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RESEARCH ARTICLE

"APPROACH TO ANTI-CCP AS A NEW SEROLOGICAL MARKER IN THE EARLY DETECTION OF RHEUMATOID ARTHRITIS"

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Abstract

Rheumatoid arthritis (RA) a severe, progressive, systemic inflammatory autoimmune disease of unknown etiology with commonly occurring severe joint damage and overall debility. Furthermore treatment of RA is limited till date. Hence the necessities of early diagnosis and treatment have been emphasized to search better molecular marker, so that effective treatment can be initiated before irreversible erosive damage occurs. Anti-cyclic citrullinated peptide (anti-CCP) antibody in comparison to RF is particularly useful in the early diagnosis of rheumatoid arthritis, with high specificity and sensitivity. Moreover significantly elevated levels of Anti-CCP antibodies have not been found in other diseases. Thus this review focuses on the diagnostic and prognostic potential of Anti-CCP with relevance to possible etiopathogenesis in RA.

INTRODUCTION

Rheumatoid arthritis (RA), most common systemic autoimmune disorder characterized by chronic inflammation of multiple joints. It mainly occurs due to synovial cell proliferation with subsequent T-lymphocyte accumulation leading to the destruction of both cartilage and bone (Sharma PK, et al. 2004). In fact RA is a complex multifactorial disease having both environmental and genetic factors, with the later being a substantial contributory factor in its Pathogenesis (John, S. et al. 2001). It affects approximately 0.5–1.0% of the world population (Werner MH, 2002).

The American Rheumatism Association had given seven criteria for the classification of RA: 1) morning stiffness, 2) arthritis of 3 or more joint areas, 3) arthritis of hand joints, 4) symmetric arthritis, 5) rheumatoid nodules, 6) serum rheumatoid factor (RF), and 7) radiographic changes. A patient should have four of the seven criteria to be diagnosed with RA and the first four criteria should be present for at least six weeks (Arnett, FC. et al. 1988).

Recent studies suggest that joint injury in RA patient progress within 2 yrs from onset, and aggressive treatments from the early stage can prevent the progression of disease. Hence, the need of early diagnosis and treatment has been emphasized. However, RA patients do not always show typical sign and symptoms at their early stage, and are often difficult to be diagnosed since they may not fulfill the classification criteria for RA.

During recent decades the RF autoantibody system, directed against the Fc part of immunoglobulin (Ig) G molecules, played a central role in the diagnosis and prognosis of RA because RF can be detected in the majority of RA patients (Mageed, RA. 1992). However the sensitivity of RF is 60-80% in RA, with relatively low specificity as RF is also detected widely and frequently in several other conditions including various connective tissue diseases, chronic liver diseases and infectious diseases and even in a few healthy people (Dorner, T. 2004).

Therefore, despite of the adoption of RF into the criteria for classification of RA, its diagnostic value is unsatisfactory especially in early disease. Anticyclic- citrullinated-peptide (anti-CCP) antibodies work effectively in earlier and more accurate diagnosis and prognosis of disease, and have been implicated in RA pathogenesis. Thus anti-CCP provides better opportunity for cure before irreversible damage occurs, and it may be even more useful, when used in combination with other diagnostic features. Thus this review focuses mainly on the prognostic and diagnostic potential of Anti-CCP along with its etiopathogenic significance in RA.

ARRIVAL OF Anti-CCP

The discovery of first citrulline-binding autoantibodies in RA sera were done by Nienhuis et al. in 1964, named as antiperinuclear factor (APF), by indirect immunofluorescence (Nienhuis et al. 1964). APF was found in 48% of patients with RA, and only 1% of healthy control. But the specificity of APF for citrulline was not appreciated for several years. In 1979, Young et al. reported that RA sera contained antibodies react with keratinized layer of epithelium and called them as antikeratin antibodies (AKA), found only in RA patients (Young, BJ. et al. 1979).

In 1993, Simon et al recognized a 40kDa protein in 75% of RA patient sera from human skin tissue (Simon, M et al. 1993). This was later on identified as target antigen of AKA by absorption study, and filaggrin by peptide mapping, which was involved in the aggregation of intracellular cytokeratin filaments. They further recognized that AKA and APF had almost the same specificity, because the target molecule of APF was pro-filaggrin, the precursor molecule of filaggrin (Sebbag, M. et al. 1995).

Further subsequent studies demonstrated that anti-keratin antibodies and antiperinuclear factor recognized a similar epitope, and were perhaps the same antibody (Simon, M. et al. 1993) (Sebbag, M. et al. 1995). It was also discovered that conversion of arginine to citrulline on peptides was essential for anti-keratin antibody and perinuclear factor binding (Schellekens, GA. et al, 1998). Therefore, antiperinuclear factor and anti-keratin antibodies can be broadly categorized as anti-citrullinated-peptide antibodies. The citrulline moiety, which is the essential part of antigenic determinant in these antigens, is post-translationally generated by peptidylarginine deiminases (PAD; EC 3.5.3.15). Multiple evidence for abnormal citrullination of various peptides are seen in various human diseases, including RA, psoriasis, and multiple sclerosis (Vorsenear, ER. et al. 2003).

ASSAYS FOR THE DETECTION OF Anti-CCP

Anti-cyclic citrullinated peptide (CCP) antibodies against synthetic citrullinated peptides are specific markers of rheumatoid arthritis. Standardization of assays for the detection of anti-citrullinated peptide antibodies against linear stretches of citrullinated peptide proved difficult, but against cyclic citrullinated peptide (CCP) resulted in greater reproducibility (Schellekens, GA. et al, 2000). This test is commercially available, and is known as the anti-CCP1 assay. Further a second-generation assay was devised by screening a large library of citrulline-containing peptides with RA sera to identify the epitopes with the highest yield. This assay is now known as the anti-CCP2 assay, currently the most widely used anti-citrullinated peptide assay and has slightly better results than anti-CCP1 (van Gaalen FA, et al. 2005).

In 2005, a third generation of anti-cyclic citrullinated peptide (CCP3) was made available for the laboratory diagnosis of RA. These assays have been reported to recognize additional citrulline epitopes that are not identifiable with the second-generation CCP assays. The CCP3 assays provide results up to 5% increased sensitivity compared to the CCP2 assays (Vieira LMEA, et al. 2007).

PROGNOSTIC VALUE OF Anti-CCP

Several studies suggest the role of anti-CCP as highly predictive marker with good sensitivity and specificity, helpful in reaching early diagnosis due to the genetic predisposition of RA in individuals with family history. According to the study performed by Nielen, *et al* on 79 RA patients who donated blood prior to symptom onset observed that 40.5% of patients became anti-CCP-positive prior to symptom onset with the median time of 4.8 yrs from first anti-CCP positivity to symptom onset (range from 0.1 to 13.8 years). They analyzed that the 5 year positive predictive value (PPV) for anti-CCP in the RA blood donor patient population was 96.6%. They also calculated the risk of developing RA within 5 years for the general population in comparison to those at "high risk," i.e those having 2 or more first-degree relatives with RA and found that the 5 year PPV for anti-CCP in the "high risk" population was 69.4%, and in the general population it was 5.3% (Nielen, MW. et al. 2004).

In a study using banked sera from the Nurses' Health Study, anti-CCP antibodies were detected up to 12 years prior to diagnosis, and were found associated for developing RA after adjusting for hormonal status and other confounding variables. This suggest that anti-CCP antibodies can appear years in advance of actual disease, and may

simultaneously allow for identification of individuals likely to develop disease (Mandl, LA. et al, 2005). Schellekens et al described that anti-CCP antibodies were detected in 68% of RA patients with decreased sensitivity up to 48% in early RA cases, and high specificity up to 96%. In particular, the combination of anti-CCP and IgM-RF at the first visit predicted erosive change at 2 yrs follow up in RA patients with 91% of positive predictive value (Schellekens, GA. et al, 2000).

Follow-up study conducted by van Gaalen and colleagues using serological markers predict the progression of undifferentiated arthritis (UA) to RA within the next few years in 318 undifferentiated arthritis (UA) patients attending an early arthritis clinic. They clearly showed that after 1 yr of follow-up, 75% of the UA patients who were anti-CCP2- positive at baseline had already progressed to RA and later on this percentage increased to 93% after 3 yrs. On the contrary in UA group who were anti-CCP2 negative at baseline, only 25% were classified RA after 3 yr (van Gaalen FA, et al. 2004). Similar results were reported by Vittecoq and colleagues in a cohort of 314 early arthritis patients, 90% of the anti-CCP2-positive patients were classified as RA patients at the 1-yr follow-up (Vittecoq, O. et al. 2004). So, collectively these predictive studies demonstrate the importance of Anti-CCP as an intermediate step in the development of RA due to the genetic predisposition through environmental risk and autoimmune response into a full blown disease.

DIAGNOSTIC VALUE OF Anti-CCP

Several cohort studies performed to establish the role of anti-CCP2 as a diagnostic marker showed its additive RF-like sensitivity with almost absolute specificity for RA. According to these multicentre studies, just like RF, anti-CCP2 antibodies are present in about 80% of established RA patients and was only positive in maximally 1 and 5% of healthy control and non-RA disease control respectively (Vasishta, A. 2002).

Study performed by Suzuki and colleagues showed higher discriminative ability of CCP2 in comparison to RF test in a cohort of 549 RA patients (Suzuki, K. et al. 2003). Another study conducted by Dubucquoi et, al reported sensitivity of 65% (at 96% specificity) in a group of RA patients with recent onset of disease and sensitivity of 77% in their established RA patient group (Dubucquoi, S. et al. 2004). Likewise in a recent study performed by Rycke and colleagues to detect the diagnostic performance of CCP2 test over RF resulted in a sensitivity of 12.8% for RF in comparison to that of 73.7% for anti-CCP2 in RA (Setting the specificity values at 98.5%) (de Rycke L. et al. 2004).

Together all these studies show that anti-CCP2 test not only equals the RF level for sensitivity, but combines this with far better specificity. The fact that around 40% of RF seronegative patients appear to be anti-CCP-positive substantiates the additional diagnostic potential of CCP (Kroot, EJ. et al. 2000). The anti-CCP test also enables effectively in differential diagnosis of RA patients from other arthritic diseases where the RF is not always discriminative. Mediwake and colleagues (Mediwake, R. et al. 2001) showed that anti-CCP (in this case CCP1) can be used to distinguish RA patients from SLE patients presented with erosive polyarthritis, which is often accompanied by RF seropositivity. Another disease that can readily be misdiagnosed because it often reveals RA-like arthropathies is chronic hepatitis C virus (HCV) infection, which is often accompanied by a positive RF. Wener et al. reported a good discriminative ability of anti-CCP2 over RF in a group of randomly selected HCV patients (44% RF+, none CCP2+) (Werner, MH. et al. 2004). Confirmations of these studies were done by Bombardieri and colleagues by assessing Anti-CCP2 level in sera from 39 patients with chronic hepatitis C virus infection. Out of 39, 8 were having articular involvement thought to be related to hepatitis C and 10 patients having RA with chronic hepatitis C. Study reported positive test in few of the established RA patients with chronic hepatitis C but none of them were found positive in only hepatitis C patients, regardless of articular involvement. Thus, anti-CCP antibodies may be useful in discriminating hepatitis-C-related arthropathy from RA (Bombardieri, M. et al. 2004). Collectively, these studies clearly outline the diagnostic strength of the anti-CCP test for RA over other diseases

ASSOCIATION OF Anti-CCP WITH INCREASING SEVERITY OF DISEASE

Several follow up studies indicate that Anti-CCP might be a predictive marker for the progression of joint destruction. Kroot et al reported that anti-CCP was positive in 70% of 273 early RA patients who were having disease symptoms for less than 1 yr. Later on 6 yrs follow up study suggested that they developed significantly more severe radiological damage that were significantly predicted by IgM-RF, radiological score at entry and anti-CCP status in multiple regression analysis (Kroot, EJ. et al. 2000). In a similar study, anti-CCP status was correlated with disease activity parameters in 379 early RA patients. Statistically significant correlations were seen between anti-CCP positivity and higher CRP, ESR, and disease activity measurements and concluded that anti-CCP as well as the

baseline Larsen score and ESR was an independent predictor of radiological damage and progression in multiple regression analysis (Forslind, K. et al. 2004).

In another study, 104 early RA patients had anti-CCP antibodies and hand radiographs done at inception. Anti-CCP antibodies were positive in 36/67 (54%) of those who had at least one erosion at inception, compared with 8/37 (22%) with non-erosive disease. Rheumatoid factor was positive in 39/67 (58%) with erosive disease, vs. 11/37 (30%) with non-erosive disease. Among seropositive patients, 22% had only anti-CCP antibodies. Thus, anti-CCP antibodies may be useful in identifying a group of RA patients who are more likely to develop damage, and who may not be identified by rheumatoid factor testing alone (Vencovsky, J. et al. 2003). Thus, as reviewed here, all the studies pertaining to anti-CCP indicates a positive correlation with radiological progression suggesting an increasing significance of Anti-CCP with the increased severity of the disease.

POSSIBLE PATHOGENESIS OF RA

Citruullinated proteins are observed in the synovial tissue but not in normal joints, mainly in the linning and sublinning layers intracellularly or found in interstitial amorphous deposits of RA synovium (Baeten, D. et al. 2001). Conversion of peptidylarginine to peptidylcitrulline is catalyzed by PAD enzymes that are found to be expressed in five different tissue specific isoforms (Vossenear, ER. et al. 2003). PAD enzymes are found in the inflamed synovium and require relatively high Ca2+ concentrations. Moreover, their activity is regulated at both transcriptional and translational levels (Vossenaar, ER. et al. 2004). Interestingly, citrullination occurs primarily in inflammed cells dying due to apoptosis or necrosis (Chapuy-Regand S, et al. 2005).

According to a recently, published report significant correlation was observed between the gene polymorphism of the citrullinating enzyme, PADI and RA susceptibility (Suzuki, A. et al. 2003). In this study PADI type 4 gene was identified as the locus for RA susceptibility gene and one of the haplotypes of PADI4 was found more frequently in RA patients (32%) than in the normal controls (25%). The PADI4 was mainly expressed in bonemarrow cells and peripheral leukocytes and monocytes as well as RA synovium. It was also demonstrated that m-RNA expressed from the RA suusceptible form of PADI4 had a longer half life than m-RNA from the non-susceptible PADI4, and RA patients who had the homoygous RA-susceptible haplotyoe developed more frequent anti-filaggrin antibodies in comparison to heterozygous form. These data suggest a possibility that proteins might be easily citrullinated in RA patients. On the contrary no correlation was observed between RA patients in UK and the PADI4 polymorphism in a study performed by Barton et al (Barton, A. et al. 2004).

According to Hill and colleagues certain HLADR alleles particularly the HLADRB1*0401 and *0404 contain the so-called shared epitope (SE) motif. Conversion of arginine to citrulline increases the affinity of a peptide for binding to HLA-DRB1 and can lead to activation of CD4+ T cells in DR4-transgenic mice (Hill, JA. et al. 2003). Thus, these results indicate that the production of anti-citrullinated protein antibodies is dependent on the presence of certain susceptibility genes for RA and also these antibodies stimulate the ongoing inflammation and severity of the disease.

CONCLUSION

Till to date, available medication and therapies for RA are mainly anti-inflammatory that are unable to cure this disease permanently. To achieve the ultimate goal of curative treatment, early diagnosis of the disease is must before erosive joint damage occurs. Anti-CCP antibodies are especially noteworthy because of their high sensitivity and high specificity. These antibodies may serve as a powerful serological marker for early diagnosis and prognostic prediction of RA. Anti-citrullinated protein antibodies are locally produced in RA joints which suggest a possibility that local citrullination of intra-articular proteins might be the initial event leading to autoantibody production in RA. Moreover certain genetic factors such as HLA and various isoforms of PAD might be associated with the induction of auto-immunity against citrullinated proteins. Further research however will be necessary to elucidate the role of protein citrullination in etiopathogenesis of RA to discover new screening technologies that will further increase the diagnostic potential of autoantibodies.

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