Anti Carbamylated Protein Antibodies as Adiagnostic Marker in Patients with Rheumatoid Arthritis and its Association with Disease Activity

Thesis

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Magda Medhat Awad El Debsy

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List of Abbreviations

Abb.	Full term
ACPA	*Anti-citrullinated protein antibodies*
ACR	*American College of Rheumatology*
AMPAs	*Anti-modified protein antibodies*
AP-1	*Activator protein1*
apo-AST	*Apo-aspartate aminotransferase*
Ca-FCS	*Carbamylated fetal calf serum*
Ca-Fib	*Carbamylated fibrinogen*
CarbVim	*Carbamylated vimentin*
CBC	*Complete blood count*
CRP	*C-Reactive Protein*
cs-DMARDs	*Conventional synthetic disease-modifying antirheumatic drugs*
DAMP	*Damage-associated molecular pattern*
DAS28	*Modified Disease Activity 28*
DC	*Dendritic cells*
DHODH	*Dihydroorotate dehydrogenase*
DMARD	*Disease modifying anti rheumatic drugs*
ESR	*Erythrocyte Sedimentation Rate*
EULAR	*European league against rheumatism*
FcγR	*Fc-gamma receptor*
FLSs	*Fibroblast-like synoviocytes*
GC	*Glucocorticoids*
GC	*Glucocorticoids*
IL	*Interleukin*
JAK	*Janus–Kinase*
MAPKs	*Mitogen-activated kinases*
M-CSF	*Macrophage colony-stimulating factor*
MHC	*Major histocompatibility complex*

List of Abbreviations *(Cont...)*

Abb.	Full term
MMPs	*Matrix metalloproteinases*
MPO	*Myeloperoxidase*
MTX	*Methotrexate*
NETs	*Neutrophil extracellular traps*
NETs	*Neutrophils extracellular traps*
NFATc1	*Nuclear factor of activated T-cell c1*
NF-kappa B	*Nuclear factor-kappa B*
NK	*Natural killer cell*
NSAID	*Non-steroidal anti-inflammatory drugs*
PAD	*Peptidyl arginine deiminase*
PTM	*Post-translational modification*
PTPN22	*Protein tyrosine phosphatase, nonreceptor type 22*
RA	*Rheumatoid arthritis*
RANKL	*Receptor activator of nuclear factor kappa-B ligand*
RF	*Rheumatoid factor*
STAT	*Signal transducer and activator of transcription*
TNF-α	*Tumor necrosis factor alpha*
TRAF	*TNF receptor-associated factor*
Tregs	*T regulatory cells*

heterogeneous and sometimes difficult to diagnose due to the lack of specific autoantibodies ***(Nordberg et al., 2017).***

Several autoantibodies against proteins with other post-translational modifications have recently been reported, and are called anti-modified protein antibodies (AMPAs), Alongside ACPA, autoantibodies directed toward carbamylated antigens are the most studied AMPAs ***(Trouw et al., 2017).***

Carbamylation is a non-enzymatic and irreversible post-translational modification (PTM) that mainly results from interaction between cynate and amino groups of proteins mainly lysine residues within polypeptide chains, producing ε-carbamyl-lysine (i.e., homocitrulline). Cynate is mainly produced from the spontaneous decomposition of urea and may also be generated from thiocyanate metabolism. Neutrophil-derived myeloperoxidase (MPO) catalyzes the oxidation of thiocyanate in the presence of hydrogen peroxide. This occurs at sites of inflammation and atherosclerotic plaque ***(Verbrugge et al., 2015).***

It has been shown that homocitrulline- containing proteins are present in the RA joint and that they may affect T-cell triggering and possibly autoantibody formation. Anti-CarP antibodies have been shown to be associated with the development of RA in patients with arthralgia and more severe radiographical progression in the total and ACPA-negative RA population ***(Ajeganova et al., 2016).***

Anti-Carp antibodies have been attracting increasing attention as a new diagnostic biomarker of RA because they are detected in 8–16% of ACPA-negative RA patients ***(Jiang et al., 2014).***

High specificity was achieved using a healthy population as the control, efficacy needs to be assessed in these patients and the prevalence of anti- Carp antibodies needs to be clarified. The diagnostic efficacy of the combined measurement of anti-Carp Ab, ACPA and RF in patients with arthritis in at least one joint must be assessed ***(Shi et al., 2015).***

Aim of the Work

This study is designed to evaluate levels of anti-carbamylated protien (anti-Carp) antibodies in rheumatoid arthritis (RA) patients in order to detect its role as a diagnostic marker and its possible association with disease activity and severity.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, progressive, systemic inflammatory disease of unknown etiology with a worldwide prevalence estimated at 0.3% to 1.2% ***(Halabi et al., 2015)***.

RA affects around 1% of the population with a female-to-male ratio of approximately 2.5–1. The incidence of RA increases with age and it most commonly affects women aged 40–60 years ***(Smolen et al., 2016).***

RA primarily affects the lining of the synovial joints and can cause progressive disability, and socioeconomic burdens. The clinical manifestations of symmetrical joint involvement include arthralgia, swelling, redness, and even limiting the range of motion ***(Cho et al., 2017).***

Autoantibodies are important biomarkers of RA. Anti-citrullinated protein/ peptide antibodies (ACPA) are most widely used in daily clinical practice, along with rheumatoid factor (RF). They are the antibodies of proteins with citrullination, which is one of the post-translational modifications of proteins in which arginine is converted to citrulline by the catalysis of peptidyl arginine deiminase (PAD) ***(Trouw et al., 2017).***

Approximately one-third of patients with established RA do not express RF or ACPAs. Seronegative RA is more

Chapter 1

RHEUMATOID ARTHRITIS

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 ***(Yoshida et al., 2015).***

RA primarily affects the lining of the synovial joints causing progressive disability, premature death, and socioeconomic burdens. The clinical manifestations of symmetrical joint involvement include arthralgia, swelling, redness with limitation for the range of motion. Early diagnosis is considered as the key improvement index for the most desirable outcomes (i.e., reduced joint destruction, less radiologic progression, no functional disability, and disease modifying anti rheumatic drugs (DMARD)-free remission) ***(Cho et al., 2017).***

RA is also associated with a reduced life expectancy. The clinical presentation of rheumatoid arthritis appears between the age of 20 and 40 years old, more commonly in women than in men with a 2–3:1 ratio. the most common age group that suffers from RA are those in the 30–50, at least 50% of these patients are unable to hold down a full-time job, presumably due to the disability that ensues ***(Garneau et al., 2018).***

Genetic and environmental factors:

Genetic predisposition is linked to more than 100 HLA and non HLA susceptibility loci, and overall 2/3 of the risk for the disease development is genetically determined ***(Yarwood et al., 2016).***

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. The most important genetic risk allele for RA resides in the class II major histocompatibility (MHC) locus, accounting for about 40% of the genetic influence ***(Angelotti et al., 2017).***

The human leucocyte antigen (HLA)-DRB1 locus, one of the oldest to have been identified, is strongly associated with RA risk, and in particular HLA-DRB1*01, *04 and *10 alleles are correlated with a high risk of developing the disease in ACPA-positive patients ***(Angelotti et al., 2017).***

These HLA-DRB1 alleles share in the peptide-binding groove an identical amino acid sequence, also known as shared epitope (SE). Since the correlation with ACPA-positive patients is high, it has been suggested that the peptides presented by the SE alleles may be citrullinated ***(Derksen et al., 2017).***

Several non HLA loci have been associated with RA susceptibility, including protein tyrosine phosphatase, nonreceptor type 22 (PTPN22), CTLA4, and loci for cytokines,