Anti-Carbamylated Protein (Anti-CarP) and Juvenile Idiopathic Arthritis

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Abstract

Background: Juvenile idiopathic arthritis is a heterogeneous group of diseases characterised by arthritis of unknown origin with onset before age of 16 years. Pivotal studies in the past 5 years have led to substantial progress in various areas, ranging from disease classification to new treatments. Gene expression profiling studies have identified different immune mechanisms in distinct subtypes of the disease, and can help to redefine disease classification criteria. Moreover, immunological studies have shown that systemic juvenile idiopathic arthritis is an acquired autoinflammatory disease, and have led to successful studies of both interleukin-1 and interleukin-6 blockade. In other forms of the disease, synovial inflammation is the consequence of a disturbed balance between proinflammatory effector cells (such as T-helper-17 cells), and anti-inflammatory regulatory cells (such as FOXP3-positive regulatory T cells). Moreover, specific soluble biomarkers (S100 proteins) can guide individual treatment. Altogether these new developments in genetics, immunology, and imaging are instrumental to better define, classify, and treat patients with juvenile idiopathic arthritis. Anti-carbamylated protein (CarP) antibodies have been studied as novel markers to aid in the diagnosis and prognosis of rheumatoid arthritis.

Keywords: Anti-Carbamylated Protein, Anti-CarP, Juvenile Idiopathic Arthritis

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Introduction

Carbamylation is a post-translational modification in which cyanate binds to primary amino or thiol groups. The binding of cyanate to either amino groups or thiol groups is specified as N-carbamylation or S-carbamylation. Beside the N-terminus of all proteins, the amino acids lysine, arginine and cysteine contain side chains that can react with cyanate . However, since carbamylation on side chains of cysteine and arginine, the N-terminus of proteins and free amino

acids is rarely reported we therefore in this review refer to carbamylation as cyanate binding on peptidyl-lysine without further specification. Urea is a source of cyanate in all individuals and is present in body fluids in equilibrium with ammonium cyanate . The equilibrium ratio between cyanate and urea has been suggested to be around 1 to 500.000 . Despite the low concentration of cyanate, trace amount of carbamylation can be detected in healthy individuals . As expected, elevated carbamylation was extensively reported among patients with renal dysfunction and elevated blood urea nitrogen (BUN) levels .(1)

In addition to renal insufficiency, inflammation is another factor which can stimulate the degree of carbamylation. Wang and Holzer et al. demonstrated that inflammation can enhance carbamylation via a mechanism which depends on myeloperoxidase (MPO) . MPO is mainly stored in granules of neutrophils and it can generate cyanate using hydrogen peroxide and thiocyanate as substrates. Thiocyanate, derived from e.g., food or smoke exposure , can be oxidized by hydrogen peroxide with the help of MPO, resulting in the formation of hypothiocyanate which decomposes to cyanate and other ions . In addition MPO can also catalyze the reactions between hydrogen peroxide and chloride that via a series of reactions leads to increased levels of cyanate . The marked increased levels of MPO in inflammation therefore stimulates the formation of cyanate. These findings indicate that MPO released from neutrophils can further increase the level of carbamylation during inflammation.(2)

Cyanate (~ 200 parts-per-trillion volume) can also be directly inhaled from urban air. A five times higher concentration, one parts-per-billion volume of cyanate in inhaled breath, can already generate an aqueous solution of 100 μM . This concentration is equal to or higher than the effective dose of cyanate which is able to cause notable effects in several in vitro studies . Cyanate in air can be derived from various sources such as biomass burning, coal burning, biofuel usage, cooking, tobacco usage and wild fire. Even in the absence of inflammation the direct exposure to air borne cyanate can be sufficient to generate low levels of carbamylation.(2)

Besides abovementioned mechanisms, carbamylation on free amino acids can also be catalyzed by the enzyme lysine carbamyltransferase. This enzyme converts the free amino acid lysine and carbamyl phosphate to homocitrulline . Whether this enzyme is able to catalyze the reaction between carbamyl phosphate and peptidyl-lysine has, to the best of our knowledge, not been studied. Carbamyl phosphate injected in rats caused extensive carbamylation . Thus leaking of carbamyl phosphate or lysine carbamyltransferase due to apoptosis or necrosis of cells might potentially be a currently unexplored source of introducing carbamylation. At this stage, protein carbamylation mediated by enzymes seems unlikely but clearly requires further investigation.(3)

Anti-Carbamylated Protein (Anti-CarP) Antibodies as a Predicting Marker of Disease Activity and Joint Damage in Juvenile Idiopathic Arthritis Patients

As a non-enzymatic post-translational modification, carbamylation participated in the pathogenesis of rheumatoid arthritis (RA). In 2011, Shi J. et al have found that anti-carbamylated proteins antibodies (anti-CarP) were detected in the serum of patients with RA in addition to anti-CCP . The formation of immune complexes in RA drives carbamylation, thereby disrupting tolerance and inducing the production of anti-CarP antibodies . They also found that anti-CarP antibodies were detected in blood donors before the appearance of clinical symptoms of RA . Actually, Anti-CarP antibodies could exist several years before the start of RA symptoms .

Additionally, it has been found that in patients not diagnosed with rheumatoid arthritis, anti-CarP antibodies were mainly present in patients with undifferentiated arthritis and primary Sjögren's syndrome . (4)

A group of researchers from Spain believed that anti-CarP antibodies were different from anti-CCP risk factors, but were related to radiological damage of RA, so anti-CarP antibodies could be used as independent RA autoantibodies . Although anti-CarP antibodies are increased in RA patients, increased carbamylation is not sufficient to result in immune responses against carbamylated proteins. Therefore, after the process of carbamylation, genetic or environmental factors may still be required to induce the production of anti-CarP antibodies. Similarly, several studies have confirmed that anti-CarP antibodies are widely present in RA patients from US military personnel, indigenous North Americans, Japanese, French, Swedish and Dutch descent as well as more common in first-degree relatives of RA patients compared to normal controls .(5)

14-3-3η proteins are a group of highly conserved protein families, composed of seven isoforms (β, γ, ε, η, σ, θ, and ζ) . Serum 14-3-3η increased in arthritic patients and was significantly associated with two biomarkers of rheumatoid arthritis. As a novel RA biomarker with a higher specificity, serum 14-3-3η was significantly elevated in patients with aggravated RA disease progression as well as involved in the pathogenesis of RA . Serum 14-3-3η levels are associated to some extent with RA activity and inflammatory responses . 14-3-3η-positive can also be detected in arthralgia patients with anti-CCP and/or RF-positive before the onset of RA and is related to the progression of arthritis . In RA patients treated with Tofacitinib (TOF), 14-3-3η reduction is associated with remission of disease progression and can therefore be used as a biomarker for monitoring the effect of TOF .(6)

The anti-CarP antibodies target proteins that have experienced a post-translational modification, the carbamylation of lysine residues into homocitrullin. However, we do not know yet the proteins that are targeted in patients. In their place, the antibodies are assayed against in vitro carbamylated fetal calf serum (FCS). These assays have shown that the anti-CarP antibodies are specific of RA, and precede clinical onset of the disease by many years (similar to the anti-CCP antibodies). In addition, the anri-CarP antibodies are associated with bone erosions, disease activity, disability and mortality in RA with independence of the anti-CCP antibodies. All these characteristics indicate that the anti-CarP antibodies could add to the classification of RA patients. In previous studies, one with arthralgia patients20, the other with early arthritis (EA) patients, the anti-CarP antibodies were associated with the RA outcome with independence of other autoantibodies. In addition, the anti-CarP antibodies seemed to contribute to classification in, at least, some subsets of patients. However, their value is still unclear because the extent of this contribution was modest and not quantified.(4)

Our results indicate anti-CarP-FCS and anti-CarP-Fib are present in prediagnosis serum of RA cases. Both anti-CarP-FCS and -Fib exhibited lower sensitivity (<30%) than anti-CCP2 or RF, although the specificity for anti-CarP was comparatively high (>95%). Anti-CarP-FCS exhibited a greater sensitivity and the same specificity as anti-CarP-Fib. Furthermore, anti-CarP-FCS was significantly associated with future RA, while anti-CarP-Fib only trended towards a significant association, which influenced our decision to consider calculations of diagnostic accuracy for future RA using only anti-CarP-FCS in antibody combinations. While we did not observe significant

differences in AUCs with the addition of anti-CarP-FCS to combinations of anti-CCP and/or RFs, we did observe a modestly increased sensitivity and decreased specificity for future RA. This could suggest utility of anti-CarP in assays that test for multiple antibodies at once, or for assessment of risk of future erosive disease in individuals who exhibit anti-CarP.(7)

Several features of the antibodies tested herein are of interest in the pathophysiology of RA development. There was a non-significant trend for anti-CCP2 to appear prior to anti-CarP-FCS and Fib, and in some cases both anti-CCP2 and anti-CarP appeared prior to RFs. Given the relatively close temporal relationship between initial positivity of the anti-CCP2 and anti-CarP, it is possible the immune processes driving the break in tolerance to these structurally distinct autoantigens are similar in time and mechanism, or could suggest a degree of cross-reactivity between ACPA and anti-CarP in some of the patients at this early time point in the evolution of disease. The higher sensitivity for disease of anti-CCP could represent a dominant autoimmune response to citrullinated antigens; alternatively, differences in the assay sensitivity between a commercially developed, optimized and validated assay compared to a preclinical research-based method may underlie this difference.(8)

These studies above have focused on the diagnostic value of 14-3-3η or Anti-Carp for RA. However, in order to better assist diagnosis and treatment, the diagnostic value of 14-3-3ηand Anri-Carp combined with other biomarkers for RA needs to be further investigated. In our present study, serum levels of anti-CCP, RF, 14-3-3η and anti-CarP antibodies were detected in RA patients, patients with autoimmune diseases except RA, and healthy subjects recruited from Han population of Northern China. We have taken clinical diagnosis as the standard to determine the application value of 14-3-3η protein, anti-CarP antibodies, as well as their potential role to diagnose RA together with RF or anti-CCP.(9)

In 2011, antibodies directed against carbamylated protein antigen (anti-CarP antibodies) were identified in RA patients, and subsequent studies have established the predictive and prognostic value of this antibody system . Unlike citrullination, which is an enzyme-mediated modification of arginine to citrulline, carbamylation occurs through the chemical modification of lysine residues with cyanide to form homo-citrulline. Homocitrulline is very similar to citrulline, with the only difference being the addition of one CH2 to its side chain (Figure 1).(10)

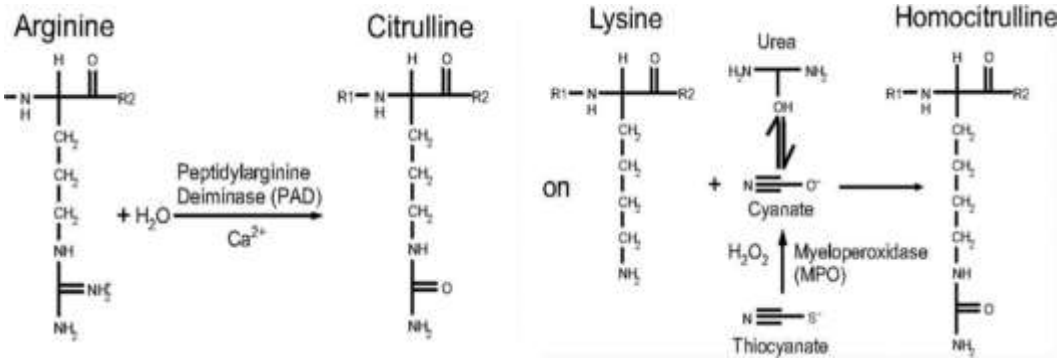

Figure 1. Citrullination (a) and carbamylation (b) occurring on different amino acids via different mechanisms but yielding similar end-products (4).

In addition, evidence is mounting in support of the value of combined testing for anti-CCP3.1, 14-3-3 eta, RF, and anti-CarP antibodies, and especially for triple positivity (anti-CCP, RF and anti-CarP), conferring a very high likelihood ratio for RA . Most previous reports describing anti-CarP assay performance characteristics have been derived from European populations using a two-step research assay in which specimens need to be tested on one microwell plate coated with carbamylated fetal calf serum (FCS) and one with uncarbamylated FCS . The final value is obtained by subtracting the value obtained on the uncarbamylated FSC from that obtained on the carbamylated plate .(10)

Anti-mutated citrullinated vimentin (anti-MCV), an antibody in the ACPA family, has a similar specificity for RA as anti-CCP2. However, systematic review and meta-analysis of the literature did not reveal superior diagnostic accuracy to anti-CCP2, ultimately limiting the adaptation of anti-MCV testing in routine clinical practice. No study has specifically addressed whether adding anti-MCV testing to RF and anti-CCP2 testing would improve overall diagnostic accuracy for RA.(11)

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