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Title: 'A New Window of Opportunity in Rheumatoid Arthritis: Targeting At Risk Individuals'

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Abstract

Purpose of review – Progress in our understanding of the preclinical events in rheumatoid arthritis (RA) has provided important insights into disease pathogenesis. Studying prospective cohorts of individuals at risk for RA development offers the opportunity to accurately characterise the sequence of events in preclinical disease as well as quantify the risk of different preclinical phenotypes. These data may provide the basis for preventive strategies in RA.

Recent findings – RA-related systemic autoimmunity and inflammation occur long before clinical arthritis. There is growing evidence that initiating events may occur at mucosal surfaces including the periodontium, lung and gut and may be influenced by the local microbiome. For potential preventive strategies to be feasible it is important that individuals at high risk for RA development can be readily identified from the general population. To this end, studying multiple biomarkers in prospective cohorts of at risk individuals enables risk prediction in different at risk phenotypes. RA prevention using immunomodulation is currently being investigated in individuals at high risk of RA development.

Summary – The prospective study of at risk individuals can provide invaluable aetiological insights as well as facilitating accurate risk prediction data. In this way, high risk individuals may be identified for preventive interventions.

Key words: rheumatoid arthritis; preclinical; autoimmunity; prevention

Introduction

The early treatment of rheumatoid arthritis (RA) is associated with better outcomes. This observation supports the concept of a 'window of opportunity' for therapy in the initial phases after clinical arthritis develops [1]. In recent years, there has been significant progress in our understanding of the preclinical phase of RA. Much of this information has come from the retrospective analysis of stored serum samples in patients that have later gone on to develop RA. However, prospective cohorts of at risk individuals are now providing invaluable insights into the sequence of events that underpin the transition from preclinical disease to clinical arthritis. These insights are now paving the way forward towards preventive strategies in RA.

In this review we first discuss the concept of preclinical RA. Strategies for identifying individuals at risk of developing RA are then outlined and recent biomarker data from prospective cohorts of at risk individuals is reviewed. Potential treatment strategies for those individuals at high risk of RA development will also be discussed.

Preclinical RA: The development of RA-related autoimmunity and inflammation

RA-related autoimmunity occurs well before individuals present with clinical disease. Anti-Citrullinated Protein Antibodies (ACPA) and Rheumatoid Factor (RF) can be detected in the serum

retrospectively in samples taken many years before individuals have developed RA [2,3]. Similarly, prospective cohorts have shown that ACPA positive individuals are at risk of developing RA and the risk is greater in those with higher antibody titres [4,5]. In the preclinical phase, the number of autoantibodies to different citrullinated epitopes increases in the run up to disease onset [6]. This expansion in ACPA fine specificity is immunologically explained by epitope spreading and the breadth of an individual's ACPA repertoire appears prognostic for future RA development [7,8]. There is also evidence for RA-related preclinical inflammation with several of the cytokines and chemokines involved in RA pathogenesis becoming elevated prior to arthritis development [6].

It is likely that certain genetic and environmental risk factors may predispose an individual to develop RA-related autoimmunity and inflammation. Carriage of the HLA-DRB1 shared epitope (SE) alleles and smoking are the most robust genetic and environmental risk factors respectively. Indeed the two are thought to interact synergistically in ACPA-positive disease [9]. A detailed discussion of these and various other risk factors is beyond the scope of this review but there are a few general considerations to note. Firstly, the majority of individuals exposed to genetic and environmental risk will never develop RA- related pathology and therefore will not develop true preclinical disease. Secondly, putative associations between various environmental factors and RA have largely been derived from retrospective case-control studies. It is difficult, therefore, to translate these associations into effect sizes that can inform us prospectively about an exposed individual's risk of RA compared to the background general population. For these reasons, careful consideration must be paid to the at risk populations selected for any studies that seek to investigate risk factor modification as an interventional strategy.

In those at risk individuals who develop preclinical autoimmunity, the triggering events may occur outside the synovial joints. The observation that RA autoantibodies are present in individuals with no clinical, imaging or histological joint abnormalities provides circumstantial evidence for this [10]. Instead, studies of RA autoantibody isotypes in at risk populations indicate that preclinical autoimmune events may begin at mucosal surfaces. The observation that IgA ACPA is relatively enriched in first degree relatives (FDRs) of RA patients compared to the IgG isotype [11,12] supports the concept that localised mucosal immunity to citrullinated epitopes precedes, and indeed may trigger, systemic autoimmunity. Such mucosal autoimmune events may occur at the oral mucosa (specifically the periodontium), lung and/or the gastrointestinal tract [13].

Periodontal disease may be a forerunner of ACPA-positive RA through its association with *Porphyromonas gingivalis.* This bacterium is equipped with unique virulence factors that enable it to modify local arginine residues to citrulline and therefore potentially generate novel citrullinated epitopes. However, it is important to note that P gingivalis is a ubiquitous oral microbe that may be found in those with good and poor periodontal health. In line with this, measuring anti- P gingivalis antibodies in RA and at risk populations has yielded mixed results; some investigators have reported higher anti-P gingivalis levels in at risk individuals compared to controls [14-16] but others have found no differences in antibody levels between RA patients and controls [17,18]. One may therefore speculate that for P gingivalis to trigger ACPA responses in certain individuals it requires additional factors which may include the expression of certain virulence factors (e.g. in a virulent strain of P gingivalis), co-localisation with other bacteria and periodontal inflammation [19]. Indeed in a recent study of almost 2000 RA cases from the Swedish EIRA cohort, antibodies to the P gingivalis virulence factor arginine gingipainB (RgpB) were significantly elevated in ACPA-positive RA

patients compared to both ACPA-negative RA cases and matched controls. An additive effect between anti-RgpB levels and smoking as well as HLA-SE was also observed [20]. However, in another recent study in which stored Pre-RA serum samples were analysed, no association was found between anti-RgpB levels and Pre-RA autoimmunity or subsequent RA development [21]. Like the periodontium, citrullination may be triggered locally at the lung mucosa [22]. RA-related autoantibodies and imaging abnormalities are more prevalent in the lungs of ACPA-positive compared to ACPA-negative early RA patients [23]. Moreover, subjects at risk of RA development (FDRs) appear to have local IgA ACPA in the sputum in the absence of a detectable systemic ACPA response and also inflammatory airway changes in the absence of clinical arthritis [24,25].

The putative link between P gingivalis and ACPA highlights the potential influence of the mucosal microbiome on RA pathogenesis. Further evidence for this is found in the gastrointestinal tract where alteration of the gut microbiome, or dysbiosis, has been described in early RA patients [26-28]. These studies have used next generation sequencing (NGS) techniques to demonstrate increased abundance of Lactobacillus [27,28]and Prevotella Copri [26] in the stool samples of early RA patients. In a recent study concordance was observed in the oral and gut microbiomes sampled from dental plaque, saliva and stool in untreated early RA patients [28]. Haemophilus species were depleted in all sites in RA patients whereas Lactobacillus salivarius was relatively enriched, particularly in those with severe disease activity. Interestingly, improvement in RA disease activity with DMARD therapy was associated with partial resolution of the dysbiosis in these patients [28].

Towards Prevention: Identifying those at high risk of RA

As our understanding of the sequence of events in preclinical RA unfolds, we can now look to identify at risk phenotypes that may be appropriate for preventive interventions. For a preventive strategy to be feasible, two key criteria should be met. Firstly the target group of at risk individuals should be readily identifiable e.g. via a straightforward screening process. Secondly, the target group should be at high risk of progression to RA, such that any potential intervention would be clinically and economically justifiable.

Healthy relatives of RA patients, particularly FDRs, are a readily identifiable group of at risk subjects. Heritability of seropositive RA is around 50% and FDRs have a threefold increased risk of developing RA compared to the general population [29]. However, monitoring healthy relatives prospectively reveals that the absolute risk of RA development in these individuals is generally low. In a prospective cohort of over 800 healthy relatives, 80% of whom were FDRs, only 2.1% developed RA at 5 years. Moreover, in the 95% of relatives that were seronegative for anti-CCP and RF, the Positive Predictive Value (PPV) for RA development was only 0.4% at 5 years [30]. Thus screening for genetic risk alone in this way does not appear to be an effective strategy for identifying high risk individuals.

An alternative strategy might be to screen for RA-related autoantibodies. However, although anti-CCP antibodies may be detected in around 1% of the general population, the risk of progression to RA at 5 years in these anti-CCP positive individuals is estimated at only around 5% [2]. Therefore, as with healthy relatives, autoantibodies alone do not appear useful as a screening target. However, there are striking improvements in predictive utility when these two risk factors are combined; 61% of healthy relatives of RA patients that were also anti-CCP antibody positive developed RA at 5 years

compared to 0.4% of seronegative relatives [30]. Combining autoantibodies with clinical symptoms appears to enrich for high risk individuals in a similar fashion. In a recent cohort study, individuals with new musculoskeletal (MSK) symptoms were screened for anti-CCP antibodies. Of the 2028 individuals screened, 2.8% were anti-CCP positive, indicating that symptom-guided autoantibody testing delivers a better yield than testing in the general population. Of note 42% of those that tested anti-CCP positive progressed to RA, the majority within one year [31].

Individuals with MSK symptoms and anti-CCP antibodies, so called 'seropositive arthralgia' patients, are currently being recruited and studied in prospective cohorts [4,5]. These individuals may be identified from both primary care and general rheumatology clinics and have variable risk of progression to RA. Stratification based upon RA-autoantibody profile and symptom characteristics is possible in the clinic and allows identification of high risk subgroups where over 50% of patients progress to RA at 1 year [5]. Patients with high anti-CCP titres and those that are both anti-CCP and RF positive appear to be at higher risk of progression to RA [4,5]. Likewise, the presence of early morning stiffness (EMS) was independently predictive of arthritis development in both cohorts. In the Leeds cohort, intra-articular ultrasound Power Doppler signal was also independently predictive of arthritis development. MSK ultrasound is now routinely used in early arthritis clinics and is therefore a readily available tool that can be used alongside symptoms and serology to stratify at risk individuals. Risk prediction rules that allow clinicians to calculate an individual seropositive arthralgia patient's risk of RA development have been proposed in two independent prospective cohorts [5,32]. In this way, high risk patients may be readily identified in the clinic. Currently, as there is no evidence base for the management of these patients, frequent monitoring for the development of synovitis and consideration for clinical trials is an appropriate strategy. Clearly these clinical prediction rules will need to be validated as the cohorts grow in size and heterogeneity. It is also likely that other biomarkers, as will be discussed below, will be informative in future prediction tools. However, the independent utility of these biomarkers and their practical availability in the clinical setting will need to be carefully considered.

Biomarkers in At Risk Individuals

A diverse range of biomarkers have been investigated in individuals at risk of RA. In prospective cohorts, examining a multimodal range of biomarkers enables better phenotyping and potentially better prognostication. Clinical symptoms are important biomarkers as they are usually the reason why patients first present to clinicians. Certain pre-specified symptoms have been shown to be predictive of arthritis development in seropositive arthralgia patients [5,32]. Furthermore, exploratory qualitative work suggests that patients experience a range of symptoms prior to the development of clinical RA [33]. Reported symptoms included joint pain, stiffness, fatigue and weakness but also less obvious features including burning sensations, warmth and redness of the skin. Based on clinical experience, one may speculate that certain symptoms are more discriminatory than others [31] and this is the subject of ongoing prospective research. It is certainly important to consider symptoms as a starting point for identifying at risk individuals; patients will present with an unknown autoantibody status and it is also important to identify seronegative patients in the earliest phases of disease.

The utility of RA-related autoantibody profiles may be further improved by measurement of autoantibodies against carbamylated proteins (anti- CarP). Anti-CarP are more prevalent in seropositive arthralgia patients compared to controls (39% vs 6%) and appear to predict progression to RA independently of anti-CCP [34]. Case-control studies have confirmed that anti-CarP are present in preclinical RA specimens and are associated with future development of RA [35,36]. However, in the most recent study the sensitivity of anti-CarP was low (<30%) and anti-CarP did not improve the predictive accuracy achieved by anti-CCP and RF any further [35]. Thus, although anti-CarP appear relevant in preclinical disease pathogenesis the clinical utility of this biomarker in at risk individuals remains to be determined.

In addition to measuring autoantibodies, one may characterise preclinical autoimmunity by identifying specific immune-related gene expression signatures. DNA microarray analysis has shown that at risk arthralgia patients who have increased expression of genes involved in type 1 interferon (IFN)-mediated immunity are at higher risk of developing RA [37,38]. The predictive ability of a type 1 IFN signature has been validated in other at risk cohorts and appears to improve predictive accuracy when added to anti-CCP and RF status [38]. Similarly, B cell related gene expression signatures are also variable in seropositive arthralgia patients [37,39]. The expression of genes involved in B cell immunity is associated with a lower risk of RA development and this may have clinical utility when combined with the type 1 IFN signature [39]. Flow cytometry analysis indicates that the measured B cell gene expression signature corresponds to circulating conventional memory B cell numbers, suggesting this B cell subset may have particular importance in preclinical disease.

Those with arthralgia and specific imaging abnormalities in the joints may be at particularly high risk of imminent RA [5,40]. The clinical utility of MSK ultrasound biomarkers has been addressed in both Dutch and UK seropositive arthralgia cohorts [5,40,41]. Van de Stadt et al reported that US abnormalities (joint effusion, grey scale synovitis, Power Doppler) were predictive of arthritis at single joint but not whole patient level [41]. In contrast, Power Doppler signal was independently predictive of arthritis development at whole patient level in the Leeds cohort (Nam J et al 2015 in press) [5,40]. A higher risk patient cohort (all anti-CCP positive) and a more comprehensive US joint dataset in the latter study may account for the discrepancy in these results. MRI abnormalities have also been consistently described in the peripheral small joints of seropositive and seronegative arthralgia patients [42-45]. However, the high sensitivity of MRI appears to somewhat compromise specificity, with a relatively high prevalence of MRI abnormalities found in pain-free healthy controls even without use of gadolinium contrast [42,45]. This may be one reason why MRI biomarkers have failed to predict arthritis development in at risk individuals in these studies. As cohorts of at risk individuals continue to develop, MRI biomarker studies will become better powered and the clinical utility of specific MRI findings e.g. bone marrow oedema will be more accuractely assessed. Other, less conventional imaging techniques have also been studied in preclinical RA; a small pilot study showed that macrophage positron emission tomography (PET) is highly specific in identifying ACPA-positive arthralgia patients that later progress to RA [46]. Although conceptually interesting, cost and radiation dose may limit the practical utility of this technique when compared with more conventional ultrasound an MRI.

The synovial features of at risk arthralgia patients have also been examined using mini-arthroscopic synovial biopsy [47,48]. These studies showed neither synovial inflammation nor inflammatory prostaglandin E2 pathway enzymes in the biopsied tissues. Furthermore, synovial histology was not

predictive for the development of future synovitis or RA. There are some caveats concerning the interpretation of these findings; synovial tissue is extremely difficult to obtain reliably from non-arthritic small joints, which is the key limitation of using this technique in at risk arthralgia patients. For this reason de Hair et al took samples only from the knee joints, where arthralgia is often more non-specific than in the hands and wrists. Furthermore, the majority of biopsied knee joints were asymptomatic in both studies [47,48].

Towards Prevention: Management of At Risk Individuals

Well characterised individuals at high risk of RA development are appropriate targets for preventive interventions. It is not currently known whether the correction of environmental and lifestyle factors that are associated with RA can prevent progression to clinical disease in those at high risk. Many of these risk factors (e.g. smoking, periodontal disease, gut dysbiosis) are thought to drive the earliest phases of RA-related autoimmunity. It is therefore difficult to speculate whether their modification would be sufficient to rescue a high risk phenotype where systemic autoimmunity is well established and clinical and/or imaging features have often already developed in the joints. A smoking history and elevated BMI were associated with an increased risk of arthritis development in seropositive at risk individuals in one small study [49]. This is in keeping with data from the US Nurses Health Studies, where elevated BMI was associated with seropositive and seronegative RA development [50]. Furthermore, in a recent study, serum levels of the adipokine vaspin were found to be higher in seropositive at risk individuals who progressed to arthritis compared to those that did not [51]. However, smoking status and BMI were not independently predictive of arthritis development in another seropositive at risk cohort [5].

The current paradigm of preclinical RA has fuelled interest in the use of immunomodulators to prevent arthritis development in high risk individuals. Only one such trial has been published; a single centre study found that intramuscular glucocorticoids could not prevent arthritis development in a small cohort of seropositive arthralgia patients [52]. Two multicentre randomised controlled trials are currently underway. The first is investigating the ability of a single infusion of rituximab to prevent the onset of arthritis in very high risk individuals (arthralgia, ACPA and RF positive and elevated CRP or subclinical synovitis on US/MRI) (PRAIRI study: NTR 2442). The second is investigating whether abatacept therapy for 12 months can prevent arthritis development in seropositive (ACPA and RF positive or high titre ACPA alone) patients with inflammatory arthralgia (APPIPRA study: ISCTRN No. 46017566).

Conclusions

RA-related autoimmunity and inflammation occur well before patients develop clinical arthritis. Retrospective case-control studies first highlighted evidence of autoimmune processes in the stored serum samples of individuals who would later develop RA. However, prospective cohorts of at risk individuals are now enabling more detailed and accurate characterisation of a range of important biomarkers and their influence on disease progression. The next goal is to use data from these cohorts to better characterise the sequence of events that occurs on the journey from risk factors to preclinical disease right through to clinical arthritis. Detailed multimodal phenotyping of these cohorts has facilitated risk prediction tools and the identification of subgroups of individuals at high

risk of progression to clinical disease. It is targeted intervention in these subgroups that represents a new ‘window of opportunity’ in RA and ultimately reflects a therapeutic shift towards the prevention of this common autoimmune disease.

Key points

- RA-related autoimmunity and inflammation begins well before the onset of clinical disease and may be triggered outside the joints at mucosal surfaces including the periodontium, lung and gut.
- Prospective cohorts of at risk individuals are allowing the detailed characterisation of various at risk phenotypes including those at genetic risk, those with systemic autoimmunity, those with imaging abnormalities and those with symptoms.
- Use of multimodal phenotyping in at risk cohorts provides insights into pathogenesis and enables stratification of individuals based upon risk of future RA development.
- The ability of immunomodulators to prevent RA development in high risk individuals is currently being investigated in multicentre randomised controlled trials.

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