INTRODUCTION

Rheumatoid arthritis (RA) is a common autoimmune disease that causes chronic inflammation of the synovium. RA synovitis evokes arthritis symptoms and leads to destruction of cartilage and bone in joints. Once RA has developed, autoimmunity is sustained and leads to persistent synovitis, which in turn causes destruction of bone and cartilage ***(Yoshida Y et al., 2015).***

RA is characterized by destructive polyarthritis and extra-articular organ involvement, including the skin, eye, heart, lung, renal, nervous and gastrointestinal systems. The extra-articular manifestations of RA can occur at any age after onset and is more frequently seen in patients with severe, active disease and is associated with increased mortality ***(Cojocaru M et al., 2010)***.

Atherosclerotic cardiovascular disease is the leading cause of mortality and disability in most countries. Approximately 30% of first acute events are fatal, and survivors often experience sequelae and a shortened life expectancy. Primary prevention is thus the best approach to fighting this pandemic disease ***(Laclaustra M et al., 2016).***

Evidence for increased risk for cardiovascular disease (CVD) in RA has accumulated during the last two decades. It has been suggested that the prevalence of CVD in patients with

RA is as high as in patients with type 2 diabetes mellitus (T2DM). Traditional cardiovascular risk factors, such as hypercholesterolaemia and hypertension, and RA-specific risk factors, such as RA disease activity, erythrocyte sedimentation rates (ESRs) and CRP levels may play a role in the overall CVD risk of RA patients ***(Deborah F et al., 2016).***

Tight control of disease activity, including monitoring of acute-phase reactants, is important to reduce joint destruction and disability in RA. Indeed, measurement of C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR) is incorporated into the Disease Activity Score based on 28 joints (DAS28), the core set of RA disease activity measures proposed by the American College of Rheumatology and The European League Against Rheumatism RA remission criteria ***(Felson DT et al., 2011).***

The markers of inflammation most commonly used to assess RA disease activity as ESR and CRP have disadvantages. For example, ESR is altered by non-inflammatory conditions such as chronic kidney disease, pregnancy, anemia, abnormal red blood cell shape or size, and serum protein concentrations ***(Ormseth M et al., 2015)***.

GlycA is emerging as a new marker of inflammation and cardiovascular (CV) risk. GlycA concentrations were associated with known inflammatory markers, such as CRP,

interleukin-6 (IL-6) and fibrinogen in 5,537 participants of the Multi-Ethnic Study of Atherosclerosis ***(Otvos JD et al., 2015).***

Moreover, GA was associated with incident CV events. For example, for every 1 standard deviation (SD) increase in baseline GA concentration, there was a 34 % increased incidence of CV events within the first 6 years of follow-up, independent of traditional risk factors, in over 27,000 women from the Women's Health Study **(Akinkuolie AO et al., 2014).**

GA can be measured in the nuclear magnetic resonance (NMR) spectra acquired for the quantification of serum or plasma lipoprotein particle numbers. The majority of circulating glycosylated proteins are acute-phase proteins, and the main contributors to the GA signal are α1-acid glycoprotein, α1-antitrypsin, haptoglobin, α1-antichymotrypsin and transferrin. Glycosylated immunoglobulin is not a main contributor to the GA signal ***(Lauridsen MB et al., 2010).***

Atherosclerosis most often develops gradually and slowly, starting from childhood and proceeding into adulthood with varying velocity and susceptibility to complications. The first structural change that can be detected in atherosclerosis is an increase in Intima-media thickness (IMT). Intima-media thickness is an important atherosclerotic risk marker. However, this increase is not synonymous with subclinical atherosclerosis, but is related to it. Indeed, increase in IMT is also the result of non-atherosclerotic processes such as smooth muscle cell

hyperplasia and fibrocellular hypertrophy leading to medial hypertrophy and compensatory arterial remodeling. Therefore this process may be an adaptive response to changes in flow, wall tension, or lumen diameter ***(Simova I, 2015).***

Intima-media thickness (IMT) measurement is advised in a search for target organ damage; asymptomatic vascular damage could be detected with ultrasound scanning of carotid arteries searching for vascular hypertrophy or asymptomatic atherosclerosis. Damage is defined as the presence of IMT >0.9 mm or plaque. The other markers of asymptomatic vascular (target organ) damage are: pulse pressure ≥ 60 mmHg, carotid-femoral pulse wave velocity > 10 m/s and ankle-brachial index < 0.9 ***(Mancia G et al., 2013).***

B-mode ultrasonography is a noninvasive, safe, easily performed, reproducible, sensitive, relatively inexpensive and widely available method for detection of early stages of atherosclerosis and is accepted as one of the best methods for evaluation of arterial wall structure. Intima-media thickness is accepted as a marker of subclinical atherosclerosis and IMT screening can help the clinician to reclassify a substantial proportion of intermediate cardiovascular risk patients into a lower or higher risk category ***(Simova I, 2015).***

Aim of the Work

This study is designed to detect the value of Serum Glycated Albumin measurement as a marker for RA associated coronary artery atherosclerosis and also a marker for RA disease activity.

Chapter 1

RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic, systemic, progressive autoimmune disease, that principally attacks the joints in a systemic pattern producing an inflammatory synovitis that often progresses to destruction of the articular cartilage and ankylosis of the joints ***(Hekmat et al., 2011)***.

RA affects almost 1-2% of the population and it is two to three times more prevalent in women than in men. RA may begin as early as infancy, but onset usually occurs in the 3rd or 4th decade ***(Turesson C and Matterson EL 2004).*** It has an impact on health causing pain, fatigue, radiological damage, functional disability and reduced life expectancy ***(Cohen et al., 2007).***

Rheumatoid Arthritis may affect many tissues and organs (lungs, heart, blood vessels, skin and muscles) but principally attacks the joints, producing a non-suppurative proliferative and inflammatory synovitis that often progress to destruct the articular cartilage and lead to joint ankylosis ***(Kumar et al., 2014).***

Extra-articular complications also often occur, leading to a worsening of the prognosis ***(Okuda, 2008).***

The presence of traditional cardiovascular risk factors were also found. The risk for myocardial infarction in female RA patients is twice that of women without RA, and in long-standing disease of at least 10 years, the risk is 3 times higher. Pericaditis is the most common cardiac manifestation in RA ***(Turesson C et al., 2007)***. Although symptomatic pericarditis is relatively uncommon, autopsy studies revealed evidence of pericardial inflammation in 50% of the patients. It usually occurs in RF-positive patients with nodules and analysis of pericardial fluid reveals changes similar to those found in rheumatoid pleural effusions ***(Van Doornum et al., 2002).***

Pathophysiology of RA

Various immune cells and cytokines are involved in RA pathogenesis, and they may also increase the risks of extra-articular complications of RA ***(Feldmann et al., 1996, Zwerina et al., 2005).***

Preclinical RA:

In most patients, the pathogenesis of RA begins years before clinical disease is evident, although acute onset reflecting immediate immune perturbation is also possible. Thus, RA is considered to be a continuum that begins with a high-risk or susceptibility stage that is primarily based on genetic factors, and proceeds through preclinical RA before articular inflammation (early RA) develops ***(Smolen J et al., 2018).***

RA development is determined by a predisposing genotype upon which environmental and genetic factors operate to ultimately result in the inflammatory and destructive synovial response. How the environmental risk factors contribute to disease is incompletely understood. However, it seems that stressors in, for example, cigarette smoke can act on cells in mucosal sites and promote post-translational conversion of the amino acid arginine to citrulline in a range of proteins, including intracellular proteins (such as histones) and matrix proteins (for example, fibronectin, collagen, fibrinogen, enolase and vimentin) via induction of peptidyl arginine deiminases in a process called citrullination (also known as deimination) ***(Makrygiannakis et al., 2008)*** **(Figure 1).**

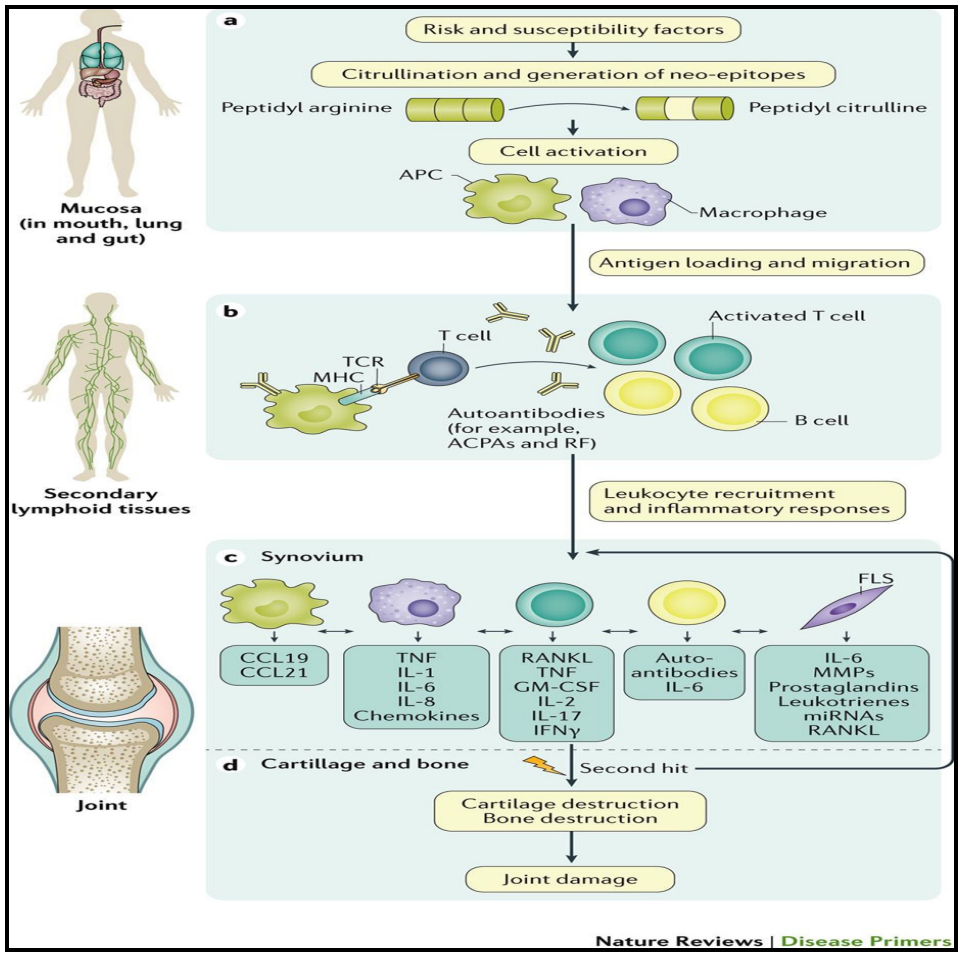

Figure (1): Mechanisms involved in initiation and progression of rheumatoid arthritis ***(Smolen J et al., 2018).***

Citrullination may also be induced by the microbiota: P. gingivalis, which is common in periodontal disease, expresses peptidyl arginine deiminases and can induce citrullination and thereby promote anti-citrullinated protein antibodies (ACPA) generation. In addition, A. actinomycetemcomitans, which produces a toxin that increases calcium influx into neutrophils, can lead to citrullination of peptides and has been recently implicated in RA aetiology ***(Konig et al., 2016).***

Following citrullination or other post-translational modifications (for example, acetylation or carbamylation), the altered peptides bind to MHC protein heterodimers, especially those containing the shared epitope, leading to antigen presentation to T cells, which in turn stimulate B cells to synthesize a range of antibodies that recognize self-proteins, including RF (targeting IgGs) and ACPAs ***(Muller S & Radic M., 2015).***

The presence of circulating ACPAs, other antibodies (such as RF) and circulating pro-inflammatory cytokines and chemokines can be detected up to 10 years before clinical disease onset and can point to immune activation during the preclinical period is associated with a more aggressive disease course and can, therefore, be used not only as a diagnostic marker but also as a prognostic marker ***(Laurent et al., 2015).***

The presence of ACPAs alone is not sufficient to cause synovitis; an additional hit like immune complex formation, complement activation or microvascular insult is likely required to initiate clinical synovitis characterized by increased vascular permeability and influx of inflammatory cells into the synovium ***(Arend and Firestein, 2012).***

Early RA is characterized by synovial inflammation based on mononuclear cell infiltration, dominated by $CD4^+$ T cells and macrophages, together with early stromal cell activation. Within 1 week of the onset of symptoms synovial

biopsy show high expression of matrix-degrading enzymes (such as matrix metalloproteinases (MMPs) in the synovial intimal lining. In addition to ACPAs, other autoantibodies that recognize immunoglobulins like RF, type 2 collagen (particularly in oxidized form), glucose-6-phosphate isomerase, proteoglycans, nuclear antigens and other joint autoantigens expand the pathways whereby autoantibodies likely contribute to pathogenesis ***(Steiner, 2007).***

Two key pathogenetic changes in the synovium are evident in RA. First, the intimal lining greatly expands owing to an increase and activation of both synoviocyte types, which are a prominent source of cytokines and proteases. The macrophage-like synoviocytes produce a variety of pro-inflammatory cytokines, including IL-1, IL-6, tumour necrosis factor (TNF) and others ***(McInnes and Schett, 2011)***.

The second change associated with RA is infiltration of adaptive immune cells into the synovial sublining. About half of the sublining cells are CD4+ memory T cells that can either diffusely infiltrate the tissue or, in 15–20% of patients, form ectopic germinal centres in which mature B cells proliferate, differentiate and produce antibodies. B cells, plasmablasts and plasma cells are also present, many of which produce RF or ACPAs ***(Humby et al., 2009).***

Cytokine and signalling networks.

Cytokine networks integrate pro-inflammatory and tissue-damaging cellular activities in synovitis The role of cytokines in disease pathogenesis was prominently established for TNF α by the advent of TNF-targeting agents on the basis of prior studies elucidating a pro-inflammatory role for TNF in leukocyte activation, MMP production, angiogenesis and promoting pain. Later studies targeting other cytokines, notably IL-6, demonstrated that the hierarchy of cytokines in patients with RA varies widely ***(Smolen J et al., 2018)*** **(Fig. 2).**

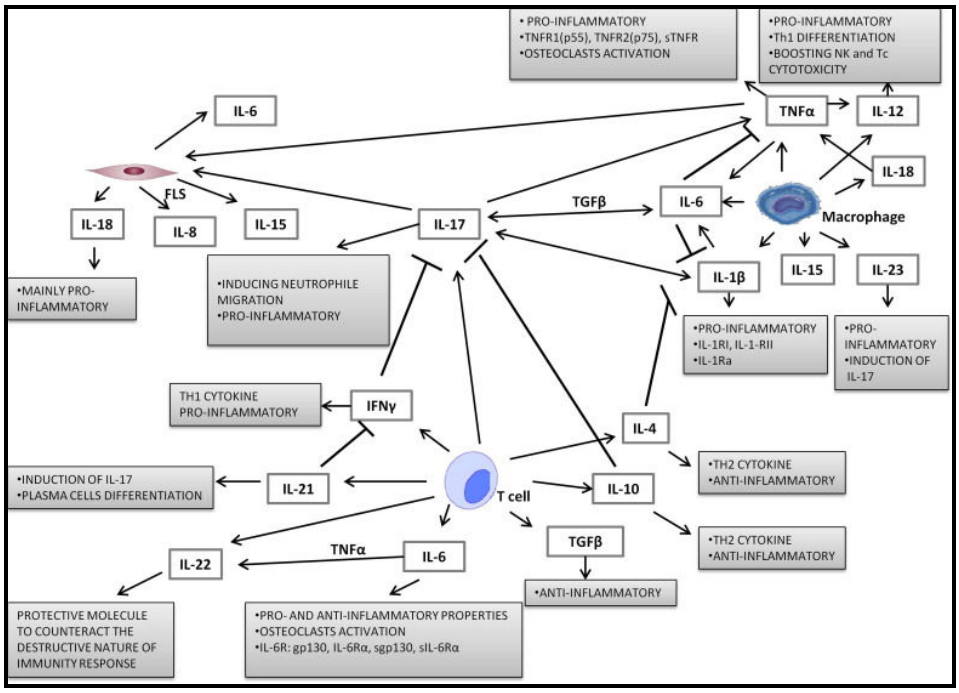

Figure (2): Cytokine network in RA ***(E Brzustewicz and E Bryl, 2015).***

Synovial cells produce cytokines that act in a paracrine or autocrine fashion and can enhance and perpetuate

inflammation in RA. For example, macrophages produce cytokines that activate adjacent fibroblast-like synoviocytes (FLS), T cells and dendritic cells. These cells in turn produce additional cytokines that can activate other cells in the joint environment. ACPA-induced IL-8 release from osteoclasts might play a particularly important part in early disease by driving neutrophil recruitment to the synovial fluid and activating and triggering subsequent responses ***(Krishnamurthy et al., 2016).***

Although a great deal of critical pathological mechanisms still remains vague, currently the Th cells are clearly placed at the center of RA pathogenesis, as they are involved in the initiation and sustainment of the disease. Proportions of Th1/Th17/Treg lymphocyte subsets are altered in peripheral blood of RA patients – the level of disarrangement depends on the stage of the disease ***(Kosmaczewska et al., 2014)***.

In RA, the inflammatory response is mainly mediated by TNFa, whereas cartilage and bone destruction is mostly directed by IL-1 ***(Vervoordeldonk et al., 2002).***

TNFa is the most important, primary cytokine of the cascade regulating production of other pro-inflammatory cytokines in the rheumatoid synovial tissue. TNFa is mostly produced by activated macrophages but it is also expressed by neutrophils, NK-cells, endothelial cells and activated

lymphocytes. The cytokine elicits biological effects by two specific receptors: TNFR1 (p55) – present on all kinds of cells, and TNFR2 (p75) – more restricted, with the highest expression on Treg lymphocytes ***(Monaco et al., 2015).***

TNFa promotes activation of synovial fibroblasts and consequently production of CTGF (connective tissue growth factor), which promotes aberrant activation of osteoclasts and disturbs the homeostasis of the cartilage, ultimately resulting in the destruction of the joint ***(Nozawa et al., 2014).***

Moreover, TNFa inhibits functions of Treg cells and induces effector T cells resistance to Treg-mediated suppression ***(Komatsu et al., 2015).*** TNFa mediates recognizable spectrum of RA elements especially: articular destruction and bone resorption-via activation of osteoclasts and chondrocytes, pannus formation-by inducing endothelial cell activation and amplification of chemokines, induction of pain and fever-by PGE2 synthesis promotion and nociceptor sensitization. Some pro-inflammatory activities are shared between TNFa and IL-1. Both cytokines promote activation of T cells and induce synthesis of other pro-inflammatory proteins ***(Brennan and McInnes, 2008).***

However, IL-1 does not promote production of TNFa. IL-1 is produced by various immune cells; in the inflamed synovium these are mainly monocytes and macrophages. This cytokine is more potent than TNFa in inducing cartilage destruction. There

are two IL-1 receptors: IL-1RI which transduces the signal and IL-1RII – which is a non-transducer, acting as a decoy receptor. The ultimate response to IL-1 is limited by natural IL-1 receptor antagonist (IL1Ra) ***(Chizzolini et al., 2009).*** An imbalance between IL-1/IL-1Ra has been demonstrated in the RA synovium ***(Vervoordeldonk and Tak, 2002)***.

IL-6 is produced mainly by activated macrophages and FLS. In RA the level of IL-6 in the synovial fluid is elevated and correlates with radiological joint destruction. IL-6 demonstrates contradictory activities – cytokine mainly recognized as a pro-inflammatory molecule, under some circumstances can exhibit anti-inflammatory properties ***(Vervoordeldonk and Tak, 2002)***.

Other Pro-inflammatory IL-12, IL-18, IL-15, IL-17

IL-12 is produced by macrophages, dendritic cells (DCs) and granulocytes and is present in RA synovial tissue. This cytokine elevates the level of IFNγ and induces Th1 differentiation. Moreover, IL-12 boosts cytotoxic functions of Tc – (CD8+ T cytotoxic lymphocytes) and NK lymphocytes ***(Krausz et al., 2012).***

IL-18, which is the IL-1 family member, acts synergistically with IL-12. IL-18 is produced primarily by activated macrophages, other sources include DCs, FLS and chondrocytes. Although IL-18 has some anti-inflammatory activities-such as inhibition of COX-2 or prevention of