B27 is strongly associated with AS (Cauli *et al.*, 2002). The HLA-B27 gene is found with highest prevalence in patients with AS (>90%) (Gonzalez-Roces *et al.*, 1997). Definitive diagnosis of AS and its subsequent association with HLA-B27 is therefore important.

In this study, the first of its kind in Ghana, the Modified New York criteria for diagnosing AS and the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) were used and it was observed that the mean age for AS patients was 49 years and this is contrary to Feldtkeller *et al.*, (2003) who reported that in Germany, the mean age for AS patients was 27.7 years. This difference could be attributed to the fact that most of the diagnosis were done late when the disease had already advanced. Early diagnosis of AS is required to overcome the delay in diagnosis. The delay in the diagnosis of AS is because there is no unique clinical symptom or laboratory test to make the diagnosis in the early stages of the disease, until the advent of Magnetic Resonance Imaging (MRI). The presence of radiological sacroiliitis is essential for the diagnosis of AS, according to the modified New York criteria. However, it takes many months of inflammation of the sacroiliac joint before radiological damage can be demonstrated. From this study the prevalence of HLA-B27 was found to be 4.6%. HLA-B27 positivity is one of the factors predicting a bad prognosis, (van der Heijde *et al.*, 2004) which

makes it more important to identify HLA-B27 positivity among patients than those with HLA-B27 negativity.The findings in this research are similar to those found within the sub-region. In a study conducted by Allsopp *et al.*,(1992) and Hill *et al.*,(1991), HLA-B27 was present in 2% to 3% of the western Africa population, however, in a study carried out among 82 inhabitants of Mali, HLA- B27 prevalence was 9.7% (Kalidi *et al.*, 1988). In another study involving 700 people in Gambia, a prevalence of 2.6% was obtained (Allsopp *et al.*, 1992) and 3% was found in Senegal on 100 persons surveyed (Hill *et al.*, 1991). Out of eight subjects examined by Lopez-Larrea *et al.*, (2002) in Togo, AS was associated to B27 in only one patient. However, the AS in the remaining 7 subjects was associated with B14 subtype. The prevalence of HLA -B27 among AS in this study (4.6%) was the same as black Americans (3-6%) (Khan *et al.*, 1977; Kellner and Yu, 1992). The HLA- B27 studies in Black Africa population are fragmentary. None of the seven patients examined by Stein *et al.*, (1990) had this antigen which was present only in one patient of Chalmers *et al.*, (1977).

HLA-B sub-types were not studied in this study, however, it would be necessary to determine sub-types of HLA-B in the patients who are HLA-B27 negative and still have AS. One study has shown that 94% of AS patients were HLA-B27-positive among white populace (Khan, 1997). This difference is probably attributed to variations in HLA-B27 distribution among racial groups (Brown *et al.*, 1997). In another study, HLA-B27 antigen was positive in 80-90% of Caucasian patients with AS compared with 8% of the general black populations (Khan *et al.*, 1977). This strongly suggests that even though HLA-B27 antigen is important in the pathogenesis of Ankylosing Spondylitis it is racially dependent.

5.6 EFFECT OF DISEASE ACTIVITY AMONG AS SUBJECTS

The mean BASDAI score from this study was 44.7/100. This BASDAI score of 44.7/100 indicate cervical and lumbar stiffness according to de Diego-Otero *et al.*, (2009). This severity seems characteristic of the AS of the black African patient. The result of this study is similar to those used for a review by Mijiyawa *et al.*, (1993) who reported clinical manifestations with spinal and sacroiliac joint involvement in studied patients from Zimbabwe, South Africa, and Togo; peripheral joint involvement was present in about 40% of patients; and the disease was in advanced stage and severe in 19% of patients with "bamboo" spine and 23% of patients with hip involvement. In addition, other organs, such as the eyes, lungs, and heart, can be also affected with AS (Inman, 2006).

In the present study, the characteristic early sign of AS which is radiological evidence of sacroiliac joint arthritis was present. Sacroiliitis as shown by x ray examination is still mandatory to fulfil the currently widely used modified New York criteria for the diagnosis of AS (van der Linden *et al.*, 1984). Moreover, Sieper *et al.*, (2005) revealed that radiological sacroiliitis can be regarded as predictor in about 80%, and this findings is similar to that of van Tubergen *et al.*, (2003). Disease activity as determined from the BASDAI score (table 4.5) indicates moderate disease activity among AS patients. The magnitude in which disease activity correlated with the lipid levels was significant. The increase in disease activity in AS could be as a result of increase catabolic state leading to loss of body cell mass due to an accelerated loss of skeletal muscle as a result of high TNF-α and other proinflammatory enzymes. These mediators are also associated with lowered HDL-C levels (Kotler, 2000) and as such a higher disease activity in rheumatoid conditions is accompanied with a higher TNF level. This might explain the relationship between disease activity and lipid profile. Inflammation with cellular

infiltration by lymphocytes, plasma cells, and polymorphonuclear leukocytes are associated with sacroilic erosion. The process usually starts at the sacroiliac joints. The prognosis based on life expectancy of patients with AS is the same as that of the general population, except for patients who are severely affected with the disease and in whom complications develop. The morbidity of AS is considerable, it is estimated that fewer than 20% of patients with adult-onset disease develop significant morbidity (van Tubergen *et al.*, 2003).

5.7 CARDIOVASCULAR RISK FACTORS AMONG AS AND RA SUBJECTS

5.7.1 Dyslipidaemia as a cardiovascular risk factor

Cardiovascular morbidity and mortality due to an increased prevalence of cardiovascular risk factors such as dyslipidaemia and inflammation are observed prominently in RA and AS patients. In this study cardiovascular and atherogenic lipid profile, characterized by an increase of TC, LDL-C, and triglyceride serum levels and a reduction in serum HDL-C levels were exhibited. Thus, an increase in the atherogenic ratio of TC/HDL-C or LDL-C/HDL-C was observed (Table 4.6). Thus from this study, a significantly increased cardiovascular risk in patients with chronic inflammatory rheumatic diseases such as RA and AS was shown. The lipid profile of patients with RA and AS has been evaluated in several studies. Some of these studies have reported lower levels of HDL-C, higher serum concentrations of lipoprotein and higher TC/HDL-C and LDL-C/HDL-C ratios in active and/or untreated disease than in the general population (Heldenberg *et al.*, 1983; Lorber *et al.*, 1985; Lakatos and Harsagyi, 1988; Kavanaugh, 1994; Asanuma *et al.*, 1999).

A possible explanation for this increased cardiovascular risk is a higher prevalence of conventional cardiovascular risk factors, such as a higher atherogenic lipid

profile which is an important prognostic indicator for future cardiovascular disease. The importance of small differences in lipid levels and disease activity over a prolonged period is best illustrated by studies by Pasceri and Yeh in which cardiovascular risk reduction was found by lowering lipid levels slightly but over a prolonged period (Pasceri and Yeh, 1999).

Atherosclerosis starts when LDL infiltrates the artery wall and is oxidized by reactive oxygen species to oxidized LDL (ox-LDL). Ox-LDL leads to phospholipid release, activating endothelial cells, thereby initiating an inflammatory process which leads to the formation of foam cells and subsequent fatty streaks. Normal HDL exerts its anti-atherogenic role by protecting LDL from oxidation (Ansell *et al.*, 2004), in addition to the inhibition of the expression of adhesion molecules and its role in the reverse cholesterol transport. This anti-inflammatory HDL can be distinguished from the so-called proinflammatory LDL which does not have these properties and actually may promote inflammation (Navab *et al.*, 2001). Analyses showed significant associations between disease activity parameters and lipid levels. Similar significant associations were found between other disease activity parameters and lipid levels.

Furthermore, this study advocates that this increase is caused partially by worsening of the cardiovascular risk profile and partially by a direct pro-atherogenic effect of inflammation, the distinctive feature of rheumatic diseases.

The worsening effect on the cardiovascular risk profile is supported in this study by demonstrating that inflammation deteriorates lipid levels, thus increasing the risk for CVD. This study also addresses the possibility of direct pro-atherogenic effect of inflammation on cardiovascular disease, the most prominent characteristic of rheumatic diseases. The inflammatory character of rheumatic diseases is thought to cause accelerated atherosclerosis with more and less stable

plaque formation in arteries. One should see a rheumatic, inflammatory disease as an independent risk factor for CVD, as diabetes mellitus is known to be. When this is more widely accepted this will have consequences on the management of patients at risk for CVD. Furthermore, this increased risk should encourage active treatment of the rheumatic disease itself. Lowering inflammation appears to, at least, slow down the build up of atherosclerosis in arteries and could subsequently decrease the prevalence of CVD.

This study points to inflammation as a major cause of the enhanced cardiovascular risk in rheumatic diseases. One could generalise this to all inflammatory diseases and events. In support of such a generalisation are several studies demonstrating that people with chronic inflammatory periodontal diseases, such as gingivitis, suffer greater risk for getting CVD (Heldenberg *et al.*, 1983; Kavanaugh, 1994). However, the fact that the inflammatory processes in rheumatic diseases are autoimmune driven may make it impossible to extrapolate the findings of this thesis to all inflammatory events. Furthermore, RA and AS are diseases with high-grade inflammation and it is not known whether low-grade inflammation has similar associations with CVD and its risk factors. It therefore appears that dyslipidaemia and systemic inflammation start in the preclinical phase of RA and AS prior to the fulfillment of all the criteria. RA and AS are associated with accelerated atherosclerosis and increased cardiovascular morbidity and mortality (Van Doornum *et al.*, 2002). Furthermore, this increased mortality seems to be predominantly caused by cardiovascular disease (CVD), with a twofold increased cardiovascular standard mortality (Mitchell *et al.*, 1986). Striking similarities exist in the inflammatory and immunologic response in RA and AS and atherosclerosis.

5.7.2 Pro inflammation and inflammation as cardiovascular risk factors

It has been postulated that inflammation deteriorates the lipid profile, thereby increasing cardiovascular risk (Wallberg-Jonsson *et al.*, 1997), but so far data concerning inflammation associated deterioration of the lipid profile for patients with RA and AS are lacking. Moreover, the association of systemic inflammation with atherosclerosis has not been well elucidated. In this study, ESR and TNF-α values were significantly higher ($p<0.001$) among RA and AS patients. This observed systemic inflammation is similar to that of Sattar *et al.*, (2003) who demonstrated that systemic inflammation plays a significant role in the pathogenesis of RA and AS. TNF-alpha is thus, a major factor in RA and AS related cardiovascular disease acting both by contributing to hypertriglyceridaemia and by promoting atherosclerosis-related inflammation in preclinical patients. Nevertheless Skoog *et al.*, (2002) demonstrated that tumour necrosis factor-α inhibitor (TNFi) treatment in RA patients generally leads to increases in total cholesterol (TC), high-density lipoprotein (HDL), and triglyceride (TG) levels.

The serum TC and LDL-C levels in rheumatoid conditions correlate with disease activity suggesting a potential role for inflammation in the atherogenic profile and the higher atherosclerotic risk observed in RA and AS. An increase in disease activity was observed to be associated with an increase in total cholesterol levels and a more pronounced decrease in HDL-c levels, subsequently resulting in a more atherogenic lipid profile. The magnitude in which disease activity influences the lipid levels was limited. However, although the observed influence is small, it should be noted that RA and AS are chronic inflammatory conditions, which means that this small detrimental influence has a potential clinically relevant effect and act as a cardiovascular risk for many years.

5.8 OXIDATIVE STRESS

Oxidative stress is caused by an imbalance between the production of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage (Ozkan *et al.*, 2007). In humans, oxidative stress is involved in many diseases, such as atherosclerosis, RA, AS, and chronic fatigue syndrome, but short-term oxidative stress may also be important in prevention of aging by induction of a process named mitohormesis (de Diego-Otero *et al.*, 2009). In this study, parameters such as Malondialdehyde (MDA), a marker of free radical mediated tissue destruction and vitamin C, a water soluble antioxidant were assayed to gain insight into the oxidative stress status in AS and RA patients. Increased MDA and decreased vitamin C levels were observed among AS and RA patients (figures 4.5 and 4.6). These findings indicate that vitamin C is effective in inhibiting lipid peroxidation initiated by peroxyl radical initiator.

MDA levels were increased significantly ($p<0.001$) in patients with Rheumatoid Arthritis and Ankylosing Spondylitis. Similar reports of elevated MDA levels have been reported in patients with rheumatic diseases (Mezes and Bartosiewicz, 1983; Ozkan *et al.*, 2007). In contrast to this study, Kajanachumpol *et al.*,(2000) reported no significant change in MDA levels in patients with rheumatoid arthritis compared to controls, nevertheless, other studies have reported increased oxidative stress and decreased levels of antioxidants in synovial fluid of rheumatoid arthritis patients (Bowie and O'Neill, 2000). Similarly, MDA have been reported to be higher or normal in AS and RA patients, or there were increases depending on disease activity. The variability in the MDA results of studies may be due to the short plasma half-lives of lipid peroxidation products

(Bowie *et al*., 1997).The increase in MDA is strongly related to lipid peroxidation caused by oxidative stress, and is expected to affect various tissues and organ systems, including vascular endothelium. When oxidative stress reaches a certain level, cellular damage occurs, including structural damage in cellular membranes, in mitochondrial and nuclear DNAs and impairment of enzymatic functions at multiple levels. Rise in MDA could also be due to increased generation of reactive oxygen species (ROS) due to the excessive oxidative damage generated in these patients. These oxygen species in turn can oxidize many other important biomolecules including membrane lipids.

Nevertheless, MDA does not correlate with markers of disease activity in RA like joint counts, duration of morning stiffness, and erythrocyte sedimentation rate. Increased serum MDA levels suggest the role of free radicals in the pathogenesis of this inflammatory arthropathy and support the need for further studies assessing the therapeutic role of free radical scavengers in RA. A study by Bowie *et al*., (1997), has indicated that increased oxidative stress and or defective antioxidant status contribute to the pathology of RA and AS.

Plasma vitamin C had also been reported to be significantly lower in patients with RA and AS (Cass *et al.,* 1954). Vitamin C is known to boost the immune system and one report found that pain was significantly reduced in elderly people suffering from arthritis when vitamin C was added to their diet (Cass *et al.,* 1954). Oxidative stress due to damage brought about by free radicals is also known to influence the response of these patients to therapy. Moreover the body's defense mechanisms would play an important role in the form of antioxidants and try to minimize the damage, adapting itself to the above stressful situation. One other study suggested that low vitamin C status may be a risk factor for rheumatoid factor-negative RA (Windrow *et al*., 1993). Comparison of changes in the oxidative stress indices of the

blood serum of RA and AS patients and control group revealed an increase in lipid oxidation. Activation of cell inflammation and hyper production of free radicals take place, thereby having an impact on the circulation of lipid peroxidation products among AS and RA patients.

The results of this study are in agreement with other studies that indicate that oxidative stress generated within an inflamed joint can produce connective tissue destruction leading to joint and periarticular deformities in rheumatoid conditions (Farrell *et al.,* 1992; Chaturvedi *et al.*, 1999). The level of oxidative stress is found to be much higher in patients with active disease. Further indepth knowledge about this aspect of RA and AS may lead to therapeutic protocols involving correction of oxidative stress levels.

5.9 LIMITATIONS OF THE STUDY

There were two limitations that need to be acknowledged and addressed regarding the present study;

1. The first limitation concerns the sample size of the AS subjects. The time frame for the study, bad record keeping and difficulty in retrieving folders at the various hospitals imparted negatively on the AS sample size.
2. The second limitation has to do with the study's inability to determine disease activity among RA subjects since the methodology; DAS-28 score is very clinical and getting a rheumatologist to attend to all the subjects from different parts of the country was difficult. Thus the extent to which the findings can be generalized beyond the cases studied was limited.

However, all other parameters obtained are relevant in answering the research questions and gives a good perspective to rheumatoid conditions in Ghana. Further empirical evaluations are needed to replicate the findings in different contexts and surroundings.

Chapter 6

CONCLUSION

6.1 GENERAL CONCLUSION

This study has demonstrated that the combination of anti-CCP and RF-IgM assays are highly specific and moderately sensitive for RA, making this combination of autoantibodies a powerful serologic tool in the serologic assessment of RA in Ghana. Anti-CCP alone is also highly specific and moderately sensitive for RA. A positive anti-CCP result in seronegative RA strongly supports the diagnosis of RA serologically.

The presence of anti-CCP at disease onset means that they have a high positive predictive value for the development of erosive joint lesions and the detection of these antibodies can therefore be used in clinical practice to help plan a therapeutic strategy. In view of the high specificity of these antibodies, the test is particularly useful in differential diagnosis between RA and other clinically similar conditions. Overall, the discriminative ability of the anti-CCP test is impressive.

HLA-B27 antigens were found to be present in 4.6% of AS patients, but not in as much proportion as among white populace. The prevalence of HLA-B27 in Ghana is similar to those within the subregion. The BASDAI scores and other clinical features clearly denote the presence of the disease with moderate disease activity and no extra articular manifestations.

Dyslipidaemia is present in early RA and AS. It starts in the preclinical phase, and that inflammation deteriorates the lipid profile, thereby increasing cardiovascular risk. Systemic inflammation plays a pivotal role in the development of atherosclerosis as evidenced in the increase in TNF-alpha and ESR. Findings from this study inform rheumatologists that they should be

aware of the increased risk for CVD in their patients and so they should easily be convinced to actively screen their patients for CVD and cardiovascular risk factors. Secondly, cardiologists should be able to see that rheumatic inflammatory diseases are important independent risk factors for CVD. Thirdly, besides awareness of clinicians, patients should be made aware of the increased cardiovascular risk associated with rheumatic diseases, so they can take the appropriate measures to try to minimise their cardiovascular risk for the future.

Increased oxidative stress in RA and AS is evidenced by raised MDA and decreased vitamin C. This postulates that, lipid peroxidation is a giant distracter in RA and AS. It has shown that ascorbic acid is effective in inhibiting lipid peroxidation.

RECOMMENDATIONS

- To use anti-CCP assays as a screening method for early detection of RA.
- Inclusion of anti-CCP antibodies among the classification criteria of RA.
- Investigations into new and more specific HLA-B subtypes, since the majority of Sub-Saharan African patients with AS are HLA-B27 negative.
- The general public, healthcare providers and state policy makers should recognize RA and AS as a major public health problem and sufficient resources made available and accessible to reduce the significant physical, social, and financial impact of RA and AS in Ghana.

REFERENCES

Allain C.C., Poon L.S., Chan C.S., Richmond W. and Fu P.C. (1974) Enzymatic determination of total serum cholesterol. *Clin Chem* 20, 470-475.

Allbutt C. and Rolleston H. (1907) *A system of medicine*. London: Macmillan.

Allsopp C.E., Harding R.M., Taylor C., Bunce M., Kwiatkowski D., Anstey N., Brewster D., McMichael A.J., Greenwood B.M. and Hill A.V. (1992) Interethnic genetic differentiation in Africa: HLA class I antigens in The Gambia. *Am J Hum Genet* 50, 411-421.

Ansell B.J., Navab M., Watson K.E., Fonarow G.C. and Fogelman A.M. (2004) Anti-inflammatory properties of HDL. *Rev Endocr Metab Disord* 5, 351-358.

Arnett F.C., Edworthy S.M., Bloch D.A., McShane D.J., Fries J.F., Cooper N.S., Healey L.A., Kaplan S.R., Liang M.H. and Luthra H.S. (1988) The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 31, 315-324.

Asanuma Y., Kawai S., Aoshima H., Kaburaki J. and Mizushima Y. (1999) Serum lipoprotein(a) and apolipoprotein(a) phenotypes in patients with rheumatoid arthritis. *Arthritis Rheum* 42, 443-447.

Atta M.S., Lim K.L., Ala'deen D.A., Powell R.J. and Todd I. (1995) Investigation of the prevalence and clinical associations of antibodies to human fibronectin in systemic lupus erythematosus. *Ann Rheum Dis* 54, 117-124.

Ball E.J. and Khan M.A. (2001) HLA-B27 polymorphism. *Joint Bone Spine* 68, 378-382.

Barham D. and Trinder P. (1972) An improved colour reagent for the determination of blood glucose by the oxidase system. *Analyst* 97, 142-145.

Barland P., Novikoff A.B. and Hamerman D. (1962) Electron microscopy of the human synovial membrane. *J Cell Biol* 14, 207-220.

Bas S., Genevay S., Meyer O. and Gabay C. (2003) Anti-cyclic citrullinated peptide antibodies, IgM and IgA rheumatoid factors in the diagnosis and prognosis of rheumatoid arthritis. *Rheumatology (Oxford)* 42, 677-680.

Benjamin M. and McGonagle D. (2001) The anatomical basis for disease localisation in seronegative spondyloarthropathy at entheses and related sites. *J Anat* 199, 503-526.

Benjamin M., Toumi H., Suzuki D., Redman S., Emery P. and McGonagle D. (2007) Microdamage and altered vascularity at the enthesis-bone interface provides an anatomic explanation for bone involvement in the HLA-B27-associated spondylarthritides and allied disorders. *Arthritis Rheum* 56, 224-233.

Bhatia A., Blades S., Cambridge G. and Edwards J.C. (1998) Differential distribution of Fc gamma RIIIa in normal human tissues and co-localization with DAF and fibrillin-1: implications for immunological microenvironments. *Immunology* 94, 56-63.

Bizzaro N., Mazzanti G., Tonutti E., Villalta D. and Tozzoli R. (2001) Diagnostic accuracy of the anti-citrulline antibody assay for rheumatoid arthritis. *Clin Chem* 47, 1089-1093.

Bjornadal L., Baecklund E., Yin L., Granath F., Klareskog L. and Ekbom A. (2002) Decreasing mortality in patients with rheumatoid arthritis: results from a large population based cohort in Sweden, 1964-95. *J Rheumatol* 29, 906-912.

Blackburn W. (1994) Validity of acute phase proteins as markers of disease activity. *J Rheumatol* 21, 9–13.

Blake D.R., Hall N.D., Treby D.A., Halliwell B. and Gutteridge J.M. (1981) Protection against superoxide and hydrogen peroxide in synovial fluid from rheumatoid patients. *Clin Sci (Lond)* 61, 483-486.

Blass S., Specker C., Lakomek H.J., Schneider E.M. and Schwochau M. (1995) Novel 68 kDa autoantigen detected by rheumatoid arthritis specific antibodies. *Ann Rheum Dis* 54, 355-360.

Blass S., Union A., Raymackers J., Schumann F., Ungethum U., Muller-Steinbach S., De Keyser F., Engel J.M. and Burmester G.R. (2001) The stress protein BiP is overexpressed and is a major B and T cell target in rheumatoid arthritis. *Arthritis Rheum* 44, 761-771.

Bowie A. and O'Neill L.A. (2000) Oxidative stress and nuclear factor-kappaB activation: a reassessment of the evidence in the light of recent discoveries. *Biochem Pharmacol* 59, 13-23.

Bowie A.G., Moynagh P.N. and O'Neill L.A. (1997) Lipid peroxidation is involved in the activation of NF-kappaB by tumor necrosis factor but not interleukin-1 in the human endothelial cell line ECV304. Lack of involvement of H2O2 in NF-kappaB activation by either cytokine in both primary and transformed endothelial cells. *J Biol Chem* 272, 25941-25950.

Braun J., Bollow M., Remlinger G., Eggens U., Rudwaleit M., Distler A. and Sieper J. (1998) Prevalence of spondylarthropathies in HLA-B27 positive and negative blood donors. *Arthritis Rheum* 41, 58-67.

Braun J., Tuszewski M., Ehlers S., Haberle J., Bollow M., Eggens U., Distler A. and Sieper J. (1997) Nested polymerase chain reaction strategy simultaneously targeting DNA sequences of multiple bacterial species in inflammatory joint diseases. II. Examination of sacroiliac and knee joint biopsies of patients with spondyloarthropathies and other arthritides. *J Rheumatol* 24, 1101-1105.

Brewerton D.A., Hart F.D., Nicholls A., Caffrey M., James D.C. and Sturrock R.D. (1973) Ankylosing spondylitis and HL-A 27. *Lancet* 1, 904-907.

Brighton S.W., de la Harpe A.L., van Staden D.J., Badenhorst J.H. and Myers O.L. (1988) The prevalence of rheumatoid arthritis in a rural African population. *J Rheumatol* 15, 405-408.

Brown M.A., Jepson A., Young A., Whittle H.C., Greenwood B.M. and Wordsworth B.P. (1997) Ankylosing spondylitis in West Africans--evidence for a non-HLA-B27 protective effect. *Ann Rheum Dis* 56, 68-70.

Brown M.A., Kennedy L.G., Darke C., Gibson K., Pile K.D., Shatford J.L., Taylor A., Calin A. and Wordsworth B.P. (1998) The effect of HLA-DR genes on susceptibility to and severity of ankylosing spondylitis. *Arthritis Rheum* 41, 460-465.

Bull B., Westengard JC and Farr M (1989) Efficacy of tests used to monitor rheumatoid arthritis. *Lancet* 2, 965-967.

Calin A., Marder A., Becks E. and Burns T. (1983) Genetic differences between B27 positive patients with ankylosing spondylitis and B27 positive healthy controls. *Arthritis Rheum* 26, 1460-1464.

Calin A., Nakache J.P., Gueguen A., Zeidler H., Mielants H. and Dougados M. (1999) Defining disease activity in ankylosing spondylitis: is a combination of variables (Bath Ankylosing Spondylitis Disease Activity Index) an appropriate instrument? *Rheumatology (Oxford)* 38, 878-882.

Carmona L., Villaverde V., Hernandez-Garcia C., Ballina J., Gabriel R. and Laffon A. (2002) The prevalence of rheumatoid arthritis in the general population of Spain. *Rheumatology (Oxford)* 41, 88-95.

Carswell E., Old L., Kassel, R.,, Green N., Fiore N. and Williamson B. (1975) An Endotoxin-Induced Serum Factor that Causes Necrosis of Tumors. *Proceedings of the National Academy of Science.* 72, 3666-3670.

Cass L.J., Frederik W.S. and Cohen J.D. (1954) Chronic disease and vitamin C. *Geriatrics* 9, 375-380.

Cauli A., Dessole G., Fiorillo M.T., Vacca A., Mameli A., Bitti P., Passiu G., Sorrentino R. and Mathieu A. (2002) Increased level of HLA-B27 expression in ankylosing spondylitis patients compared with healthy HLA-B27-positive subjects: a possible further susceptibility factor for the development of disease. *Rheumatology (Oxford)* 41, 1375-1379.

Cavanagh L.L., Boyce A., Smith L., Padmanabha J., Filgueira L., Pietschmann P. and Thomas R. (2005) Rheumatoid arthritis synovium contains plasmacytoid dendritic cells. *Arthritis Res Ther* 7, R230-240.

Chalmers I.M., Seedat Y.K. and Mudaliar M.Y. (1977) Ankylosing spondylitis in three Zulu men negative for the HLA B27 antigen. *S Afr Med J* 52, 567-569.

Charles P.J. and Maini R.N. (1993) Antibodies to neutrophil cytoplasmic antigens in rheumatoid arthritis. *Adv Exp Med Biol* 336, 367-370.

Chaturvedi V., Handa R., Rao D.N. and Wali J.P. (1999) Estimation & significance of serum & synovial fluid malondialdehyde levels in rheumatoid arthritis. *Indian J Med Res* 109, 170-174.

Chu C.Q., Field M., Feldmann M. and Maini R.N. (1991) Localization of tumor necrosis factor alpha in synovial tissues and at the cartilage-pannus junction in patients with rheumatoid arthritis. *Arthritis Rheum* 34, 1125-1132.

Combe B. (2001) [Factors predictive of severity in rheumatoid arthritis]. *Ann Med Interne (Paris)* 152, 522-526.

Combe B., Landewe R., Lukas C., Bolosiu H.D., Breedveld F., Dougados M., Emery P., Ferraccioli G., Hazes J.M., Klareskog L., Machold K., Martin-Mola E., Nielsen H., Silman A., Smolen J. and Yazici H. (2007) EULAR

recommendations for the management of early arthritis: report of a task force of the European Standing Committee for International Clinical Studies Including Therapeutics (ESCISIT). *Ann Rheum Dis* 66, 34-45.

Comstock G.W., Burke A.E., Hoffman S.C., Helzlsouer K.J., Bendich A., Masi A.T., Norkus E.P., Malamet R.L. and Gershwin M.E. (1997) Serum concentrations of alpha tocopherol, beta carotene, and retinol preceding the diagnosis of rheumatoid arthritis and systemic lupus erythematosus. *Ann Rheum Dis* 56, 323-325.

Corrigall V.M., Bodman-Smith M.D., Fife M.S., Canas B., Myers L.K., Wooley P., Soh C., Staines N.A., Pappin D.J., Berlo S.E., van Eden W., van Der Zee R., Lanchbury J.S. and Panayi G.S. (2001) The human endoplasmic reticulum molecular chaperone BiP is an autoantigen for rheumatoid arthritis and prevents the induction of experimental arthritis. *J Immunol* 166, 1492-1498.

D'Elia H.F., Larsen A., Mattsson L.A., Waltbrand E., Kvist G., Mellstrom D., Saxne T., Ohlsson C., Nordborg E. and Carlsten H. (2003) Influence of hormone replacement therapy on disease progression and bone mineral density in rheumatoid arthritis. *J Rheumatol* 30, 1456-1463.

Darlington L.G. and Stone T.W. (2001) Antioxidants and fatty acids in the amelioration of rheumatoid arthritis and related disorders. *Br J Nutr* 85, 251-269.

de Diego-Otero Y., Romero-Zerbo Y., el Bekay R., Decara J., Sanchez L., Rodriguez-de Fonseca F. and del Arco-Herrera I. (2009) Alpha-tocopherol protects against oxidative stress in the fragile X knockout mouse: an experimental therapeutic approach for the Fmr1 deficiency. *Neuropsychopharmacology* 34, 1011-1026.

de Vries-Bouwstra J.K., Dijkmans B.A. and Breedveld F.C. (2005) Biologics in early rheumatoid arthritis. *Rheum Dis Clin North Am* 31, 745-762.

del Rincon I.D., Williams K., Stern M.P., Freeman G.L. and Escalante A. (2001) High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors. *Arthritis Rheum* 44, 2737-2745.

Despres N., Boire G., Lopez-Longo F.J. and Menard H.A. (1994) The Sa system: a novel antigen-antibody system specific for rheumatoid arthritis. *J Rheumatol* 21, 1027-1033.

Doran M.F., Crowson C.S., O'Fallon W.M. and Gabriel S.E. (2004) The effect of oral contraceptives and estrogen replacement therapy on the risk of rheumatoid arthritis: a population based study. *J Rheumatol* 31, 207-213.

Doran M.F., Crowson C.S., Pond G.R., O'Fallon W.M. and Gabriel S.E. (2002) Frequency of infection in patients with rheumatoid arthritis compared with controls: a population-based study. *Arthritis Rheum* 46, 2287-2293.

Drabkin D.L. and Austin J.H. (1935) Spectro-photometric studies. II.Prepareations from washed blood cells; nitric oxide haemoglobin and sulfhaemoglobin *J. biol. Chem.*

112, 51.

Eberhardt K.B., Truedsson L., Pettersson H., Svensson B., Stigsson L., Eberhardt J.L. and Wollheim F.A. (1990) Disease activity and joint damage progression in early rheumatoid arthritis: relation to IgG, IgA, and IgM rheumatoid factor. *Ann Rheum Dis* 49, 906-909.

Ebringer A. and Wilson C. (2000) HLA molecules, bacteria and autoimmunity. *J Med Microbiol* 49, 305-311.

Edwards J.C.W., Leigh R.D. and Cambridge G. (1997). Expression of molecules involved in B lymphocyte survival and differentiation by synovial fibroblasts. *Clin-Exp-Immunol* 108, 407-414.

Edwards S.W., Derouet M., Howse M. and Moots R.J. (2004) Regulation of neutrophil apoptosis by Mcl-1. *Biochem Soc Trans* 32, 489-492.

Egeland T. and Munthe E. (1983) The role of the laboratory in rheumatology. Rheumatoid factors. *Clin Rheum Dis* 9, 135-160.

Ekstrom K., Hjalgrim H., Brandt L., Baecklund E., Klareskog L., Ekbom A. and Askling J. (2003) Risk of malignant lymphomas in patients with rheumatoid arthritis and in their first-degree relatives. *Arthritis Rheum* 48, 963-970.

Emery P. and Salmon M. (1995) Early rheumatoid arthritis: time to aim for remission? *Ann Rheum Dis* 54, 944-947.

Expert Panel on Blood Rheology of the International Council for Standardization for Haematology (1993) ICSH recommendation for measurement of ESR. *J. Clin. Pathol* 46, 198-203.

Farrell A.J., Blake D.R., Palmer R.M. and Moncada S. (1992) Increased concentrations of nitrite in synovial fluid and serum samples suggest increased nitric oxide synthesis in rheumatic diseases. *Ann Rheum Dis* 51, 1219-1222.

Feldmann M., Brennan F.M. and Maini R.N. (1996) Rheumatoid arthritis. *Cell* 85, 307-310.

Feldtkeller E., Khan M.A., van der Heijde D., van der Linden S. and Braun J. (2003) Age at disease onset and diagnosis delay in HLA-B27 negative vs. positive patients with ankylosing spondylitis. *Rheumatol Int* 23, 61-66.

Fiorillo M.T., Greco G., Maragno M., Potolicchio I., Monizio A., Dupuis M.L. and Sorrentino R. (1998) The naturally occurring polymorphism Asp116->His116, differentiating the ankylosing spondylitis-associated HLA-B*2705 from the non-associated HLA-B*2709 subtype, influences peptide-specific CD8 T cell recognition. *Eur J Immunol* 28, 2508-2516.

Firestein G.S., Alvaro-Gracia J.M. and Maki R. (1990) Quantitative analysis of cytokine gene expression in rheumatoid arthritis. *J Immunol* 144, 3347-3353.

Fossati P. and Prencipe L. (1982) Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin Chem* 28, 2077-2080.

Friedewald W.T., Levy R.I. and Fredrickson D.S. (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 18, 499-502.

Fries J.F. (1990) Reevaluating the therapeutic approach to rheumatoid arthritis: the "sawtooth" strategy. *J Rheumatol Suppl* 22, 12-15.

Gabriel S.E., Crowson C.S., Kremers H.M., Doran M.F., Turesson C., O'Fallon W.M. and Matteson E.L. (2003) Survival in rheumatoid arthritis: a population-based analysis of trends over 40 years. *Arthritis Rheum* 48, 54-58.

Garrett S., Jenkinson T., Kennedy L.G., Whitelock H., Gaisford P. and Calin A. (1994) A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. *J Rheumatol* 21, 2286-2291.

Girbal-Neuhauser E., Durieux J.J., Arnaud M., Dalbon P., Sebbag M., Vincent C., Simon M., Senshu T., Masson-Bessiere C., Jolivet-Reynaud C., Jolivet M. and Serre G. (1999) The epitopes targeted by the rheumatoid arthritis-associated antifilaggrin autoantibodies are posttranslationally generated on various sites of (pro)filaggrin by deimination of arginine residues. *J Immunol* 162, 585-594.

Goemaere S., Ackerman C., Goethals K., De Keyser F., Van der Straeten C., Verbruggen G., Mielants H. and Veys E.M. (1990) Onset of symptoms of rheumatoid arthritis in relation to age, sex and menopausal transition. *J Rheumatol* 17, 1620-1622.

Goldbach-Mansky R., Lee J., McCoy A., Hoxworth J., Yarboro C., Smolen J.S., Steiner G., Rosen A., Zhang C., Menard H.A., Zhou Z.J., Palosuo T., Van Venrooij W.J., Wilder R.L., Klippel J.H., Schumacher H.R., Jr. and El-Gabalawy H.S. (2000) Rheumatoid arthritis associated autoantibodies in patients with synovitis of recent onset. *Arthritis Res* 2, 236-243.

Gonzalez-Roces S., Alvarez M.V., Gonzalez S., Dieye A., Makni H., Woodfield D.G., Housan L., Konenkov V., Abbadi M.C., Grunnet N., Coto E. and Lopez-Larrea C. (1997) HLA-B27 polymorphism and worldwide susceptibility to ankylosing spondylitis. *Tissue Antigens* 49, 116-123.

Goodnough L.T. and Marcus R.E. (1997) The erythropoietic response to erythropoietin in patients with rheumatoid arthritis. *J Lab Clin Med* 130, 381-386.

Goodson N.J. and Solomon D.H. (2006) The cardiovascular manifestations of rheumatic diseases. *Curr Opin Rheumatol* 18, 135-140.

Gorman J.D., Sack K.E. and Davis J.C., Jr. (2002) Treatment of ankylosing spondylitis by inhibition of tumor necrosis factor alpha. *N Engl J Med* 346, 1349-1356.

Gracie J.A., Forsey R.J., Chan W.L., Gilmour A., Leung B.P., Greer M.R., Kennedy K., Carter R., Wei X.Q., Xu D., Field M., Foulis A., Liew F.Y. and McInnes I.B. (1999) A proinflammatory role for IL-18 in rheumatoid arthritis. *J Clin Invest* 104, 1393-1401.

Gran J. and Husby G. (2003) *Epidemiology of ankylosing spondylitis*, 3rd edn. ed. London:: Mosby.

Gran J.T. and Skomsvoll J.F. (1997) The outcome of ankylosing spondylitis: a study of 100 patients. *Br J Rheumatol* 36, 766-771.

Hamersma J., Cardon L.R., Bradbury L., Brophy S., van der Horst-Bruinsma I., Calin A. and Brown M.A. (2001) Is disease severity in ankylosing spondylitis genetically determined? *Arthritis Rheum* 44, 1396-1400.

Harris E.J. (1997) *Rheumatoid Arthritis*. Philadelphia: Saunders WB.

Harrison B.J., Symmons D.P., Barrett E.M. and Silman A.J. (1998) The performance of the 1987 ARA classification criteria for rheumatoid arthritis in a population based cohort of patients with early inflammatory polyarthritis. American Rheumatism Association. *J Rheumatol* 25, 2324-2330.

Harvey J., Lotze M., Stevens M.B., Lambert G. and Jacobson D. (1981) Rheumatoid arthritis in a Chippewa Band. I. Pilot screening study of disease prevalence. *Arthritis Rheum* 24, 717-721.

Hassfeld W., Steiner G., Graninger W., Witzmann G., Schweitzer H. and Smolen J.S. (1993) Autoantibody to the nuclear antigen RA33: a marker for early rheumatoid arthritis. *Br J Rheumatol* 32, 199-203.

Hassfeld W., Steiner G., Hartmuth K., Kolarz G., Scherak O., Graninger W., Thumb N. and Smolen J.S. (1989) Demonstration of a new antinuclear antibody (anti-RA33) that is highly specific for rheumatoid arthritis. *Arthritis Rheum* 32, 1515-1520.

Hayem G., Chazerain P., Combe B., Elias A., Haim T., Nicaise P., Benali K., Eliaou J.F., Kahn M.F., Sany J. and Meyer O. (1999) Anti-Sa antibody is an accurate diagnostic and prognostic marker in adult rheumatoid arthritis. *J Rheumatol* 26, 7-13.

Heldenberg D., Caspi D., Levtov O., Werbin B., Fishel B. and Yaron M. (1983) Serum lipids and lipoprotein concentrations in women with rheumatoid arthritis. *Clin Rheumatol* 2, 387-391.

Helmick C.G., Felson D.T., Lawrence R.C., Gabriel S., Hirsch R., Kwoh C.K., Liang M.H., Kremers H.M., Mayes M.D., Merkel P.A., Pillemer S.R., Reveille J.D. and Stone J.H. (2008) Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part I. *Arthritis Rheum* 58, 15-25.

Higashi K., Kouba D.J., Song Y.J., Uitto J. and Mauviel A. (1998) A proximal element within the human alpha 2(I) collagen (COL1A2) promoter, distinct from the tumor necrosis factor-alpha response element, mediates transcriptional repression by interferon-gamma. *Matrix Biol* 16, 447-456.

Hill A.V., Allsopp C.E., Kwiatkowski D., Anstey N.M., Greenwood B.M. and McMichael A.J. (1991) HLA class I typing by PCR: HLA-B27 and an African B27 subtype. *Lancet* 337, 640-642.

Hoet R.M. and van Venrooij W.J. (1992) B-cell epitopes of RNA autoantigens. *Mol Biol Rep* 16, 199-205.

Hueber W., Utz P.J. and Robinson W.H. (2003) Autoantibodies in early arthritis: advances in diagnosis and prognostication. *Clin Exp Rheumatol* 21, S59-64.

Huizinga T.W., Amos C.I., van der Helm-van Mil A.H., Chen W., van Gaalen F.A., Jawaheer D., Schreuder G.M., Wener M., Breedveld F.C., Ahmad N., Lum R.F., de Vries R.R., Gregersen P.K., Toes R.E. and Criswell L.A.

(2005) Refining the complex rheumatoid arthritis phenotype based on specificity of the HLA-DRB1 shared epitope for antibodies to citrullinated proteins. *Arthritis Rheum* 52, 3433-3438.

Inman R.D. (2006) Mechanisms of disease: infection and spondyloarthritis. *Nat Clin Pract Rheumatol* 2, 163-169.

Isenberg D.A., Steiner G. and Smolen J.S. (1994) Clinical utility and serological connections of anti-RA33 antibodies in systemic lupus erythematosus. *J Rheumatol* 21, 1260-1263.

Jacobsson L.T. and Pillemer S.R. (1994) What can we learn about rheumatic diseases by studying Pima Indians? *J Rheumatol* 21, 1179-1182.

Janeway C., Travers P., Walport M. and Capra J. (1999) *Immunobiology: The Immune System in Health and Disease*. New York, N.Y: Garland Publishers.

Jang M. and Pezzuto J.M. (1998) Effects of resveratrol on 12-O-tetradecanoylphorbol-13-acetate-induced oxidative events and gene expression in mouse skin. *Cancer Lett* 134, 81-89.

Jansen A.L., van der Horst-Bruinsma I., van Schaardenburg D., van de Stadt R.J., de Koning M.H. and Dijkmans B.A. (2002) Rheumatoid factor and antibodies to cyclic citrullinated Peptide differentiate rheumatoid arthritis from undifferentiated polyarthritis in patients with early arthritis. *J Rheumatol* 29, 2074-2076.

Jimenez-Balderas F.J. and Mintz G. (1993) Ankylosing spondylitis: clinical course in women and men. *J Rheumatol* 20, 2069-2072.

Kajanachumpol S., Vanichapuntu M., Verasertniyom O., Totemchokchyakarn K. and Vatanasuk M. (2000) Levels of plasma lipid peroxide products and antioxidant status in rheumatoid arthritis. *Southeast Asian J Trop Med Public Health* 31, 335-338.

Kalidi I., Fofana Y., Rahly A.A., Bochu V., Dehay C., Gony J. and Hors J. (1988) Study of HLA antigens in a population of Mali (West Africa). *Tissue Antigens* 31, 98-102.

Kallenberg C.G., Mulder A.H. and Tervaert J.W. (1992) Antineutrophil cytoplasmic antibodies: a still-growing class of autoantibodies in inflammatory disorders. *Am J Med* 93, 675-682.

Kamal A.A., Gomaa A., el Khafif M. and Hammad A.S. (1989) Plasma lipid peroxides among workers exposed to silica or asbestos dusts. *Environ Res* 49, 173-180.

Kavanaugh A. (1994) Dyslipoproteinaemia in a subset of patients with rheumatoid arthritis. *Ann Rheum Dis* 53, 551-552.

Kelley , Harris ED Jr, Ruddy S and CB S. (1985) *Textbook of Rheumatology*, 2nd ed. ed. Philadelphia: W.B.Saunders.

Kellner H. and Yu D. (1992) The pathogenetic aspects of spondyloarthropathies from the point of view of HLA-B27. *Rheumatol Int* 12, 121-127.

Khan M.A. (1997) *Prevalence of HLA-B27 in world population. HLA-B27 in the development of spondyloarthropathies*. Austin (TX):: Landes Company.

Khan M.A., Braun W.E., Kushner I., Grecek D.E., Muir W.A. and Steinberg A.G. (1977) HLA B27 in ankylosing spondylitis: differences in frequency and

relative risk in American Blacks and Caucasians. *J Rheumatol Suppl* 3, 39-43.

Khan N.A., Yazici Y., Calvo-Alen J., Dadoniene J., Gossec L., Hansen T.M., Huisman M., Kallikorm R., Muller R., Liveborn M., Oding R., Luchikhina E., Naranjo A., Rexhepi S., Taylor P., Tlustochowich W., Tsirogianni A. and Sokka T. (2009) Reevaluation of the role of duration of morning stiffness in the assessment of rheumatoid arthritis activity. *J Rheumatol* 36, 2435-2442.

Kim T.H., Uhm W.S. and Inman R.D. (2005) Pathogenesis of ankylosing spondylitis and reactive arthritis. *Curr Opin Rheumatol* 17, 400-405.

Kim W.U., Yoo W.H., Park W., Kang Y.M., Kim S.I., Park J.H., Lee S.S., Joo Y.S., Min J.K., Hong Y.S., Lee S.H., Park S.H., Cho C.S. and Kim H.Y. (2000) IgG antibodies to type II collagen reflect inflammatory activity in patients with rheumatoid arthritis. *J Rheumatol* 27, 575-581.

Klareskog L. and McDevitt H. (1999) Rheumatoid arthritis and its animal models: the role of TNF-alpha and the possible absence of specific immune reactions. *Curr Opin Immunol* 11, 657-662.

Kobelt G., Jonsson L., Lindgren P., Young A. and Eberhardt K. (2002) Modeling the progression of rheumatoid arthritis: a two-country model to estimate costs and consequences of rheumatoid arthritis. *Arthritis Rheum* 46, 2310-2319.

Kotler D.P. (2000) Cachexia. *Ann Intern Med* 133, 622-634.

Kroot E.J., de Jong B.A., van Leeuwen M.A., Swinkels H., van den Hoogen F.H., van't Hof M., van de Putte L.B., van Rijswijk M.H., van Venrooij W.J. and van Riel P.L. (2000) The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis. *Arthritis Rheum* 43, 1831-1835.

Kurowska M., Rudnicka W., Kontny E., Janicka I., Chorazy M., Kowalczewski J., Ziolkowska M., Ferrari-Lacraz S., Strom T.B. and Maslinski W. (2002) Fibroblast-like synoviocytes from rheumatoid arthritis patients express functional IL-15 receptor complex: endogenous IL-15 in autocrine fashion enhances cell proliferation and expression of Bcl-x(L) and Bcl-2. *J Immunol* 169, 1760-1767.

Kvien T.K., Glennas A., Knudsrod O.G., Smedstad L.M., Mowinckel P. and Forre O. (1997) The prevalence and severity of rheumatoid arthritis in Oslo. Results from a county register and a population survey. *Scand J Rheumatol* 26, 412-418.

Lackner K.J., Schlosser U., Lang B. and Schmitz G. (1998) Autoantibodies against human calpastatin in rheumatoid arthritis: epitope mapping and analysis of patient sera. *Br J Rheumatol* 37, 1164-1171.

Lakatos J. and Harsagyi A. (1988) Serum total, HDL, LDL cholesterol, and triglyceride levels in patients with rheumatoid arthritis. *Clin Biochem* 21, 93-96.

Landre-Beauvais A.J. (2001) The first description of rheumatoid arthritis. Unabridged text of the doctoral dissertation presented in 1800. *Joint Bone Spine* 68, 130-143.

Lau E., Symmons D., Bankhead C., MacGregor A., Donnan S. and Silman A. (1993) Low prevalence of rheumatoid arthritis in the urbanized Chinese of Hong Kong. *J Rheumatol* 20, 1133-1137.

Leirisalo-Repo M., Hannu T. and Mattila L. (2003) Microbial factors in spondyloarthropathies: insights from population studies. *Curr Opin Rheumatol* 15, 408-412.

Lindqvist E., Eberhardt K., Bendtzen K., Heinegard D. and Saxne T. (2005) Prognostic laboratory markers of joint damage in rheumatoid arthritis. *Ann Rheum Dis* 64, 196-201.

Liu H. and Pope R.M. (2004) Phagocytes: mechanisms of inflammation and tissue destruction. *Rheum Dis Clin North Am* 30, 19-39, v.

Lopez-Larrea C., Mijiyawa M., Gonzalez S., Fernandez-Morera J.L., Blanco-Gelaz M.A., Martinez-Borra J. and Lopez-Vazquez A. (2002) Association of ankylosing spondylitis with HLA-B*1403 in a West African population. *Arthritis Rheum* 46, 2968-2971.

Lorber M., Aviram M., Linn S., Scharf Y. and Brook J.G. (1985) Hypocholesterolaemia and abnormal high-density lipoprotein in rheumatoid arthritis. *Br J Rheumatol* 24, 250-255.

Lundberg K., Nijenhuis S., Vossenaar E.R., Palmblad K., van Venrooij W.J., Klareskog L., Zendman A.J. and Harris H.E. (2005) Citrullinated proteins have increased immunogenicity and arthritogenicity and their presence in arthritic joints correlates with disease severity. *Arthritis Res Ther* 7, R458-467.

Luukkainen R., Kajander A. and Isomaki H. (1977) Effect of gold on progression of erosions in rheumatoid arthritis. Better results with early treatment. *Scand J Rheumatol* 6, 189-192.

MacNaul K.L., Chartrain N., Lark M., Tocci M.J. and Hutchinson N.I. (1990) Discoordinate expression of stromelysin, collagenase, and tissue inhibitor of metalloproteinases-1 in rheumatoid human synovial fibroblasts. Synergistic effects of interleukin-1 and tumor necrosis factor-alpha on stromelysin expression. *J Biol Chem* 265, 17238-17245.

Martinez A., Pacheco-Tena C., Vazquez-Mellado J. and Burgos-Vargas R. (2004) Relationship between disease activity and infection in patients with spondyloarthropathies. *Ann Rheum Dis* 63, 1338-1340.

Maury C.P., Liljestrom M., Laiho K., Tiitinen S., Kaarela K. and Hurme M. (2003) Tumor necrosis factor alpha, its soluble receptor I, and -308 gene promoter polymorphism in patients with rheumatoid arthritis with or without amyloidosis: implications for the pathogenesis of nephropathy and anemia of chronic disease in reactive amyloidosis. *Arthritis Rheum* 48, 3068-3076.

McGonagle D. and Emery P. (2000) Enthesitis, osteitis, microbes, biomechanics, and immune reactivity in ankylosing spondylitis. *J Rheumatol* 27, 2302-2304.

McGowan M.W., Artiss J.D., Strandbergh D.R. and Zak B. (1983) A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin Chem* 29, 538-542.

McInnes I.B., al-Mughales J., Field M., Leung B.P., Huang F.P., Dixon R., Sturrock R.D., Wilkinson P.C. and Liew F.Y. (1996) The role of interleukin-15 in T-cell migration and activation in rheumatoid arthritis. *Nat Med* 2, 175-182.

Meager and Anthony. (1991) *Cytokines*. N.J: Prentice Hall.

Menard H.A. and el-Amine M. (1996) The calpain-calpastatin system in rheumatoid arthritis. *Immunol Today* 17, 545-547.

Menard H.A., Lapointe E., Rochdi M.D. and Zhou Z.J. (2000) Insights into rheumatoid arthritis derived from the Sa immune system. *Arthritis Res* 2, 429-432.

Mezes M. and Bartosiewicz G. (1983) Investigations on vitamin E and lipid peroxide status in rheumatic diseases. *Clin Rheumatol* 2, 259-263.

Mijiyawa M. (1993) [Rheumatic diseases in black Africa]. *Rev Rhum Ed Fr* 60, 451-457.

Mijiyawa M., Koumouvi K., Segbena A., Oniankitan I., Pitche V., Napo-Koura G., Bellow A., N'Dakena K. and Doury P. (1996) [Spinal diseases in a rheumatology hospital unit in Lome (Togo)]. *Ann Med Interne (Paris)* 147, 397-401.

Mijiyawa M., Oniankitan O. and Khan M.A. (2000) Spondyloarthropathies in sub-Saharan Africa. *Curr Opin Rheumatol* 12, 281-286.

Mimori T., Hardin J.A. and Steitz J.A. (1986) Characterization of the DNA-binding protein antigen Ku recognized by autoantibodies from patients with rheumatic disorders. *J Biol Chem* 261, 2274-2278.

Miranda-Carus M.E., Balsa A., Benito-Miguel M., Perez de Ayala C. and Martin-Mola E. (2004) IL-15 and the initiation of cell contact-dependent synovial fibroblast-T lymphocyte cross-talk in rheumatoid arthritis: effect of methotrexate. *J Immunol* 173, 1463-1476.

Mishan-Eisenberg G., Borovsky Z., Weber M.C., Gazit R., Tykocinski M.L. and Rachmilewitz J. (2004) Differential regulation of Th1/Th2 cytokine responses by placental protein 14. *J Immunol* 173, 5524-5530.

Mitchell D.M., Spitz P.W., Young D.Y., Bloch D.A., McShane D.J. and Fries J.F. (1986) Survival, prognosis, and causes of death in rheumatoid arthritis. *Arthritis Rheum* 29, 706-714.

Miyasaka N. and Koike R. (2007) [Treatment guidelines for the use of biologics in rheumatoid arthritis; present and future]. *Nippon Rinsho* 65, 1169-1178.

Miyasaka N., Sato K., Goto M., Sasano M., Natsuyama M., Inoue K. and Nishioka K. (1988) Augmented interleukin-1 production and HLA-DR expression in the synovium of rheumatoid arthritis patients. Possible involvement in joint destruction. *Arthritis Rheum* 31, 480-486.

Molberg O. and Sollid L.M. (2006) A gut feeling for joint inflammation—using coeliac disease to understand rheumatoid arthritis. *Trends Immunol* 27, 188-194.

Mor A., Abramson S.B. and Pillinger M.H. (2005) The fibroblast-like synovial cell in rheumatoid arthritis: a key player in inflammation and joint destruction. *Clin Immunol* 115, 118-128.

Mottonen T., Hannonen P., Korpela M., Nissila M., Kautiainen H., Ilonen J., Laasonen L., Kaipiainen-Seppanen O., Franzen P., Helve T., Koski J., Gripenberg-Gahmberg M., Myllykangas-Luosujarvi R. and Leirisalo-Repo M. (2002) Delay to institution of therapy and induction of remission using single-drug or combination-disease-modifying antirheumatic drug therapy in early rheumatoid arthritis. *Arthritis Rheum* 46, 894-898.

Mulder A.H., Horst G., van Leeuwen M.A., Limburg P.C. and Kallenberg C.G. (1993) Antineutrophil cytoplasmic antibodies in rheumatoid arthritis. Characterization and clinical correlations. *Arthritis Rheum* 36, 1054-1060.

Murray J., Barbara J., Dunkley S., Lopez A., Van Ostade X., Condliffe I., Haslett C. and Chilvers E. (1997) Regulation of Neutrophil Apoptosis by Tumor Necrosis Factor-Alpha : Requirements for TNF-R55 and TNF-R75 for Induction of Apoptosis In Vitro. *Blood.* 90, 2772-2783. .

Naparstek Y. and Plotz P.H. (1993) The role of autoantibodies in autoimmune disease. *Annu Rev Immunol* 11, 79-104.

Natelson S. (1961) *Microtechnics of Clinical Chemistry,*, 2 ed: Springfield, Illinois.

.

Navab M., Hama S.Y., Hough G.P., Subbanagounder G., Reddy S.T. and Fogelman A.M. (2001) A cell-free assay for detecting HDL that is dysfunctional in preventing the formation of or inactivating oxidized phospholipids. *J Lipid Res* 42, 1308-1317.

Nell V.P., Machold K.P., Eberl G., Stamm T.A., Uffmann M. and Smolen J.S. (2004) Benefit of very early referral and very early therapy with disease-modifying anti-rheumatic drugs in patients with early rheumatoid arthritis. *Rheumatology (Oxford)* 43, 906-914.

Nienhuis R.L. and Mandema E. (1964) A New Serum Factor in Patients with Rheumatoid Arthritis; the Antiperinuclear Factor. *Ann Rheum Dis* 23, 302-305.

Okamoto H., Yamamura M., Morita Y., Harada S., Makino H. and Ota Z. (1997) The synovial expression and serum levels of interleukin-6, interleukin-11, leukemia inhibitory factor, and oncostatin M in rheumatoid arthritis. *Arthritis Rheum* 40, 1096-1105.

Ozgocmen S., Sogut S., Ardicoglu O., Fadillioglu E., Pekkutucu I. and Akyol O. (2004) Serum nitric oxide, catalase, superoxide dismutase, and malondialdehyde status in patients with ankylosing spondylitis. *Rheumatol Int* 24, 80-83.

Ozkan Y., Yardym-Akaydyn S., Sepici A., Keskin E., Sepici V. and Simsek B. (2007) Oxidative status in rheumatoid arthritis. *Clin Rheumatol* 26, 64-68.

Page G., Lebecque S. and Miossec P. (2002) Anatomic localization of immature and mature dendritic cells in an ectopic lymphoid organ: correlation with selective chemokine expression in rheumatoid synovium. *J Immunol* 168, 5333-5341.

Paimela L., Palosuo T., Leirisalo-Repo M., Helve T. and Aho K. (1995) Prognostic value of quantitative measurement of rheumatoid factor in early rheumatoid arthritis. *Br J Rheumatol* 34, 1146-1150.

Pap T., Claus A., Ohtsu S., Hummel K.M., Schwartz P., Drynda S., Pap G., Machner A., Stein B., George M., Gay R.E., Neumann W., Gay S. and Aicher W.K. (2003) Osteoclast-independent bone resorption by fibroblast-like cells. *Arthritis Res Ther* 5, R163-173.

Pasceri V. and Yeh E.T. (1999) A tale of two diseases: atherosclerosis and rheumatoid arthritis. *Circulation* 100, 2124-2126.

Perlman H., Georganas C., Pagliari L.J., Koch A.E., Haines K., 3rd and Pope R.M. (2000) Bcl-2 expression in synovial fibroblasts is essential for maintaining mitochondrial homeostasis and cell viability. *J Immunol* 164, 5227-5235.

Pillinger M.H. and Abramson S.B. (1995) The neutrophil in rheumatoid arthritis. *Rheum Dis Clin North Am* 21, 691-714.

Pincus T. and Callahan L.F. (1993) The 'side effects' of rheumatoid arthritis: joint destruction, disability and early mortality. *Br J Rheumatol* 32 Suppl 1, 28-37.

Pincus T., Callahan L.F., Sale W.G., Brooks A.L., Payne L.E. and Vaughn W.K. (1984) Severe functional declines, work disability, and increased mortality in seventy-five rheumatoid arthritis patients studied over nine years. *Arthritis Rheum* 27, 864-872.

Pinheiro G.C., Scheinberg M.A., Aparecida da Silva M. and Maciel S. (2003) Anti-cyclic citrullinated peptide antibodies in advanced rheumatoid arthritis. *Ann Intern Med* 139, 234-235.

PPME-GHS (2005) Ghana Health Service, Policy Planning, Monitoring and Evaluation Division Facts and figures. Accra, Ghana.

Rashid T. and Ebringer A. (2007) Ankylosing spondylitis is linked to Klebsiella--the evidence. *Clin Rheumatol* 26, 858-864.

Reveille J.D. (2006) Major histocompatibility genes and ankylosing spondylitis. *Best Pract Res Clin Rheumatol* 20, 601-609.

Reveille J.D., Ball E.J. and Khan M.A. (2001) HLA-B27 and genetic predisposing factors in spondyloarthropathies. *Curr Opin Rheumatol* 13, 265-272.

Rink L. and Kirchner H. (1996) Recent Progress in the Tumor Necrosis Factor-Alpha Field. *International Archives of Allergy and Immunology.* 111 199-209.
.

Roberts-Thomson P.J., Wernick R.M. and Ziff M. (1982) Quantitation of rheumatoid factor by laser nephelometry. *Rheumatol Int* 2, 17-20.

Roelofs M.F., Joosten L.A., Abdollahi-Roodsaz S., van Lieshout A.W., Sprong T., van den Hoogen F.H., van den Berg W.B. and Radstake T.R. (2005) The expression of toll-like receptors 3 and 7 in rheumatoid arthritis synovium is increased and costimulation of toll-like receptors 3, 4, and 7/8 results in synergistic cytokine production by dendritic cells. *Arthritis Rheum* 52, 2313-2322.

Ronnelid J., Lysholm J., Engstrom-Laurent A., Klareskog L. and Heyman B. (1994) Local anti-type II collagen antibody production in rheumatoid

arthritis synovial fluid. Evidence for an HLA-DR4-restricted IgG response. *Arthritis Rheum* 37, 1023-1029.

Rose , Ragan C, Pearce E and . L.M. (1949) Differential agglutination of normal and sentized

sheep erythrocytes by sera of patients with rheumatoid arthritis. *Proc Soc Exp Biol Med*

68.

Ruddy S. (1989) *Complement, In, Textbook of Rheumatology.*, Third Edition ed. Philadelphia.

Saraux A., Berthelot J.M., Chales G., Le Henaff C., Thorel J.B., Hoang S., Valls I., Devauchelle V., Martin A., Baron D., Pennec Y., Botton E., Mary J.Y., Le Goff P. and Youinou P. (2001) Ability of the American College of Rheumatology 1987 criteria to predict rheumatoid arthritis in patients with early arthritis and classification of these patients two years later. *Arthritis Rheum* 44, 2485-2491.

Sattar N., McCarey D.W., Capell H. and McInnes I.B. (2003) Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis. *Circulation* 108, 2957-2963.

Saulot, Yon G, Vittecoq O, Charlionnet G, Manchour N, LangeC, Marvin L, Gilbert D and X L.L. (2000) Sa, alpha-enolase and rheumatoid arthritis *Arthritis Rheum* 43.

Schaller M., Burton D.R. and Ditzel H.J. (2001) Autoantibodies to GPI in rheumatoid arthritis: linkage between an animal model and human disease. *Nat Immunol* 2, 746-753.

Schellekens G.A., de Jong B.A., van den Hoogen F.H., van de Putte L.B. and van Venrooij W.J. (1998) Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. *J Clin Invest* 101, 273-281.

Schellekens G.A., Visser H., de Jong B.A., van den Hoogen F.H., Hazes J.M., Breedveld F.C. and van Venrooij W.J. (2000) The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. *Arthritis Rheum* 43, 155-163.

Schlosstein L., Terasaki P.I., Bluestone R. and Pearson C.M. (1973) High association of an HL-A antigen, W27, with ankylosing spondylitis. *N Engl J Med* 288, 704-706.

Schnabel A., Hauschild S. and Gross W.L. (1996) Anti-neutrophil cytoplasmic antibodies in generalized autoimmune diseases. *Int Arch Allergy Immunol* 109, 201-206.

Scott D.L., Grindulis K.A., Struthers G.R., Coulton B.L., Popert A.J. and Bacon P.A. (1984) Progression of radiological changes in rheumatoid arthritis. *Ann Rheum Dis* 43, 8-17.

Scott D.L., Smith C. and Kingsley G. (2005) What are the consequences of early rheumatoid arthritis for the individual? *Best Pract Res Clin Rheumatol* 19, 117-136.

Sebbag M., Simon M., Vincent C., Masson-Bessiere C., Girbal E., Durieux J.J. and Serre G. (1995) The antiperinuclear factor and the so-called

antikeratin antibodies are the same rheumatoid arthritis-specific autoantibodies. *J Clin Invest* 95, 2672-2679.

Seemayer C.A., Kuchen S., Neidhart M., Kuenzler P., Rihoskova V., Neumann E., Pruschy M., Aicher W.K., Muller-Ladner U., Gay R.E., Michel B.A., Firestein G.S. and Gay S. (2003) p53 in rheumatoid arthritis synovial fibroblasts at sites of invasion. *Ann Rheum Dis* 62, 1139-1144.

Sengupta R. and Stone M.A. (2007) The assessment of ankylosing spondylitis in clinical practice. *Nat Clin Pract Rheumatol* 3, 496-503.

Shichikawa K., Inoue K., Hirota S., Maeda A., Ota H., Kimura M., Ushiyama T. and Tsujimoto M. (1999) Changes in the incidence and prevalence of rheumatoid arthritis in Kamitonda, Wakayama, Japan, 1965-1996. *Ann Rheum Dis* 58, 751-756.

Shiel W.C., Stopplier M.C. and Driver B.C. (2010) Living With Rheumatoid Arthritis. http://www.medicinenet.com/rheumatoid_arthritis/article.htm.

Shingu M., Nagai Y., Isayama T., Naono T. and Nobunaga M. (1993) The effects of cytokines on metalloproteinase inhibitors (TIMP) and collagenase production by human chondrocytes and TIMP production by synovial cells and endothelial cells. *Clin Exp Immunol* 94, 145-149.

Short C.L. and Bauer W. (1948) The course of rheumatoid arthritis in patients receiving simple medical and orthopedic measures. *N Engl J Med* 238, 142-148.

Sidiropoulos P.I., Hatemi G., Song I.H., Avouac J., Collantes E., Hamuryudan V., Herold M., Kvien T.K., Mielants H., Mendoza J.M., Olivieri I., Ostergaard M., Schachna L., Sieper J., Boumpas D.T. and Dougados M. (2008) Evidence-based recommendations for the management of ankylosing spondylitis: systematic literature search of the 3E Initiative in Rheumatology involving a broad panel of experts and practising rheumatologists. *Rheumatology (Oxford)* 47, 355-361.

Sieper J. and Rudwaleit M. (2005) Early referral recommendations for ankylosing spondylitis (including pre-radiographic and radiographic forms) in primary care. *Ann Rheum Dis* 64, 659-663.

Sies H. (1997) Oxidative stress: oxidants and antioxidants. *Exp Physiol* 82, 291-295.

Silman A., Kay A. and Brennan P. (1992) Timing of pregnancy in relation to the onset of rheumatoid arthritis. *Arthritis Rheum* 35, 152-155.

Silman A.J., Ollier W., Holligan S., Birrell F., Adebajo A., Asuzu M.C., Thomson W. and Pepper L. (1993) Absence of rheumatoid arthritis in a rural Nigerian population. *J Rheumatol* 20, 618-622.

Silman A.J. and Pearson J.E. (2002) Epidemiology and genetics of rheumatoid arthritis. *Arthritis Res* 4 Suppl 3, S265-272.

Simonsson M., Bergman S., Jacobsson L.T., Petersson I.F. and Svensson B. (1999) The prevalence of rheumatoid arthritis in Sweden. *Scand J Rheumatol* 28, 340-343.

Skoog T., Dichtl W., Boquist S., Skoglund-Andersson C., Karpe F., Tang R., Bond M.G., de Faire U., Nilsson J., Eriksson P. and Hamsten A. (2002)

Plasma tumour necrosis factor-alpha and early carotid atherosclerosis in healthy middle-aged men. *Eur Heart J* 23, 376-383.

Skriner K., Sommergruber W.H., Tremmel V., Fischer I., Barta A., Smolen J.S. and Steiner G. (1997) Anti-A2/RA33 autoantibodies are directed to the RNA binding region of the A2 protein of the heterogeneous nuclear ribonucleoprotein complex. Differential epitope recognition in rheumatoid arthritis, systemic lupus erythematosus, and mixed connective tissue disease. *J Clin Invest* 100, 127-135.

Smolen and J.S. (1996) *Autoantibodies in rheumatoid arthritis. In Manual of Biological Markers of Disease*: Kluwer Academic.

Smolen J.S. (1996) Autoantibodies in rheumatoid arthritis. [R.N. In: W.J. van Venrooij, Manual of Biological Markers of Disease., editor. Amsterdam: Kluwer Academic

Smolen J.S. (2007) What is the place of recently approved T cell-targeted and B cell-targeted therapies in the treatment of rheumatoid arthritis? Lessons from global clinical trials. *J Rheumatol Suppl* 79, 15-20.

Smolen J.S. and Steiner G. (1998) Are autoantibodies active players or epiphenomena? *Curr Opin Rheumatol* 10, 201-206.

Sokka T., Hannonen P. and Mottonen T. (2005) Conventional disease-modifying antirheumatic drugs in early arthritis. *Rheum Dis Clin North Am* 31, 729-744.

Sokka T., Toloza S., Cutolo M., Kautiainen H., Makinen H., Gogus F., Skakic V., Badsha H., Peets T., Baranauskaite A., Geher P., Ujfalussy I., Skopouli F.N., Mavrommati M., Alten R., Pohl C., Sibilia J., Stancati A., Salaffi F., Romanowski W., Zarowny-Wierzbinska D., Henrohn D., Bresnihan B., Minnock P., Knudsen L.S., Jacobs J.W., Calvo-Alen J., Lazovskis J., Pinheiro Gda R., Karateev D., Andersone D., Rexhepi S., Yazici Y. and Pincus T. (2009) Women, men, and rheumatoid arthritis: analyses of disease activity, disease characteristics, and treatments in the QUEST-RA study. *Arthritis Res Ther* 11, R7.

Specker C., Blass S., Meier C., Lakomek H.J., Schwochau M. and Schneider M. (1997) [RA-specific autoantibodies against a 68k antigen]. *Z Rheumatol* 56, 63-70.

Stein E.A. and Myers G.L. (1995) National Cholesterol Education Program recommendations for triglyceride measurement: executive summary. The National Cholesterol Education Program Working Group on Lipoprotein Measurement. *Clin Chem* 41, 1421-1426.

Stein M., Davis P., Emmanuel J. and West G. (1990) The spondyloarthropathies in Zimbabwe: a clinical and immunogenetic profile. *J Rheumatol* 17, 1337-1339.

Steiner G., Hartmuth K., Skriner K., Maurer-Fogy I., Sinski A., Thalmann E., Hassfeld W., Barta A. and Smolen J.S. (1992) Purification and partial sequencing of the nuclear autoantigen RA33 shows that it is indistinguishable from the A2 protein of the heterogeneous nuclear ribonucleoprotein complex. *J Clin Invest* 90, 1061-1066.

Stites D.P., Terr A.L., Parslow G. and (1994) *Basic and clinical immunologyBasic and clinical immunology*, Eighth edition ed. Norwalk CT: Appleton and Lange.

Storey G.D. (2001) Alfred Baring Garrod (1819-1907). *Rheumatology (Oxford)* 40, 1189-1190.

Strieter R., Kunkel S. and Bone R. (1993) Role of Tumor Necrosis Factor-Alpha in Disease States and Inflammation. *Critical Care Medicine* 21, S447-S463.

Symmons D.P., Barrett E.M., Bankhead C.R., Scott D.G. and Silman A.J. (1994) The incidence of rheumatoid arthritis in the United Kingdom: results from the Norfolk Arthritis Register. *Br J Rheumatol* 33, 735-739.

Szomor Z., Shimizu K., Fujimori Y., Yamamoto S. and Yamamuro T. (1995) Appearance of calpain correlates with arthritis and cartilage destruction in collagen induced arthritic knee joints of mice. *Ann Rheum Dis* 54, 477-483.

Tak P.P. and Bresnihan B. (2000) The pathogenesis and prevention of joint damage in rheumatoid arthritis: advances from synovial biopsy and tissue analysis. *Arthritis Rheum* 43, 2619-2633.

Tamaoku K., K., Ueno K., Akiura and Y. O. (1982) New water-soluble hydrogen donors for the enzymatic photometric determination of hydrogen peroxide. . *Chem. Pharm.* Bull.30, 2492–2497.

Tampoia M., Brescia V., Fontana A., Maggiolini P., Lapadula G. and Pansini N. (2005) Anti-cyclic citrullinated peptide autoantibodies measured by an automated enzyme immunoassay: analytical performance and clinical correlations. *Clin Chim Acta* 355, 137-144.

Tanaka N., Ito K., Ishii S. and Yamazaki I. (1999) Autologous blood transfusion with recombinant erythropoietin treatment in anaemic patients with rheumatoid arthritis. *Clin Rheumatol* 18, 293-298.

Tarkowski A., Klareskog L., Carlsten H., Herberts P. and Koopman W.J. (1989) Secretion of antibodies to types I and II collagen by synovial tissue cells in patients with rheumatoid arthritis. *Arthritis Rheum* 32, 1087-1092.

Teitsson I., Withrington R.H., Seifert M.H. and Valdimarsson H. (1984) Prospective study of early rheumatoid arthritis. I. Prognostic value of IgA rheumatoid factor. *Ann Rheum Dis* 43, 673-678.

Tracey K. and Cerami A. (1990) *Metabolic Response to Cachectin/TNF*. New York: New York Academy of Sciences

Trinder P. (1969) Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. *J Clin Pathol* 22, 158-161.

Turesson C., O'Fallon W.M., Crowson C.S., Gabriel S.E. and Matteson E.L. (2002) Occurrence of extraarticular disease manifestations is associated with excess mortality in a community based cohort of patients with rheumatoid arthritis. *J Rheumatol* 29, 62-67.

Uesugi H., Ozaki S., Sobajima J., Osakada F., Shirakawa H., Yoshida M. and Nakao K. (1998) Prevalence and characterization of novel pANCA, antibodies to the high mobility group non-histone chromosomal proteins HMG1 and HMG2, in systemic rheumatic diseases. *J Rheumatol* 25, 703-709.

Ulfgren A.K., Grondal L., Lindblad S., Khademi M., Johnell O., Klareskog L. and Andersson U. (2000) Interindividual and intra-articular variation of proinflammatory cytokines in patients with rheumatoid arthritis: potential implications for treatment. *Ann Rheum Dis* 59, 439-447.

Vallbracht I., Rieber J., Oppermann M., Forger F., Siebert U. and Helmke K. (2004) Diagnostic and clinical value of anti-cyclic citrullinated peptide antibodies compared with rheumatoid factor isotypes in rheumatoid arthritis. *Ann Rheum Dis* 63, 1079-1084.

van Boekel M.A., Vossenaar E.R., van den Hoogen F.H. and van Venrooij W.J. (2002) Autoantibody systems in rheumatoid arthritis: specificity, sensitivity and diagnostic value. *Arthritis Res* 4, 87-93.

van der Heijde , Wanders A, Mielants H, Dougados M and RBM. L. (2004) Prediction of progression of radiographic damage over 4 years in patients with ankylosing spondylitis. *Ann Rheum Dis* 63, (suppl I):98.

van der Heijde D., van Leeuwen MA and PL v.R. (1992) Biannual radiographic assessments of hands and feet in a three-year prospective follow-up of patients with early rheumatoid arthritis. *Arthritis Rheum* 35, 126±134.

van der Linden S., Valkenburg H. and Cats A. (1983) The risk of developing ankylosing spondylitis in HLA-B27 positive individuals: a family and population study. *Br J Rheumatol* 22, 18-19.

van der Linden S., Valkenburg H.A. and Cats A. (1984) Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. *Arthritis Rheum* 27, 361-368.

van Dinther-Janssen A.C., Pals S.T., Scheper R., Breedveld F. and Meijer C.J. (1990) Dendritic cells and high endothelial venules in the rheumatoid synovial membrane. *J Rheumatol* 17, 11-17.

Van Dongen , van Aken J, Lard LR R.H.K., Hulsmans H.M.J. and Speyer I (2007) Efficacy of methotrexate treatment in patients with probable rheumatoid arthritis: a double-blind, randomized, placebo-controlled trial. *Arthritis Rheum* 56, 1424–1432.

Van Doornum S., McColl G. and Wicks I.P. (2002) Accelerated atherosclerosis: an extraarticular feature of rheumatoid arthritis? *Arthritis Rheum* 46, 862-873.

van Jaarsveld C.H., ter Borg E.J., Jacobs J.W., Schellekens G.A., Gmelig-Meyling F.H., van Booma-Frankfort C., de Jong B.A., van Venrooij W.J. and Bijlsma J.W. (1999) The prognostic value of the antiperinuclear factor, anti-citrullinated peptide antibodies and rheumatoid factor in early rheumatoid arthritis. *Clin Exp Rheumatol* 17, 689-697.

van Tubergen , Heuft-Dorenbosch L, Schulpen G, Landewe´ R, Wijers R v. and der Heijde D (2003) Radiographic assessment of sacroiliitis by radiologists and rheumatologists: does training improve quality? *Ann Rheum Dis* 62, 519–525.

.

van Zeben D., Hazes J.M., Zwinderman A.H., Cats A., van der Voort E.A. and Breedveld F.C. (1992) Clinical significance of rheumatoid factors in early

rheumatoid arthritis: results of a follow up study. *Ann Rheum Dis* 51, 1029-1035.

Vasishta A. (2002) Diagnosing early-onset rheumatoid arthritis: the role of anti-CCP antibodies. *Am Clin Lab* 21, 34-36.

Verstraeten A. and Dequeker J. (1986) Mineral metabolism in postmenopausal women with active rheumatoid arthritis. *J Rheumatol* 13, 43-46.

Vincent C., de Keyser F., Masson-Bessiere C., Sebbag M., Veys E.M. and Serre G. (1999) Anti-perinuclear factor compared with the so called "antikeratin" antibodies and antibodies to human epidermis filaggrin, in the diagnosis of arthritides. *Ann Rheum Dis* 58, 42-48.

Vossenaar E.R., Nijenhuis S., Helsen M.M., van der Heijden A., Senshu T., van den Berg W.B., van Venrooij W.J. and Joosten L.A. (2003) Citrullination of synovial proteins in murine models of rheumatoid arthritis. *Arthritis Rheum* 48, 2489-2500.

Waaler E. (1940) On the occurrence of a factor in human serum activating the specific agglutination

of sheep blood corpuscles. *Acta Pathologica Et Microbiologica Scandinavia* 17, 172-188.

Wallberg-Jonsson S., Ohman M.L. and Dahlqvist S.R. (1997) Cardiovascular morbidity and mortality in patients with seropositive rheumatoid arthritis in Northern Sweden. *J Rheumatol* 24, 445-451.

Warnick G.R., Nguyen T. and Albers A.A. (1985) Comparison of improved precipitation methods for quantification of high-density lipoprotein cholesterol. *Clin Chem* 31, 217-222.

Weaver A.L. (2004) The impact of new biologicals in the treatment of rheumatoid arthritis. *Rheumatology (Oxford)* 43 Suppl 3, iii17-iii23.

Westedt M.L., Herbrink P., Molenaar J.L., de Vries E., Verlaan P., Stijnen T., Cats A. and Lindeman J. (1985) Rheumatoid factors in rheumatoid arthritis and vasculitis. *Rheumatol Int* 5, 209-214.

Whicher J., Bell AM, Martin MF M.L. and PA. D. (1984) Prostaglandins cause an increase in serum acute-phase proteins in man, which is diminished in systemic sclerosis. *Clin Sci (Lond)* 66, 165–117.

Wiik A.S., Gordon T.P., Kavanaugh A.F., Lahita R.G., Reeves W., van Venrooij W.J., Wilson M.R. and Fritzler M. (2004) Cutting edge diagnostics in rheumatology: the role of patients, clinicians, and laboratory scientists in optimizing the use of autoimmune serology. *Arthritis Rheum* 51, 291-298.

Wilske K.R. and Healey L.A. (1989) Remodeling the pyramid--a concept whose time has come. *J Rheumatol* 16, 565-567.

Wilson A., Yu H.T., Goodnough L.T. and Nissenson A.R. (2004) Prevalence and outcomes of anemia in rheumatoid arthritis: a systematic review of the literature. *Am J Med* 116 Suppl 7A, 50S-57S.

Windrow V., Winyard P. and Morris C. (1993) *Free radicals in inflammation: second messengers and mediators of tissue destruction; Free radicals in medicine*. New York: Churchill.Living stone.

Wolfe F., Cathey M.A. and Roberts F.K. (1991) The latex test revisited. Rheumatoid factor testing in 8,287 rheumatic disease patients. *Arthritis Rheum* 34, 951-960.

Wolfe F. and Michaud K. (1994) The clinical and research significance of the erythrocyte sedimentation rate. *J Rheumatol.* 21, 1227-1237.

Wollheim F. and Eberhardt K. (1992) The search for laboratory measures of outcome in rheumatoid arthritis. *BaillieÁ re's Clin Rheumatol* 6, 69±69.

Yamamoto S., Shimizu K., Suzuki K., Nakagawa Y. and Yamamuro T. (1992) Calcium-dependent cysteine proteinase (calpain) in human arthritic synovial joints. *Arthritis Rheum* 35, 1309-1317.

Yelin E., Meenan R., Nevitt M. and Epstein W. (1980) Work disability in rheumatoid arthritis: effects of disease, social, and work factors. *Ann Intern Med* 93, 551-556.

Youinou P., Le Goff P., Dumay A., Lelong A., Fauquert P. and Jouquan J. (1990) The antiperinuclear factor. I. Clinical and serologic associations. *Clin Exp Rheumatol* 8, 259-264.

Young B.J., Mallya R.K., Leslie R.D., Clark C.J. and Hamblin T.J. (1979) Anti-keratin antibodies in rheumatoid arthritis. *Br Med J* 2, 97-99.

Zochling J., van der Heijde D., Dougados M. and Braun J. (2006) Current evidence for the management of ankylosing spondylitis: a systematic literature review for the ASAS/EULAR management recommendations in ankylosing spondylitis. *Ann Rheum Dis* 65, 423-432.

APPENDIX

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

FACULTY OF HEALTH SCIENCES

SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF MOLECULAR MEDICINE

RESEARCH QUESTIONNAIRE

GENERAL INFORMATION

Subject Number:

Name:

Residential area:

Marital Status: married \ single\divorced\separated

Age

Educational background

Sex

Occupation

Hometown

Disease duration

COMPLAINT AND SYMPTOMS

- Morning stiffness and pain lasting longer than one hour? YES. NO.
- Arthritis involving three or more joints? YES. NO.
- Arthritis of the hand (proximal interphalangeal)? YES. NO.
- General malaise? YES. NO.
- Weight Loss? YES. NO.
- Fever of unknown origin (FUO)? YES. NO.
- Any family member with such compliant /conditions? YES. NO.
- Any previous diagnosis of SLE or autoimmune disorders? YES. NO.
- Are you on any anti-inflammatory drug? YES. NO.
- Pain and stiffness in the low back? YES. NO.
- Pain in upper buttock area, neck? YES. NO.
- Back pain, usually most severe at night during rest? YES. NO.
- Stooped posture in response to back pain? YES. NO.
- Straight and stiff spine? YES. NO.
- Inability to take a deep breath? YES. NO.
- Deformed joints (ulnar deviation)? YES. NO.
- X-ray Examination (sacroilitis)? YES. NO.
- Presence of extra articular manifestation? YES. NO.

The Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)

Please place a mark to indicate your answer to each question relating to **the past week**

1. How would you describe the overall level of **fatigue/tiredness** you have experienced?

NONE (0)_____(10) SEVERE

2. How would you describe the overall level of AS **neck, back or hip pain** you have had?

NONE (0)_____ (10) SEVERE

3. How would you describe the overall level of pain/swelling in joints other than **neck, back, hips** you have had?

NONE _(0)_____(10)_SEVERE

4. How would you describe the overall level of **discomfort** you have had from any areas tender to touch or pressure?

NONE _(0)_____(10) VERY SEVERE

5. How would you describe the overall level of **morning stiffness** you have had **from the time you wake up?**

NONE _(0)_____(10)__SEVERE

6. How long does your morning stiffness last from the time you wake up?

_____

0 hrs ½ 1 1½ 2 or more hours

The mean measurement (score) of questions 5 and 6 is added to the scores from questions 1 to 4. This total is then divided by 5 to give the average. This is the
BASDAI score.

MEAN OF 5 & 6	
TOTAL OF 1 TO 4 ADDED TO MEAN OF 5 & 6 (TOTAL OUT OF 50)	
TOTAL / 5 (BASDAI SCORE)	