ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBODY RELATED TO THE DISEASE PROGRESSION AND EXTRA-ARTICULAR INVOLVEMENT IN PATIENT WITH RHEUMATOID ARTHRITIS IN HOSPITAL

UNIVERSITI SAINS MALAYSIA

BY

DR FARAH NADIAH BINTI SULAIMAN

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF MEDICINE (INTERNAL MEDICINE)

UNIVERSITI SAINS MALAYSIA

2017

ACKNOWLEDGEMENTS

Bismillahirrahmanirrahim

In the name of Allah the most graceful and the most merciful for His blessing and guidance had shown me ways to completion of this dissertation.

I wish to express my deep gratitude to my supervisor, Dr Wan Syamimee Wan Ghazali (Rheumatologist, Medical Department of HUSM) who gave me a lot of guidance and encouragement to make this dissertation possible. She also taught me a lot throughout my academic program.

Secondly, I also want to show my appreciation to my Head of Department of Medicine, Prof Dato' Dr Zurkurnai Yusof for his assistance and guidance along my journey to become a physician.

Many thanks to Dr Najib Majdi Yaacob (Department of Biostatic, HUSM) and Dr Mohd Azimullah Abdullah@Zakaria (Department of Community Medicine) for their guidance on the statistical aspect.

My appreciation are also extended to all lecturers, specialist and colleagues in Hospital Universiti Sains Malaysia who always supported and helped me in making this dissertation real, and also taught me in my study not to forget many other priceless things in life.

To my mother, Ruhani binti Abdullah, my father Sulaiman bin Jusoh, my in-laws and sibling, I am blessed with your love and your great support during my time as a student. The kindness that all of you showed really give me strength in order for me to be a good physician.

A very special appreciation to my beloved husband, Amir Hamzah Khalid who has been together with me during my up and down in my master program, and sacrifices a lot especially

during our long distance relationship. Thank you very much dear. With your love, I gained a lot of confident to conquer all the challenges in this journey to become a physician and a good wife.

Last but not least, I would like to dedicate this masterpiece to all my true friends who stood beside me during my happiness and hardship as an MMed student.

Thank you.

Farah Nadiah Sulaiman

TABLE OF CONTENT

LIST OF ABBREVIATION

ACR	American College of Rheumatology
ADL	Activity daily living
Anti-CCP antibody	Anti-Cyclic Citrullinated Peptide antibody
ANCA	Anti-neutrophil cytoplasmic antibody
ARA	American Rheumatism Association
bDMARDs	Biological agent Disease-modifying anti-rheumatic drugs
bs-infliximab	Biosimilar infliximab
CDAI	Clinical Disease Activity Index
CRP	C-reactive protein
DAS28	Disease Activity Score – 28 joint count
DIPJ	Distal interphalangeal joint
DMARDs	Disease-modifying anti-rheumatic drugs
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
ESR	Erythrocyte sedimentation rate
EULAR	European League Against Rheumatism
FGF	Fibroblast Growth Factor
HUSM	Hospital Universiti Sains Malaysia

HRCT	High resolution computed tomography
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-1	Interleukin-1
IL-6	Interleukin-6
MCPJ	Metacarpophalangeal joint
MIF	Migration inhibitory factor
MTP	Metatarsophalangeal joints
NHS	Nurses' Health Study
NSAIDs	Non-steroidal anti-inflammatory drugs
PAS	Patient Activity Scale
PAS II	Patient Activity Scale II
PDGF	Platelet-derived Growth Factor
PIPJ	Proximal interphalangeal joint
RA	Rheumatoid arthritis
RAPID 3	Routine Assessment of Patient Index Data 3
RF	Rheumatoid factor
SDAI	Simplified Disease Activity Index
sDMARDs	synthetic chemical compounds of Disease-modifying anti-rheumatic drugs

SLE	Systemic Lupus Erythematosus
TGF-β	Transforming Growth Factor - beta
TNF- α	Tumor Necrosis Factor - alpha
VEGF	Vascular endothelial growth factor

LIST OF TABLE

LIST OF FIGURE

ABSTRAK (VERSI BAHASA MELAYU)

PENGENALAN :

Rheumatoid arthritis (Radang sendi) merupakan penyakit kronik yang kebanyakannya menyerang sendi-sendi dan tisu-tisu sekitar, dan turut menyerang struktur lain dalam badan manusia. Ia akan menyebabkan kemusnahan kepada tulang dan juga tulang rawan seterusnya menyebabkan pesakit mengalami kecacatan yang kekal. Selain 'rheumatoid factor', anti-citrate citrullinated peptide antibody ('anti-CCP antibody') merupakan satu ujian yang baru diperkenalkan, yang dapat digunakan untuk mengesahkan diagnosis ini malah dikatakan lebih tepat berbanding ujian-ujian lain.

OBJEKTIF :

Tujuan kajian ini ialah untuk menilai keberkesanan dan potensi 'anti-CCP antibody' dalam menentukan tahap keaktifan dan progres penyakit terutama dalam kontek radiologi dan penglibatan struktur badan yang lain selain sendi, semasa diagnosis RA dibuat melibatkan pesakit rheumatoid arthritis dalam populasi HUSM.

KAEDAH :

Kajian ini merupakan kajian 'cross sectional with retrospective record review'. Seramai 159 orang pesakit yang disahkan menghidap penyakit 'rheumatoid arthritis' berdasarkan '2010 ACR/EULAR Rheumatoid Arthritis Classification Criteria' dari tahun 2010 hingga 2017 yang sedang menerima rawatan di Klinik Rheumatologi di HUSM diterima masuk dalam kajian ini. Pesakit berumur kurang daripada 16 tahun semasa diagnosis, 'probable RA', dan 'overlap

syndromes' atau penyakit radang yang lain dikecualikan daripada kajian ini. Data dikumpul daripada rekod perubatan untuk 'rheumatoid factor', 'anti-CCP antibody', penilaian aktiviti penyakit melalui skor 'DAS-28 ESR', x-ray tangan dan kaki dan penilaian untuk 'rheumatoid nodule', penyakit paru-paru, 'Felty syndrome', 'vasculitis' dan penyakit kurang darah merah semasa diagnosis dilakukan.

KEPUTUSAN :

Daripada 159 pesakit, 134 orang (84.3%) merupakan perempuan, dengan majority etnik adalah Melayu (87.4%). Purata umur ialah 48.3 tahun; iaitu di antara 17 hingga 79 tahun. 'Prevalence 'anti-CCP antibody' adalah 52.2% (95% CI 0.44 hingga 0.60) manakala peratus untuk 'rheumatoid factor' ialah 62.3%. Purata markah DAS28-ESR untuk keseluruhan pesakit ialah 4.74 di mana untuk peratusan kumpulan penilaian aktiviti penyakit, kebanyakan pesakit terletak dalam kumpulan sederhana dan tinggi. 36.5% pesakit menunjukkan perubahan pada ujian radiologi manakala 30.8% pesakit pula mempunyai tanda-tanda 'extra-articular' iaitu 'rheumatoid nodule', penyakit paru-paru, dan kurang darah merah.

Pesakit yang menunjukkan keputusan yang positif dalam ujian 'rheumatoid factor' dan 'anti-CCP antibody' adalah lebih ramai berbanding pesakit yang menunjukkan keputusan yang berbeza (n=83; 73.5%) (p-value 0.002). Untuk penilaian melibatkan 'anti-CCP antibody' positif dan negatif, Perubahan radiologi didapati lebih tinggi di dalam kumpulan 'anti-CCP antibody' positif berbanding kumpulan 'anti-CCP antibody' negative. Namun,.didapati tiada perbezaan ketara antara purata dan kumpulan penilaian aktiviti penyakit, dan tanda-tanda 'extra-articular'. Tiada perbezaan di antara tahap 'disease activity' yang berbeza dan tanda-tanda 'extra-articular'.

KESIMPULAN :

Sebagai kesimpulan, pesakit yang mempunyai ‘anti-CCP antibody’ positif didapati mempunyai kaitan yang ketara dengan ‘rheumatoid factor’ dan perubahan radiologi, tetapi tidak menunjukkan sebarang perbezaan ketara dalam tahap keaktifan penyakit dan tanda-tanda ‘extra-articular’ semasa pesakit didiagnosis sebagai rheumatoid arthritis.

ABSTRACT (ENGLISH VERSION)

BACKGROUND :

Rheumatoid arthritis (RA) is a chronic debilitating inflammatory disease affecting mainly the joint and surrounding tissue, and also involved other extra articular structures in the body. It can lead to destruction of bone and cartilage which may cause severe disability to the patient. Other than rheumatoid factor, anti-cyclic citrullinate peptide (anti-CCP) antibody is another biomarker that can be used to diagnose RA with higher sensitivity and specificity.

OBJECTIVE :

To evaluate the significant of anti-CCP antibody in predicting the disease activity, and disease progression in term of radiological and extra articular manifestation upon diagnosis in patient with rheumatoid arthritis in HUSM.

METHODS :

This was a cross sectional study with retrospective record review. A total of 159 patients who were diagnosed as rheumatoid arthritis based on 2010 EULAR criteria for RA since 2010 until 2017 under rheumatology clinic HUSM follow up were included in this study. Exclusion criteria include age of 16 years old or less at the onset of diagnosis, probable RA, and overlap syndromes or related to other connective tissue disease. The data was retrieved from medical record for rheumatoid factor, anti-CCP antibody, assessment of disease activity using DAS28 ESR calculator, plain radiograph of hands or feet, and extra-articular manifestation in term of

assessment of rheumatoid nodule, pulmonary involvement, Felty syndrome, vasculitis and anemia upon diagnosis.

RESULTS :

From a total of 159 patients, 134 (84.3%) patients were female with majority ethnic group was Malay (87.4%). The mean for age was 48.3 years old; ranging from 17 to 79. The prevalence of anti-CCP antibody was 52.2% (95% CI 0.44 to 0.60) and the percentage of positive rheumatoid factor was 62.3%. Mean DAS28-ESR score for total patients was 4.74 which medium and high disease activity were predominant upon diagnosis. 36.5% of patients had radiological involvement and 30.8% of patients had extra-articular involvement manifested by rheumatoid nodule, pulmonary involvement, and anemia.

Patients with both positive rheumatoid factor and anti-CCP antibody was significantly higher than other groups (n=83; 73.5%) (p-value 0.002). For radiological involvement, it was significantly higher in anti-CCP antibody positive group. However, there was no significant difference between mean and classes of disease activity score, and extra-articular manifestations between different anti-CCP antibody positivity group. Extra-articular manifestations was not associated with high disease activity upon diagnosis of RA.

CONCLUSION :

In conclusion, there was significant association between anti-CCP antibody positivity and positive rheumatoid factor and radiological involvement, however no significant difference found in mean disease activity and extra-articular manifestation upon diagnosis in patient with

RA in HUSM. Extra-articular manifestations was not associated with high disease activity upon diagnosis of RA.

CHAPTER ONE

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic debilitating inflammatory disease affecting mainly the joint and surrounding tissue. It is characterized by chronic inflammatory process in the synovium which is caused by infiltration by the immune and inflammatory cells; particularly macrophages, B- and T-lymphocytes, dendritic cells and plasma cells. This process leads to increase in the levels of cytokines which contains destructive enzymes, specifically matrix metalloproteinase (Feldman et al., 1996). As a result, sustained recruitment, inappropriate retention and impaired apoptosis may occur which can cause destruction of the bone, cartilage and soft tissue and subsequently leads to severe disability to the patient (Morovic et al., 2003). The etiology or precipitating factor of this disease is unknown; however interaction between both genetic and environmental factors are said to contribute to its occurrence (Alamanos et al., 2005). RA is a systemic disease, in which can also involve variety of extra-articular organs such as lung, eye, vessels, blood cells and many others. Early and aggressive intervention with establish or even new drugs can reduce the pathogenic process, improve the function of the joint, and increase the quality of life (Niewold et al., 2007).

RA is affecting approximately 0.5% to 1% of the world population based on the geographical distribution and ethnically diverse population (Gabriel et al., 2009). In 2010, Egyptian Society for Rheumatology had reported the prevalence of RA was 0.1%, in comparison to previous study more than 10years ago in Northwest Greece by Drosos et al., (1997) which showed the prevalence of 0.2%. On the other hand, the majority of

studies carried out in Northern European and North American areas estimate a higher prevalence rate of 0.5 to 1%, and a mean annual incidence of 0.02-0.05% (Alamanos et al., 2005). Female was found to be affected higher than male (Drosos et al., 1997, Carmona et al., 2003).

1.1 Pathophysiology of Rheumatoid Arthritis

The pathogenesis of RA is not well understood. Some theories suggest autoimmune reaction triggered by an external circumstance such as trauma, smoking, infection, or stress, leading to inflammation of the synovial membrane. Inflammatory cells and cytokines play a major roles in the pathophysiology of RA. CD4 T cells, mononuclear phagocytes, osteoclasts, neutrophils, and fibroblast may infiltrate the synovial tissue while together with abnormal cytokines, chemokines and other inflammatory mediators such as tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), interleukin-6, transforming growth factor beta (TGF-β), fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF), cause destruction of various tissues including cartilage, bones, tendons, ligaments and blood vessels.

Angiogenesis also contributes to the development of chronic inflammation and plays an important role in the pathogenesis of RA (Eker et al., 2014). Vascular endothelial growth factor (VEGF) has been detected to be present in very large amounts in the inflammatory synovium in RA. Various studies showed that both VEGF and macrophage migration inhibitory factor (MIF) are associated with disease activity parameters and with each other in RA

It is believed that genetic factors also contributed to the formation of RA (Alamanos et al., 2005). A family history of RA increases the risk around three to five times; as of 2016 it was estimated that genetics factors may account for between 40 and 65% of cases of seropositive RA, but only around 20% for seronegative RA.

Smoking is the strongest known environmental risk factor for RA especially in Caucasian populations. It is increasing the risk three times compared to non-smokers, particularly in men. This association was first described over a decade ago but has been further characterized recently with the use of anti-CCP antibody assay. A recent study found that tobacco smoking was specifically associated with an increased risk of ACPA-positive and not ACPA-negative RA. As the majority of RA patients who are ACPA positive are also rheumatoid factor positive, these findings concur with previous studies which show an overall risk of RA for smokers, specifically for rheumatoid factor-positive RA (Katherine et al, 2009). The risk of RA increases with amount and duration of cigarette use. Findings from a large prospective cohort study, the Nurses' Health Study (NHS), showed a linear relationship between smoking and risk of RA whereby increasing doses of cigarettes (pack-years of smoking) was associated with an increased risk of RA. The heaviest smokers with more than 40 pack-years had approximately two-fold increase of risk for RA than those who had never smoked. Furthermore, an individual remains at increased risk even after cessation for 20 years or more.

Silica exposure has been linked to RA. A recent review of the epidemiologic evidence of environmental factors in human autoimmune diseases concluded that exposure to crystalline silica contributes to the development of a number of autoimmune diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), systemic

sclerosis, and anti-neutrophil cytoplasmic antibody (ANCA) related vasculitis (Pollard K. M., 2016)

1.2 Clinical Presentation Of Rheumatoid Arthritis

The hallmark feature of rheumatoid arthritis is the symmetrical polyarthritis involving small joints of hands or feet. A patient may complaint of pain and stiffness of the joints especially early in the morning which usually improve after movement. The number of joints involved is variable; it can be either single small joint involvement at first then spread to another joints, and typically affected more than five joints. Any synovial joint can become involved in RA. The joints most frequently involve are metacarpophalangeal joint (MCPJ) and proximal interphalangeal joint (PIPJ) of the hands, wrist, and small joints of the feet including the metatarsophalangeal joints (MTP). Sparing of the distal interphalangeal joints (DIPJ) is the most characteristic feature of RA. Though presented often in textbooks as features of ‘swan neck’ and ‘boutonniere’ deformity of the digits, it appears late in disease and is a feature of chronic disease. They are not usually seen at initial presentation where signs of synovitis and joint damage may be subtle. Swan neck deformity is a condition which the DIPJ is flexed while PIPJ is hyperextended. Boutonniere deformity occur when there is flexion of PIPJ with hyperextension of DIPJ. Another features that often found in RA patients is “Z-thumb" or "Z-deformity" of the thumb. It consists of hyperextension of the interphalangeal joint, fixed flexion and subluxation of the metacarpophalangeal joints and gives a "Z" appearance to the thumb. Not to forget other joints involvement such as elbows, shoulders, knees, and also the hips and temporomandibular joints. RA also affects the clavicular joints and the crico-

arytenoid joints. A mono- or bilateral arthropathy of the shoulder or wrist may account for up to 30 to 40% of initial presentations while 5% of initial presentations involve the knee.

The pain and stiffness may lead to difficulty in performing activities of daily living (ADL) such as combing hair, turning the doorknob, dressing, or even walking. A patient who already has deformity of the hand performs worse. Acute inflammatory arthritis is reversible, and it is very important to detect it early to prevent chronic inflammation which can cause severe irreversible disability which may affect their ADL. In addition to these, nonspecific systemic symptoms such as myalgia, fatigue, fever, reduce in appetite and loss of weight may also present. RA usually has insidious onset of symptoms. Initial symptoms can be systemic or mild arthritis without significant interruption in daily activity before the appearance of overt joint pain and swelling.

1.3 Diagnosis of Rheumatoid Arthritis

1.3.1 1987 ACR Classification Criteria of Rheumatoid Arthritis

In 1956, a committee of the American Rheumatism Association (ARA) had proposed a criteria for the diagnosis of RA. From this criteria, eleven components had been identified. This criteria had been revised few times in 1958, 1966, and 1983 to improve the sensitivity and specificity of the criteria in order to aid in the diagnosis and management of RA.

The revised criteria for the classification of RA (Arnett et al., 1988) was published in 1987. There were a total of seven component in this criteria, listed as follows: 1) morning

stiffness in and around the joints lasting at least one hour before maximal improvement; 2) soft tissue swelling (arthritis) of three or more joint areas observed by a physician; 3) swelling (arthritis) of the PIPJ, MCPJ, or wrist joints; 4) symmetrical swelling (arthritis); 5) rheumatoid nodules; 6) presence of rheumatoid factor; and 7) radiographic erosions and/or peri articular osteopenia in hand and/or wrist joints. Criteria 1 to 4 must have been present for at least 6 weeks. To diagnose RA, a patient must have at least 4 or more criteria, and no further classifications (eg: classic, definite, or probable) are required. This criteria had demonstrated 91% to 94% sensitivity and 89% specificity for RA subject in comparison to non-RA control subjects.

Symptoms of early morning stiffness for at least one hour represent inflammatory process that accumulate much more while the joint is less mobile. Total joint involvement must be more than three, which most of the time is symmetrical and typically involve small joint of the hands or feet compared to larger joints. Rheumatoid factor is the only laboratory test included in this criteria while rheumatoid nodule is the only extra-articular manifestation that is required. For radiological changes, the expected finding includes erosions or unequivocal bony decalcification localized in the involved joints. Over the last decades, this 1987 classification criteria is well accepted as a benchmark for disease definition, however it had been criticized for lack of sensitivity in diagnosing early disease (Aletaha et al., 2010). This criteria had been recognized for the diagnosis of establish RA but unable to discriminate those who are in early disease whom would benefit from early intervention especially with the raised of biologic agents.

1.3.2 2010 ACR/EULAR Rheumatoid Arthritis Classification Criteria

In order to facilitate the diagnosis of early RA, American Colleges of Rheumatology (ACR) and the European League against Rheumatism (EULAR) had been joining hand to form a working group in order to develop a new classification criteria for RA. Hence, in 2010, this new ACR/EULAR Rheumatoid Arthritis Classification criteria had been introduced (Aletaha et al., 2010). In this new criteria, it redefines RA on the condition at earlier stages, compared to the previous 1987 ACR criteria that predict more of erosive disease.

2010 ACR/EULAR classification criteria for RA had classify definite RA based on four main categories; A) joint involvement; B) serological test; C) acute phase reactants and D) duration of symptom. Patients who need to be tested must have at least one joint involvement with definite clinical synovitis, and has no other alternative diagnosis to explain the synovitis. Definite RA is confirmed by having a score of 6 or more from a total score of 10. In this criteria, the first domain calculate the number and site of joint involvement either small or large joint which give a score of 0 to 5, while second domain is comprise of serological test such as rheumatoid factor or anti-CCP antibody assay based on titre with the score of 0 to 3. The third domain include acute phase reactant which is either Erythrocyte Sedimentation Rate (ESR) or C - Reactive Protein (CRP) that give a score of 1 if positive and 0 if negative. Fourth domain is about the duration of symptoms; with the score of one for duration of 6 weeks or more.

In comparison with the 1987 ACR criteria, the 2010 ACR/EULAR criteria have higher sensitivity but lower specificity, especially in patients aged 60 years or more.

1.3.3 Rheumatoid Factor

Rheumatoid factor is the immunoglobulin M (IgM) autoantibody against denatured immunoglobulin G (IgG) Fc fragment which is secreted by B cells and can be found circulating in the blood. About 60–80% RA patients had positive rheumatoid factor during the course of their disease (Nell V.P. et al, 2005). However, rheumatoid factor results were positive in less than 40% of patients with early RA. Rheumatoid factor is also present with other connective-tissue diseases, autoimmune disorders, and pro-inflammatory states such as systemic lupus erythematosus (SLE), polymyositis, tuberculosis, syphilis, viral hepatitis and many others. It is also observed in 1-5% of healthy individuals and can be found up to 20% in older population. Thus, rheumatoid factor is not considered specific for RA.

The normal reference range for RF is less than 15 IU/mL or less than 1:16.

Rheumatoid factor had been use as the only serological marker for the diagnosis of rheumatoid arthritis since many years. It has assumed a pivotal role in subcategorizing patients into ‘seropositive’ and ‘seronegative’ groups, despite incomplete knowledge of the role of rheumatoid factor in disease pathogenesis, its frequent absence in early stages of disease, its suppression by disease modifying drugs and its occurrence in hidden form.

In patients with rheumatoid arthritis, some studies showed that RA patients with positive rheumatoid factor had higher proportion of complicated with extra-articular manifestations, had more aggressive radiological progress, and had worse prognosis than the RF-negative RA (Edelman et al, 1983). Greiner and colleagues (2005) followed up 135 RA patients for 6 years and the result showed that the disease activity was higher in

the persistent RF-positive group, and the patients need more intensive treatment in the persistent RF-positive group than the persistent RF-negative group.

1.3.4 Anti Cyclic Citrullinated Peptide Antibody

The introduction of anti-CCP antibody assay as a new serological marker for RA is a significant advance in rheumatological field. Anti-CCP antibody is principally an autoantibodies directed to citrullinated proteins in the synovium of patients with RA (Szekanecz et al., 2008). The first citrulline-binding autoantibodies in RA sera were discovered by Nienhuis et al. in 1964, and were named as anti-perinuclear factor. In 1979, Young et al. found that RA sera contained anti-keratin antibodies that reacted to the keratinized layer of epithelium. Uniquely these antibodies were only found in patients who had been diagnosed with RA. Subsequent studies demonstrated that anti-keratin antibodies and anti-perinuclear factor recognized a similar epitope, and therefore, can be categorized as anti citrullinated peptide antibodies (Niewold et al., 2007). Subsequently, anti-CCP 1 assay was made commercially available followed by the second generation of anti-CCP 2 assay that was made using combination of synthetic cyclic citrullinated peptides selected through a screening process. It was built to increase the sensitivity over anti-CCP 1 antibody while maintaining the specificity (Taylor et al., 2011). Now anti-CCP 2 antibody assay is been used widely in the diagnosis of RA.

Many studies had been done to assess the effectiveness of this new serology marker in the diagnosis of RA and also in the progression of the disease, either related to the disease activity or in the prognostication of RA. The sensitivity and specificity of anti-CCP antibody for the diagnosis of rheumatoid arthritis (RA) were 66.0% and 90.4%,

respectively (Niewold et al., 2007). In an early arthritis study, which was conducted in The Netherlands, Jansen et al (2002) found that having values of IgM rheumatoid factor more than 40 or anti-CCP of more than 50 predicts which patients with early arthritis will be diagnose as RA with a sensitivity of 55.4% and a specificity of 96.7%. Saraux et al (2003) studied two hundred and seventy patients with early arthritis and found anti-CCP antibody sensitivity of 47% and specificity of 93%. Anti-CCP antibody was proven to be useful in identifying those with significant disease activity and more severe radiological outcome (van der Helm-Van Mil et al., 2005., Chou et al., 2007).

The reference ranges for blood test for anti-CCP antibody are negative if less than 20 EU, low or weak positive if ranging between 20 to 39 EU, moderate positive if fall in range of 40 to 59 and high or strong positive if the level is more than 60 EU.

1.4 Assessment Of Disease Activity

In daily clinical practice, assessment of disease activity is important especially to determine the requirement for treatment. There are many criteria that can be used to determine patient's disease activity but out of 63 criteria, ACR had recommended six with the best psychometric properties. The criteria recommended by ACR are Patient Activity Scale (PAS), Patient Activity Scale II (PAS-II), Routine Assessment of Patient Index Data with 3 measures (RAPID 3), Clinical Disease Activity Index (CDAI), Disease Activity Score with 28-joint counts (ESR or CRP) (DAS-28 ESR or CRP), and Simplified Disease Activity Index (SDAI). All these criteria had been chosen as they accurately reflex the disease activity, categorized well in different states of remission, mild,

moderate and high disease activity, and easy to perform in clinical setting. The aim of treatment is to get patients into remission or at least mild disease activity.

The DAS28 is an index similar to the original DAS, modified from the original 44 joint count in DAS index into simplified 28 tender and swollen joint count (range 0-28), ESR, and an optional general health assessment on a visual analogue scale (range 0-100). Because of the use of reduced and non-graded joint counts, the DAS28 is easier to complete than the DAS. The DAS28 has a continuous scale ranging from 0 to 9.4, and usually shows a Gaussian distribution in RA populations. DAS and DAS28 values cannot be directly compared, but a formula to transform DAS28 into DAS values is available. This is important especially in research purposes.

Disease activity had been studied widely in relationship with serology markers like rheumatoid factor and anti-CCP antibody. Many studies showed anti-CCP antibody titres are positively correlated with the disease activity (Drosos et al., 1997, Kastbom et al., 2004)

1.5 Radiological Manifestation of Rheumatoid Arthritis

Other than plain radiograph, there are few other imaging modalities available to assess the radiographic progression in RA patient. Magnetic Resonance Imaging (MRI) have the capability to image the joint in great detail and is more sensitive than other modalities to detect joint inflammation and destruction. However, the main disadvantages is it is quite expensive especially for monitoring as it need to be done from time to time, regularly. Furthermore, it also has no standardize scoring method available between different centres. Ultrasonography can detect early synovitis by detection of effusion,

synovial hypertrophy, and signal from power Doppler, other than bony erosion and cartilage thickness. It is easy to be used in clinical setting and become more popular nowadays among rheumatologist. The main disadvantages include inability to assess certain sites due to acoustic window and because it is operator dependent, it requires training for a significant period of time.

Plain radiograph, although seems to be old fashion compared to these newer modalities, is still the investigation of choice as it is readily available, cheap and easy to perform. Assessment of structural damage to look for bony erosion and joint space narrowing may suggest establish disease and failure to detect early changes makes plain radiograph less favourable. One large cohort study showed that radiographically demonstrable erosions were present in 30% of patients at diagnosis, and in 70% three years later support the importance of this modalities in identifying establish RA disease. The radiographic hallmarks of rheumatoid arthritis are soft tissue swelling, which are fusiform and periarticular to represents a combination of joint effusion, oedema and tenosynovitis, osteoporosis which initially juxta-articular, and later generalised; compounded by corticosteroid therapy and disuse, joint space narrowing which can either symmetrical or concentric, and marginal erosions due to erosion by pannus of the bony bare areas. In the hands, a patient can demonstare late changes such as subchondral cyst, subluxation causing ulnar deviation of the MCPJ, swan neck deformity and boutonniere's deformity, hitchhiker's thumb deformity, carpal instability manifested by scapholunate dissociation and ulnar translocation, and ankylosis.

From most of the study, they found that RA patients with positive anti-CCP antibody have more radiographic joint damage as compared to those without these antibodies (Meyer et al., 2003, Forslind et al., 2004).

1.6 Extra Articular Manifestation

Although joint surface is the primary site involved, tissues in the body other than the joint can still be affected. It can manifest as rheumatoid nodule, vasculitic lesion, pulmonary involvement, pericarditis, peripheral neuropathy, benign lymphadenopathy or haematological manifestation like anemia. The frequency of extra-articular manifestation is difficult to estimate. Most studies have been performed at highly specialized centres, with poorly defined catchment areas. Population studies of extra-articular manifestation on the other hand, are difficult to perform since they require uniform and consistent ascertainment of such complications in a defined catchment area population (Turesson et al, 1999).

Extra articular manifestation can develop at any time during the course of the disease, even in the early stages. Extra articular manifestation of RA are thought to be particularly frequent in severe, active disease. They are known as a serious condition and has been shown to be associated with significant morbidity and high mortality.

1.6.1 Rheumatoid Nodule

Rheumatoid nodule is the most frequent skin manifestation of RA, occur in about 20-30% of RA patients (Vela et al., 2014, Cojocaru et al., 2010). It has variable consistency, but usually rather firm to touch. The nodule is related to poor prognostic factor and almost exclusively occur in rheumatoid factor positive patient (Richman et al., 2013, Cojocaru et al., 2010). It is most commonly seen on pressure area such as olecranon process and proximal ulna, but can also be seen in finger joints, occiput, sacral prominences and Achilles tendon. Rarely does it occurs in the main organ of the body such as the lung,

heart valves, or at diverse sites on body (e.g. upper eyelid, distal region of the soles of the feet, vulva and internally in the gallbladder, larynx, and spine). The occurrence of nodules in the lung of miners exposed to silica dust was known as Caplan's Syndrome. About 5% of RA patients have such nodules within two years of disease onset. In the great majority of cases nodules are not painful or disabling in any way, being more of an unsightly nuisance, but in some cases they can be painful, especially if the overlying skin breaks down.

1.6.2 Pulmonary involvement

Pulmonary involvement in RA can be manifested in few different ways, includes parenchymal pulmonary nodules, pleural effusion, interstitial lung disease (ILD), pulmonary vasculitis and small airway disease. Pulmonary nodules are usually asymptomatic, but can cause cavitation and had increased risk of infection. ILD is the most important pulmonary manifestation of RA, as it leads to high morbidity and mortality. It is among the commonest pulmonary cause of death in RA. Types of ILD that most commonly occur are usual interstitial pneumonia (UIP) (44% to 56%), non-specific interstitial pneumonia (NSIP) (33% to 44%), mixed disease (0% to 12%) and obliterative bronchiolitis. Pathophysiology related to this pulmonary manifestation is by early inflammatory phase associated with pulmonary mononuclear cell infiltrates which later form a fibrosis. This condition usually occur in male patient with positive rheumatoid factor and had long standing nodular disease. Clinical presentations are similar to idiopathic pulmonary fibrosis but the respond to immunosuppressant is usually better. Diagnosis must be based on clinical presentation, blood gases, lung function test,

and high resolution computed tomography (HRCT) or lung biopsy. Six minute walking test is a simple bedside test that can be done if a patient is suspected to have this pulmonary involvement.

1.6.3 Felty Syndrome

Felty syndrome is defined as combination of RA with splenomegaly and neutropenia. It is a rare disease which usually occur less than 1% in elderly woman at the age of around 60. It is usually associated with severe long standing articular disease and also positive rheumatoid factor and anti-CCP antibody. In most patient with Felty Syndrome, other extra-articular features such as lymphadenopathy, hepatomegaly, vasculitis and leg ulcer will also present. Felty syndrome can lead to severe infection due to neutropenia which can be recurrent, and sometimes fatal. A careful evaluation should be made before the diagnosis of Felty Syndrome being made to exclude other malignant haematological causes of neutropenia; with the fact that this syndrome has been associated with an increased risk of malignant lympho proliferative disease. The clinical significant of Felty syndrome is commonly was not driven so much attention as it usually occur during inactive disease of RA.

1.6.4 Anemia

Anemia is among the most common haematological changes presents in patient with RA. It is mostly related to anemia of chronic illness, which is normocytic normochromic type of anemia, but can also occur as secondary to other causes. Decreased iron absorption

was shown to be the result of active RA rather than a cause of anemia of chronic disease or iron deficiency anemia. It has been hypothesized that iron availability in the bone marrow decreases due to decreased in iron release by the mononuclear phagocyte system. Another postulation is that the anemia in anemia of chronic diseae is due to ineffective erythropoiesis, however these remain controversial theories. Studies considering a decreased erythropoietin responsiveness have not produced consistent results. Erythroid colony growth is suppressed in vitro by interleukins 1 and interleukin 6 and tumour necrosis factor alpha but their role in vivo in anemia of chronic disease is unknown. The diagnosis of anemia of chronic disease is made by exclusion. Iron deficiency is detected by transferrin, ferritin, and cellular indices after adaptation of their normal values. This is proven by a study by Papadaki et al (2002), saying that treatment with anti-TNF therapies results in significant improvement in the anemia associated RA.

1.6.5 Cutaneous vasculitis

Vasculitis in RA can manifested in many organs including skin, neuron, or specific organ vasculitis such as renal or pulmonary. The most common manifestation of cutaneous vasculitis are small digital infarction along the nail bed, skin ulcerations or gangrene. As is true with other forms of vasculitis that involve the skin, cutaneous lesions can erupt on various areas of the body in RV, with a predilection for the lower extremities. Typical findings include ulcers concentrated near the ankles. The manifestations of RV in other body's different organ systems, including peripheral nervous system is manifested as numbness to the hands and feet, while arteries of the fingers and toes involvement can cause digital ischemia, and eyes involvement are typically with scleritis. This vasculitic

lesion is related to the deposition of immune complexes intravascularly by rheumatoid factor and immunoglobulins, which leads to damage and destruction to the vessels. The syndrome usually emerges after years of seropositive RA which is persistently in active disease. However, vasculitis can also occur when joints are in inactive state.

1.6.6 Other extra-articular manifestation of RA

There are many other signs of extra-articular involvement that can be elicited in a patient with RA related to any organ in the body. Ocular involvement occurs in 27% of RA patients. Keratoconjunctivitis of Sjogren's syndrome is the most common benign eye manifestation that presented as dry eyes (sicca). Episcleritis may occur occasionally with the main complaint of mild pain and intense redness of the eye. Scleritis and corneal ulcerations are rare but requires urgent attention and treatment with DMARDS. Schirmer test is a simple bedside test that can be done for screening of dry eyes.

Sjogren's syndrome occur in approximately 10-15% of RA patients. It is a chronic inflammatory disorder that affects exocrine gland function especially lacrimal and salivary gland. It leads to reduction of tear (keratoconjunctivitis sicca), dry mouth (xerostomia) and reduce vaginal secretions.

Cardiovascular manifestation in RA patients include pericarditis, myocarditis, cardiac amyloidosis, coronary vasculitis, arrhythmia, valve disease and most importantly ischemic heart disease and congestive cardiac failure. The last two diseases has high mortality risk in RA patient in comparison to general population. RA is associated with a 1.5-fold increased incidence of coronary heart disease (CHD), stroke, total cardiovascular disease, fatal cardiovascular disease, and total mortality. The

pathogenesis is said to be related to acceleration in the atherogenesis that cannot be explained by the classical risk of atherosclerosis. Despite improved treatment, there is little evidence of reduction in coronary heart disease or cardiovascular morbidity or mortality. Risk factors for incident of coronary heart disease in RA include traditional cardiovascular disease risk factors (e.g., cigarette smoking, hypertension, diabetes mellitus, and elevated low-density lipoprotein cholesterol) and markers of RA severity, including inflammatory markers (e.g., ESR and CRP), joint pain, and disability. Pericarditis is also common, occur in 1% to 4% of patients especially those with positive rheumatoid factor.

The most common neurological manifestation of RA is primary sensory peripheral neuropathy, which is usually mild and involved lower extremities. This nerve damage can cause foot or wrist drop, as a cause of mononeuritis multiplex. This condition, which may be significantly disabling, is often preceded by a change in sensation in the same area which can be either numbness, tingling, burning, or pain. These abnormal sensations can progress to muscle weakness, focal paralysis, and eventually to muscle wasting. Recovery from this condition, caused by nerve infarction, can take months. In some cases, recoveries from mononeuritis multiplex are incomplete. Other neurological manifestations include cervical myelopathy, vasculitis, and nodule within the central nervous system, meningitis or stroke.

Nephropathy in RA can occur due to various causes. Several potential causes that had been identified are drug induce, renal amyloidosis, and various type of glomerulonephritis. Gold or penicillamine can cause membranous type of glomerulonephritis. Other medications such as non-steroidal anti-inflammatory drugs (NSAIDs), sulphasalazine or methotrexate were also found to cause serious adverse

effect especially in patient with underlying kidney disease. Renal involvement in RA is clinically meaningful because it worsen the course of primary disease and increases mortality.

1.7 Management of Rheumatoid Arthritis

The goals of treatment in rheumatoid arthritis include reduction in joint pain and inflammation, prevention of joint destruction or permanent damage and the most important thing is the improvement in the quality of life. The principle of treating rheumatoid arthritis is based on treat-to-target recommendation as proposed by 2014 Update of the Recommendations of an International Task Force (Smolen et al., 2015). The target for the treatment is remission or low disease activity in every follow up which are proven as the best and second best outcome in RA.

Disease-modifying anti-rheumatic drugs (DMARDs) is a group of medication that is widely used as the primary agent for the treatment of RA. These agents are capable of slowing down the disease progression thus able to improve patient's quality of life. DMARDs can be divided into synthetic chemical compounds DMARDs (sDMARDs) and biological agent DMARDs (bDMARDs). Methotrexate is the most commonly used agent for sDMARDs other than sulfasalazine, hydroxychloroquine and leflonamide, whereas tofacitinib is a new agent targeting Janus kinases.

Although originally designed as a chemotherapy drug (using high doses), in low doses, methotrexate is a generally safe and well tolerated drug in the treatment of certain autoimmune diseases. Because of its effectiveness, low-dose methotrexate is now first-line therapy for the treatment of rheumatoid arthritis. Weekly doses are beneficial for 12

to 52 weeks duration therapy, although discontinuation rates are as high as 16% due to adverse effects. Although methotrexate for autoimmune diseases is taken in lower doses than it is for cancer, side effects such as hair loss, nausea, headaches, and skin pigmentation are still common. Use of low doses of methotrexate together with NSAIDS such as aspirin or analgesics such as paracetamol is relatively safe in people being treated for rheumatoid arthritis, if adequate monitoring is done.

Methotrexate toxicity can lead to stomatitis, gastro intestinal disturbance, and also alopecia. This effect can be reduced by the addition of folic acid daily, without loss of therapeutic effect. Parenteral administration is a useful option if oral treatment is not tolerated, and there is also some evidence that parenteral administration can improved the efficacy of methotrexate compared with oral administration. Another rare but important complication of methotrexate are pneumonitis and pulmonary fibrosis. These complications should not deter the physician from using methotrexate in aggressive systemic and skeletal disease. Rare, life-threatening, pulmonary toxicity can occur at any time but is most common in the first year and is not directly related to dose or duration of treatment. A chest radiograph should be taken before methotrexate is commenced. Mild drug-induced hepatitis is relatively common and is often corrected by the addition of folic acid. Liver function abnormalities fluctuate and may require the drug to be stopped for a short period if transaminase levels rise above 2–3 times the upper limit of normal, with possible re-introduction after a period of time when levels have normalized, with close monitoring. Patients are advised that they should abstain from alcohol use completely.

Sulfasalazine and hydroxychloroquine are often used initially in mild RA, partly because of their relative safety and convenience. Both agents are generally well-tolerated and take

effect within 1 to 3 months. Many clinicians now choose MTX first if there is evidence of early aggressive disease. Leflunomide is also effective for the treatment of RA. It is an inhibitor of the enzyme dihydro-orotate dehydrogenase and shows anti-proliferative activity, inhibiting pyrimidine synthesis. It has a long half-life of 2 weeks.

IM and oral steroids are very effective in active RA, reducing active disease in an acute crisis or while waiting for a DMARD to take effect.

Biological DMARDs consist of TNF inhibitor such as adalimumab, certolizumab pegol, etanercept, golimumab and infliximab, T cell costimulation inhibitors such as abatacept, anti-B cell agent such as rituximab, interleukin-6 receptor blocking monoclonal antibody such as tocilizumab and interleukin-1 inhibitor such as anakinra. With all the above anti-TNF-α agents, co-administration with methotrexate is recommended when tolerated as this has been shown to increase efficacy, and use with infliximab also reduces the production of anti-infliximab and antinuclear antibodies. Another type of agent that had been recently approved by the European Medicines Agency (EMA) is a biosimilar type of DMARD, for example is the biosimilar infliximab (bs-infliximab). This agent may help in reducing the treatment costs associated with biologics.

Damage to joints, with associated pain and loss of function remains a familiar feature of chronic RA. Surgical intervention may have a place in such situations, although certain procedures such as shoulder replacement may only be effective in reducing pain and may not necessarily improve the joint function. Synovectomy is less frequently performed now, although tenosynovectomy is common, and a quick and safe relief of nerve entrapment. Other common surgical procedures include decompression of the carpal tunnel, reconstructive arthroplasty of hip and knee which less often done if involve the

shoulder, elbow, and small joints of the hand, corrective arthrotomies of the metatarsals, stabilization of the cervical spine and tendon release and transfer and also arthrodesis, particularly of the ankle joint.

1.8 Prognosis

Identification of poor prognostic factors in RA patient is important as it may help in providing appropriate and adequate treatment to the patient in order to prevent further complication. Intervention with DMARDs in early RA may help in retarding progression to joint destruction. Few conditions associated with unfavourable prognosis are HLA-DRB 1 genotype, high titre of rheumatoid factor and anti-CCP antibody, onset at younger age which is usually less than 30years old, female, high number of joint involvement, insidious onset of symptom, and presence of extra-articular manifestations. Other markers that are related to poor prognosis are early radiological involvement, presence of anemia and antikeratin antibodies.

RA gives rise to higher morbidity and mortality rates; it cause 60% of the patients with RA unable to work after 10 years (Quinn et al., 2005). The development of extra–articular manifestation in RA patients can have significant impact on their daily activity, including increased mortality compared to RA patients in general. That is the reason why early detection of RA and early treatment is very crucial in order to prevent devastating deformity.

1.9 Study Background and Rationale

Anti-CCP antibody has not been used widely in most of the centre in Malaysia. Many physician still depends on rheumatoid factor to diagnose RA. This is most likely because they do not understand and see the importance of this assay in the diagnosis and management of RA. Because of this, we are not only missed the chance to detect patient with early disease, we also unable to prognosticate them and overlooked those whom would benefit from early intervention especially with the raised of biologic agents. In this study, we want to prove that anti-CCP antibody had significant importance in different aspect of disease activity, radiological involvement, and extra-articular manifestation in the diagnosis of RA. We sought to assess the efficacy of this anti-CCP antibody in patient with RA and also assess the association between variety of presentation upon diagnosis so that anti-CCP antibody assay can be utilized as optimum as possible.

Extra-articular manifestation was not being investigate thoroughly in our daily practice. Many extra-articular manifestation were misdiagnosed as other disease not related to RA because they can develop at any time during the course of the disease, even in the early stages. Furthermore, there were not many studies done in Malaysia assessing the importance of this assay particularly in the occurrence of extra-articular manifestation. Other than that, in this study, we also wanted to assess the association between extra-articular manifestation and disease activity upon diagnosis.

CHAPTER TWO

OBJECTIVE AND RESEARCH QUESTIONS

2.1 General Objective

To evaluate the significant of anti-CCP antibody in predicting disease activity and disease progression upon diagnosis in patient with rheumatoid arthritis in HUSM.

2.2 Specific Objectives

2.2.1 To determine the prevalence of patient with positive anti-CCP antibody in RA population in HUSM.

2.2.2 To determine the association between anti-CCP antibody and rheumatoid factor in RA patients in HUSM.

2.2.3 To compare the mean of DAS28 disease activity score in RA patients with positive and negative anti-CCP antibody upon diagnosis.

2.2.4 To determine the association between DAS28 classes of disease activity, radiological involvement and extra-articular manifestation in RA patients with anti-CCP antibody positivity upon diagnosis.

2.2.5 To determine the association between disease activity and extra-articular manifestations in patient with RA upon diagnosis.