Cover Page

Universiteit Leiden

The handle http://hdl.handle.net/1887/44019 holds various files of this Leiden University dissertation.

Author: Steenbergen, H.W. van
Title: The course of clinically suspect arthralgia and early rheumatoid arthritis : clinical features, imaging and genetics
Issue Date: 2016-11-08

Part I

**Phase of
Clinically Suspect Arthralgia**

Review: The preclinical phase of rheumatoid arthritis. *What is acknowledged and what needs to be assessed?*

Hanna van Steenbergen, Tom Huizinga,
Annette van der Helm-van Mil

2

Introduction

Rheumatoid arthritis (RA) affects 1% of the population worldwide and is characterised by persistent inflammation and joint damage. It has repeatedly been observed that early initiation of disease-modifying therapy reduces the severity of the disease course, as measured by fewer signs and symptoms and less structural damage. Early treatment is associated with less severe joint damage progression and increased chances of achieving disease-modifying antirheumatic drug-free sustained remission [1,2]. Observations that treatment in the very early phase of RA is more effective, conceivably because the load of disease cells is smaller or because disease mechanisms are not yet settled, have led to increased interest in the earliest disease phases. Ideally, this period is used to modify the disease course and improve the outcome of RA. The timeframe of this treatment-susceptible period is, however, unknown. Several recent studies have provided data indicating that disease processes are already active in the preclinical phase (the period before arthritis becomes clinically detectable). Consequently, it is at present unclear when RA actually starts. The concept that the disease starts when arthritis has become (clinically) detectable is no longer valid.

Herein we systematically review what is presently known of the preclinical phase of RA. With the assistance of a medical librarian, we performed a search for the central terms "rheumatoid arthritis," "preclinical," "autoantibody-positive arthralgia," and "developing RA" in the medical literature databases Medline (Ovid version), PubMed, EMBase (Ovid version), and Web of Science up to December 2012 (Figure 1). Findings from the identified articles, combined with additional hand-searched articles from the reference lists of the identified articles, are summarized here. This overview will lead to the identification of research items that need to be explored in order to identify patients in the preclinical phase who will develop RA. This may ultimately allow individualized interventions during the preclinical phase.

Basis of pre-RA

Although interest in the preclinical phase has increased considerably during the last few years, the idea that disease processes related to RA occur before arthritis is clinically detectable was proposed more than two decades ago. The prevalence of rheumatoid factor (RF) in the preclinical phase of RA was first observed in Finnish and Icelandic patients with RA [3,4]. Increased prevalences of antifilaggrin and antiperinuclear antibodies were also reported [5,6]. The first large longitudinal population study of pre-RA was performed in the high-risk population of Pima Indians, showing that the presence of RF was a risk factor for the development of RA, and that this risk increased in parallel with the RF level [7]. Similar observations were noted in a longitudinal study that evaluated another high-risk population, multicase families [8,9]. These studies have formed the basis for what is now called pre-RA.

Approximately 15 years later, the pre-RA phase received renewed attention. Rantapää-Dahlqvist et al [10] and Nielen et al [11] studied serum samples from RA patients collected serially

in the preclinical phase and observed that the prevalence of autoantibodies increased over time and that this increase can take place even years before RA becomes clinically evident. These two studies served as subsequent landmark studies, after which the number of publications on systemic and local responses and symptoms in the preclinical phase of RA rapidly increased (Figure 1).
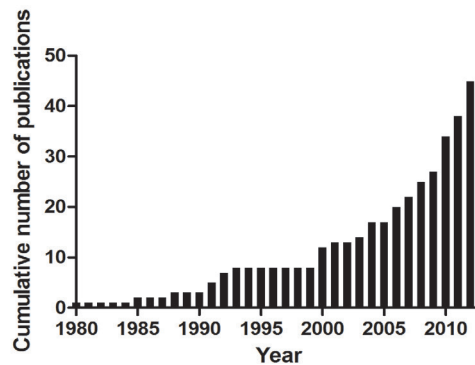
Figure 1. Number of original publications on the preclinical phases of RA. In total, 964 references were extracted from the medical databases Medline (Ovid version), PubMed, EM-Base (Ovid version), and Web of Science. Animal studies, reviews, conference abstracts, case reports, case series, studies including patients 18 years of age, and studies in languages other than English were excluded. A total of 66 unique publications on systemic autoimmunity (phase C) and other systemic or local responses associated with RA and symptoms without clinical arthritis (phase D) published before December 1, 2012 were identified.

Definition of pre-RA

Many different terms are used to describe the phases that occur before clinically manifest RA. These include "pre-RA," "preclinical RA," and "(very) early RA." In order to achieve homogeneity in terminology, in 2011 the study group for risk factors for RA, established by the European League Against Rheumatism (EULAR) Standing Committee on Investigative Rheumatology, formulated a recommendation for terminology to be used with regard to the preclinical and earliest clinically apparent phases of RA [12]. Six phases (phases A-F) of RA development were formulated. These are phase A, genetic risk factors for RA; phase B, environmental risk factors for RA; phase C, systemic autoimmunity associated with RA; phase D, symptoms without clinical arthritis; phase E, unclassified arthritis; and phase F, RA (Figure 2A). It was emphasized that patients do not have to pass through all phases and that the phases do not necessarily occur in the same order before RA eventually develops. In addition, a patient can be in two phases concurrently. Importantly, it was also recommended that the term "pre-RA" only be used retrospectively. This recommendation was made since if all persons who carry certain genetic risk factors or who are exposed to certain environmental risk factors were labeled as having pre-RA, many persons who will never develop RA would inappropriately have been classified as being in a predisease stage. The proposed phases are relevant since they form a framework for future research. Below we review the data available on preclinical arthritis within the framework of these study group formulated phases.

Genetic risk factors for RA

The first phase at which individuals develop an increased risk of RA is at conception, when a subject inherits risk alleles for RA from his or her parents (phase A). More than 40 such risk alleles are currently known, and the majority of these variants are commonly present. When evaluating the frequencies of these risk alleles in the population, the chance that an individual carries none of the RA risk alleles is $7.1 \times 10^{-13}\%$; in other words, almost everyone carries one or several of these risk alleles. This calculation underlines the relevance of using the term "pre-RA" only retrospectively. These genetic variants have small effect sizes, and a large proportion of the population carrying risk alleles never develops RA.
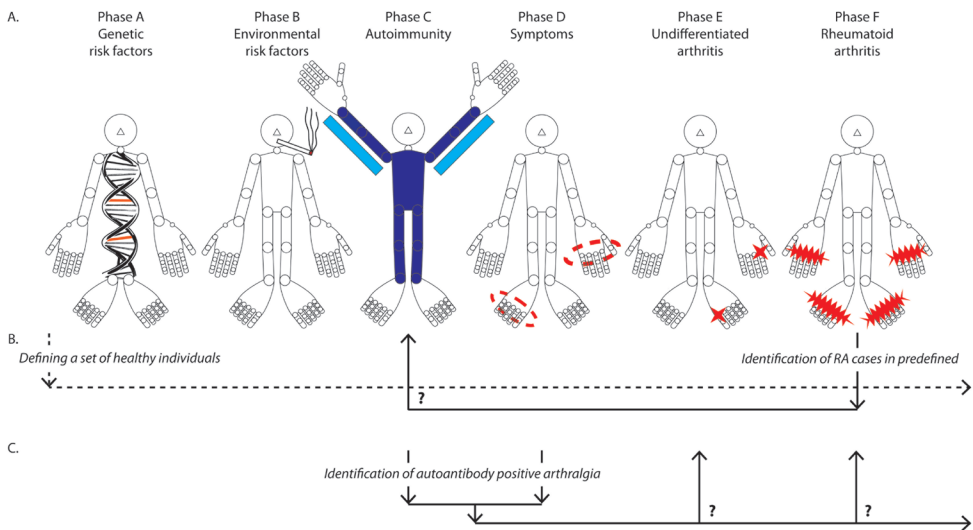

Figure 2. Overview of the preclinical phases of RA and the designs of the studies of the preclinical phases that have been performed. (A) The six phases of preclinical and earliest clinically apparent RA, as defined by the EULAR study group for risk factors for RA. (B) Nested case-control study design. A predefined set of subjects (e.g., blood donors) is followed up. From this set of subjects, RA cases are identified and for each case, a specified number of matched controls who have not developed RA is selected. Biomarkers are compared between preclinically collected samples from these cases and the controls. (C) Prospective cohort study design. Autoantibody-positive arthralgia patients are identified and followed up prospectively.

Environmental risk factors for RA

The heritability of RA has been estimated at 60% [13], implying that 40% of the variance in developing RA might be explained by environmental risk factors (phase B). Many environmental risk factors have been studied, and smoking is the best replicated environmental risk factor [14,15]. Smoking predisposes to RA particularly in patients who carry specific HLA-DRB1 alleles, e.g., smokers carrying two HLA-DRB1 alleles have a 21-fold increased risk of developing anti-citrullinated peptide antibody (ACPA)-positive RA [16]. Despite the high odds ratio in this subgroup, a large majority of smokers do not develop RA. Weaker interactions have also been demonstrated between other genes and smoking [17].

The genetic and environmental risk factors conceptually constitute the earliest preclinical phases of RA. These risk factors have already been known for some time, and an extensive discussion of these risk factors is beyond the scope of this review.

The next two preclinical phases, "developing systemic autoimmunity associated with RA" (phase C) and "symptoms without clinical arthritis" (phase D), have been studied in the last few years. This has been done using mainly two different study designs: nested case-control studies and prospective cohort studies. As will be discussed, the design of the study determined the sort of outcome that was obtained and the conclusions that can be drawn.

From RA back to pre-RA systemic autoimmunity

Studies associating RA with systemic autoimmune responses in the preclinical phase (phase C, with or without phase A and B) were mainly performed using a nested case-control study design (also called case-control studies in a cohort) (Figure 2B). In this type of study, RA cases were identified who were members of a predefined dataset, e.g., a cohort of blood donors from whom blood samples were obtained at least once [10,18]. For each RA patient, a specified number of matched controls who had not developed RA was selected from the same dataset. Consequently, blood samples from RA cases that were collected and stored years before the onset of arthritis could be compared with blood samples from matched controls.

Compared to full prospective cohort studies, the main advantage of this study design is the smaller number of study subjects that is required, which coincides with lower efforts and costs. However, this study design also has limitations. The cases and controls are selected, which may give rise to sampling error and bias. Second, the time of onset of symptoms and arthritis in the cases is not exactly known, leaving the timing of the appearance of autoantibodies in relationship to the onset of symptoms unexplored. Furthermore, in several studies the healthy controls were not carefully evaluated for rheumatic diseases; this might have led to an overestimation of the prevalence of autoantibodies in controls and an underestimation of the specificity. Using nested case-control studies, the biomarkers described below have been identified to be abnormally regulated in the preclinical phases of RA (see also Table 1).

Autoantibodies - In the nested case-control studies by Rantapää-Dahlqvist et al [10] and Nielen et al [11], 83 Swedish patients with RA (73.1% IgM-RF-positive and 70.1% ACPA-positive) and 79 Dutch patients with RA (frequencies of autoantibodies at diagnosis not reported), respectively, who were donors to a blood bank before symptom onset were studied [10,11]. A total of 98 pre-RA blood samples were available for the patients in the Swedish study, and a total of 1,078 pre-RA blood samples were available for the patients in the Dutch study. In the Swedish study, the presence of IgM-RF and ACPA was reported in 19.3% and 33.7%, respectively, of the RA cases within 10 years before RA diagnosis, compared to 6.0% and 1.8%, respectively, of the matched controls [11]. Similarly, in the Dutch dataset, 27.8% of the patients

Table 1. Results of the nested case-control studies of abnormalities in the preclinical phase of RA*

Author, year	Cohort	No. of cases (total no. of samples)	Time between (first) sample and RA	Measured factors	Main result
Autoantibodies					
Rantapää-Dahlqvist et al, 2003 [10]	Northern Sweden health and disease study and Maternity cohort of Northern Sweden	83 (98)	Median 2.5 years	IgG-RF, IgM-RF, IgA-RF, ACPA; sensitivity, specificity, PPV and NPV of autoantibodies	Increased prevalence of all autoantibodies (IgM-RF 19%, ACPA 34%) in pre-RA. Sensitivity: ACPA 34%, IgM-RF 20% Specificity: ACPA 98%, IgM-RF 95% PPV†: ACPA 16%, IgM-RF 4% NPV†: ACPA 99%, IgM-RF 99%
Nielen et al, 2004 [11]	Sanquin blood bank, the Netherlands	79 (1,078)	Median 7.5 years	IgM-RF and ACPA; sensitivity, specificity, PPV and NPV of autoantibodies	Increased prevalence of IgM-RF (28%) and ACPA (41%) in pre-RA. Sensitivity: ACPA 29%, IgM-RF 21% Specificity: ACPA 99%, IgM-RF 100% PPV‡: ACPA 5%, IgM-RF 2%
Majka et al, 2008 [22]	Department of defense serum repository, US	83 (243)	Mean 6.6 years	IgM-RF and ACPA	Increased prevalence of IgM-RF (57%) and ACPA (61%) in pre-RA. Period of time that autoantibodies are present before diagnosis lengthens as the age at time of diagnosis increased.
Chibnik et al, 2009 [23]	Nurses' Health Study	93 (93)	Mean 5.6 years	ACPA level; sensitivity, specificity and hazard for various ACPA thresholds.	Higher ACPA levels were associated with shorter time to RA diagnosis; lower threshold for ACPA positivity more sensitive in predicting RA development.
Van der Woude et al, 2010 [25]	Northern Sweden Health and Disease Study and Northern Sweden Maternity cohort	36 (36)§	Median 2.5 years	Recognition of 5 citrullinated peptides	Number of recognized peptides increased in the pre-RA period.
Kolfenbach et al, 2010 [62]	Department of defense serum repository, US	83 (243)	Mean 6.6 years	Anti-PAD-4	Anti-PAD-4 prevalence of 18.1% in pre-RA and its presence was associated with ACPA-positivity.

Study	Source	N (n)	Time	Markers	Findings
Van de Stadt et al, 2011 [26]	Sanquin blood bank, the Netherlands	53 (374) §	Median 1.0 years	Recognition of 5 citrullinated peptides	Number of recognized peptides increased over time in the pre-RA phase without a dominant epitope spreading pattern.
Autoantibodies & markers of systemic inflammation					
Jørgensen et al, 2008 [18]	Blood bank, Norway	49 (49)	Median 9.3 years	IgM-RF, ACPA, 16 cytokines and related markers	Increased prevalence of IgM-RF (20%) and ACPA (31%) in pre-RA; increased prevalence of raised TNFα levels within 5 years pre-RA.
Kokkonen et al, 2011 [33]	Northern Sweden Health and Disease Study and Northern Sweden Maternity cohort	71 (71)	Median 2.5 years	Isotypes of ACPA (IgG, IgM, IgA) and 29 cytokines and chemokines	Increased prevalence of mainly IgG-ACPA and IgA-ACPA in pre-RA; different pattern of up-regulated chemokines in IgG-ACPA and IgA-ACPA-positive pre-RA.
Sokolove et al, 2012 [24]	Department of defense serum repository, US	81 (±283)	Mean 6.4 years	ACPA reactivity 48 cytokines	Gradual increase in number of ACPA subtypes is followed by a parallel increase in cytokines in pre-RA approaching symptom onset.
Autoantibodies & markers of bone metabolism					
Turesson et al, 2011 [21]	Malmö Diet and Cancer Study, Sweden	169 (169)	Median 5 years	IgM-RF, ACPA, anti-MCV and COMP	Increased prevalence of IgM-RF (19%), ACPA (22%) and anti-MCV; no increased COMP level in preclinical phase of RA cases; increased prevalence of elevated COMP level within 3 years prior ACPA-negative RA.
Markers of systemic inflammation					
Aho et al, 2000 [28]	Community cohort, Finland	124 (124)	Upper limit 20 years	CRP	No increased CRP levels in pre-RA.
Masi et al, 2001 [27]	Community cohort, US	18 (18)	Median 12 years	CRP and acute SAA	Increased CRP levels in men with pre-RA.
Nielen et al, 2004 [32]	Sanquin blood bank, the Netherlands	79 (1,078)	Median 7.5 years	CRP	Increased CRP levels, mainly within 2 years prior symptom onset in pre-RA.
Shadick et al, 2006 [20]	Women's health study, US	90 (90)	Mean 6.6 years	CRP	No association between increased CRP levels in pre-RA and development of RA
Nielen et al, 2006 [34]	Sanquin blood bank, the Netherlands	79 (1,078)	Median 7.5 years	sPLA2, CRP, IgM-RF and ACPA	No time lag between development of acute phase reactants and autoantibodies in pre-RA.

Study	Cohort	N (controls)	Time	Biomarkers	Findings
Rantapää-Dahlqvist et al, 2007 [29]	Northern Sweden Health and Disease Study and Northern Sweden Maternity cohort	92 (92)	Median 3.3 years	sPLA2, CRP, IL-6 and MCP-1	Increased MCP-1 level in IgM-RF or ACPA-positive pre-RA.
Karlson et al, 2009 [30]	Nurses' health study and Women's health study, US	170 (170)	Mean 5.2 years	CRP, IL-6 and sTNFRII	Association between sTNFRII level in pre-RA and development of RA.
Kokkonen et al, 2010 [19]	Medical biobank Northern Sweden	85 (85)	Median 3.3 years	29 cytokines and related factors and chemokines	Increased levels of 18 of the analytes in pre-RA, particularly in ACPA or IgM-RF-positive RA.
Deane et al, 2010 [31]	Department of Defense Serum Repository, US	73 (212) ¶	Mean 6.6 years	CRP and 14 cytokines and chemokines	Increased levels of CRP, cytokines and chemokines in pre-RA. Predicted time to diagnosis based on number of elevated cytokines/chemokines increased with age.

Biomarkers of bone metabolism

Study	Cohort	N (controls)	Time	Biomarkers	Findings
Schaardenburg et al, 2011 [35]	Sanquin blood bank, the Netherlands	79 (191)	1, 2 and 5 years	Osteocalcin, P1NP, β-CTX, osteoprotegerin and RANKL	Increased levels of P1NP and osteoprotegerin and no increased levels of osteocalcin, β-CTX and RANKL in pre-RA.

Lipid profile and cardiovascular disease

Study	Cohort	N (controls)	Time	Biomarkers	Findings
Maradit-Kremers et al, 2005 [38] #	Population-based incidence cohort, Minnesota, US	603	NA	Hospitalized MI, unrecognized MI, coronary revascularization procedures, angina pectoris, out-of-hospital sudden death	Increased number of hospitalized and unrecognized MIs and decreased number of angina pectoris cases in pre-RA.
Van Halm et al, 2007 [36]	Sanquin blood bank, the Netherlands	79 (1,078)	Median 7.5 years	Total cholesterol, HDL, triglycerides, Apo A-I, Apo B, Lp(a)	Increased levels of total cholesterol, triglycerides and Apo B and decreased level of Apo A-I in pre-RA.

Study	Description	N	Time period	Outcomes	Results
Holmqvist et al, 2009 [39]	National Early Arthritis Register and Epidemiologic Investigation of RA case-control study of incident RA, Sweden	10,579	NA	CHD, MI and angina pectoris	No increased occurrence of CHD, MI or angina pectoris in pre-RA.
Myasoedova et al, 2010 [37]	Population-based inception cohort Minnesota, US	577 (3,048)	Within 5 years pre-RA to 5 years post-RA	Total cholesterol, HDL, LDL and triglycerides	Lower prevalence of increased total cholesterol or LDL in pre-RA; significant decline in total cholesterol and LDL levels in the 5 years pre-RA.
Kerola et al, 2012 [40] #	Based on a nationwide register on special reimbursements for medication costs, Finland	7,209	NA	CHD and chronic hypertension	Slightly increased prevalence of CHD and no increased prevalence of chronic hypertension at the time of RA diagnosis.

*All results are based on comparing cases with pre-RA to matched non-RA controls. †Positive predictive value (PPV) and negative predictive value (NPV) for, respectively, developing and not developing RA at any point in life; the population-based pretest frequency of RA was set as a pretest probability. ‡PPV for developing RA within 5 years; the population-based pretest frequency of RA was set as a pretest probability. §All ACPA-positive. ¶ All IgM-RF and/or ACPA-positive. #These studies are not defined as nested case-control studies, but as retrospective cohort studies in which the prevalence of cardiovascular disease in the pre-RA period was retrospectively examined in RA patients and compared to matched controls in the same population. Anti-PAD-4=anti-peptidylarginine deiminase 4; anti-MCV=anti-mutated and citrullinated vimentin; COMP=cartilage oligomeric matrix protein; SAA=serum amyloid A; sPLA2=secretory phospholipase A2; MCP-1=monocyte chemotactic protein 1; sTNFRII=soluble tumor necrosis factor receptor type II; PINP=N-terminal type I procollagen propeptide; β-CTX=C-terminal crosslinking telopeptide of type I collagen; MI myocardial infarction; Apo A-I apolipoprotein A-I; Lp(a) lipoprotein(a); CHD coronary heart disease.

were IgM-RF-positive and 40.5% were ACPA-positive within 15 years prior to RA diagnosis, compared to 1.1% and 0.6%, respectively, of the matched controls [10]. Similar frequencies of IgM-RF and ACPA in the pre-RA phase were reported in several cohorts in Norway, Sweden, and the US [18–21]. Even higher frequencies were reported in the pre-RA phase in a military cohort in the US (57% IgM-RF-positive and 61% ACPA-positive) [22]. Comparison of these frequencies with the prevalence of IgM-RF and ACPA observed in other patients with early arthritis (58% and 53%, respectively [2]) is potentially scientifically incorrect; nonetheless, this comparison suggests that in a large proportion of autoantibody-positive RA patients the autoantibodies are already present in the preclinical period.

Based on the proportion of RA cases with autoantibodies in the preclinical phase (sensitivity) and the proportion of controls without autoantibodies (specificity) in the studied cohorts, the risk of developing arthritis for individuals with autoantibodies in the general population was estimated. In these calculations the population-based frequency of RA was set as a pretest probability. The risk of developing RA within 5 years [11] and at any time in life [10] was estimated at 1.5% and 4%, respectively, in subjects who were IgM-RF-positive and 5.3% and 16%, respectively, in subjects who were ACPA-positive.

Regarding the time course of autoantibody development, the frequency of autoantibody positivity as well as the levels of these autoantibodies increased approaching the onset of symptoms [10,11,18,22,23]. It is unclear whether IgM-RF or ACPA appears earlier in time. Although in the Swedish cohort IgM-RF could be detected earlier than ACPA [10], in the Dutch cohort ACPA was detected earlier than IgM-RF (median duration from seroconversion to symptom onset 2.0 years for IgM-RF and 4.8 years for ACPA) [11]. In a US cohort, IgM-RF and ACPA appeared concurrently in the pre-RA phase (6.0 years for IgM-RF and 5.4 years for ACPA) [22]. With regard to the ACPA response, the number of epitopes to which the response is directed increased over time and in parallel with the increase in ACPA level approaching the onset of symptoms, revealing that this response matures in the preclinical disease phases [24–26].

Markers of systemic inflammation - Various acute-phase reactants, cytokines, cytokine-related factors, and chemokines were measured in serum samples that were collected once (one sample per case) or serially from RA patients prior to symptom onset. In the studies in which serum was obtained at a single time point, the period between sample collection and RA diagnosis was variable, with a maximum of 20 years; only a small number of samples were obtained within one year of symptom onset. These studies with single samples do not allow drawing conclusions with regard to the evolution of systemic markers of inflammation over time before arthritis onset, and negative findings are difficult to interpret since they might potentially be the result of analyzing samples that were obtained too early.

None of the single serum sample-based studies, except for one [27], demonstrated increased levels of C-reactive protein (CRP) or other acute-phase reactants (i.e., secretory

phospholipase A2) during the pre-RA phase, irrespective of autoantibody status or time to diagnosis [20,27–30]. However, a study evaluating serially collected serum samples from autoantibody-positive RA cases demonstrated increased levels in significantly more cases than controls [31]. Other investigators measured CRP serially over time in serum samples collected from RA patients during the preclinical period and observed a statistically significant increase in median CRP levels, although within the normal range, in the periods 4-5 years, 1-2 years, and 0-1 years prior to diagnosis [32]. A gradual increase in CRP level was observed both in autoantibody-positive and autoantibody-negative cases, with the highest level observed at the time closest to arthritis onset. Furthermore, at all preclinical time points studied, the autoantibody-positive patients had slightly higher CRP levels than the autoantibody-negative patients [32].

Over 30 different cytokines have been studied using different techniques with different sensitivities; the majority of these were measured in the single serum sample-based studies. Since the time period between collection of serum sample and RA onset was variable in these studies, the results cannot be easily compared to look for replication. Nonetheless, the levels of some cytokines (tumor necrosis factor α [TNFα] and/or the soluble TNF receptor type I/II, which parallels TNFα levels, and interleukin-6 [IL-6]) were increased during the preclinical phase in several studies. Both TNFα and IL-6 levels were significantly increased during the pre-RA phase in most [18,19,24,30,31,33] but not all studies [18,29,33]. More variable results were found for other markers, including different interleukins, granulocyte-macrophage colony-stimulating factor, monocyte chemotactic protein 1, and interferon-γ. In the presence of increased cytokine levels, these results were most often found in both autoantibody-positive and autoantibody-negative cases, but autoantibody-positive cases had generally higher levels than the autoantibody-negative cases [19,24,29]. These levels were also the highest close to the diagnosis of RA [19].

It remains unclear whether levels of the inflammatory markers increase before, after, or simultaneously with the development of autoantibodies. No time lag was found between the increase in CRP level and the presence of autoantibodies in some studies [32,34], whereas another study suggested a longer predating period for autoantibodies than for increased cytokine levels [18]. Consistent with this finding, a recent study showed that the increase in ACPA level was followed by an elevation in cytokine levels [24]. Interestingly, Deane et al studied serum samples from cases that were ACPA-negative but would later in the pre-RA period become ACPA-positive and showed higher proportions of positive cytokines in these samples than in control samples, suggesting that the increase in autoantibodies occurred later in time than the change in the levels of inflammatory markers [31].

Biomarkers of bone metabolism - Two nested case-control studies examined some markers of bone metabolism during the preclinical phase, but no definitive conclusions could be drawn. One study reported a higher prevalence of increased levels of cartilage oligomeric

Table 2: Results of the longitudinal cohort studies in autoantibody-positive arthralgia*

Author, year	Cohort†	No. of cases	Progression to arthritis (% of subjects)	Median duration from study entry to diagnosis of arthritis, months	Median duration of follow-up, months	Measured factors	Main result
Autoantibodies							
Bos et al, 2010 [41]	Amsterdam, the Netherlands (cohort 1)	147	20	11	28	IgM-RF and ACPA levels, CRP and shared epitope	The presence of ACPA, but not IgM-RF was associated with progression to arthritis.
Autoantibodies & markers of systemic inflammation							
Van de Stadt et al, 2011 [45]	Amsterdam, the Netherlands (cohort 1)	244	28	11	36	IgM-RF and ACPA levels, reactivity to 5 citrullinated peptides and shared epitope	Broader ACPA repertoire in case of development to arthritis. Similar CRP levels in patients with and without arthritis development.
Markers of systemic inflammation							
Van Baarsen et al, 2010 [42]	Amsterdam, the Netherlands (cohort 1)	109	18	7	NP	Gene expression profile	Signatures associated with arthritis development were involved in IFN-mediated immunity, hematopoieses and chemokine/cytokine activity.
Limper et al, 2012 [47]	Amsterdam, the Netherlands (cohort 1)	137	26	11	21	CRP, PCT, sPLA2, TNFα, IL-6, IL-12p70, IL-10, IFN-γ and 21 mRNA biomarkers	Similar biomarker levels in patients with and without progression to arthritis during follow-up.
Lipid profile & cardiovascular risk							
Van de Stadt et al, 2012 [48]	Amsterdam, the Netherlands (cohort 1)	348	33	12	24	Total cholesterol, HDL, LDL, triglycerides, Apo A-I and Apo B	After adjusting for ACPA status, only an association was seen between decreased level of Apo A-I and progression to arthritis.

De Hair et al, 2012 [49]	Amsterdam, the Netherlands (cohort 2)	55‡	27	13	27	BMI ≥25 kg/m² and smoking	Association between overweight on smoking on progression from arthralgia to arthritis.
Local inflammation on imaging							
Van de Stadt et al, 2010 [44]	Amsterdam, the Netherlands (cohort 1)	192	23	11	26	Joint effusion, arthritis, tenosynovitis and power Doppler signal on ultrasonography of tender and contralateral joints	Significant association at joint level between ultrasonography abnormalities and progression from arthralgia to arthritis, only a positive trend was seen at the level of the patient.
Van de Sande et al, 2011 [43]	Amsterdam, the Netherlands (cohort 2)	13	31	3	37	Dynamic contrast-enhanced MRI and synovial biopsy of knee	No differences on MRI and immuno-histochemical findings synovial biopsy in cases with and without progression to arthritis and controls.
Gent et al, 2012 [46]	Amsterdam, the Netherlands (cohort 1)	29§	31	NP	24	Macrophage PET MCPs, PIPs and wrists	All of the 4 cases with increased uptake on PET and another 5 cases without increased uptake progressed from arthralgia to arthritis.
Combining risk factors							
Van de Stadt et al, 2013 [63]	Amsterdam, the Netherlands (cohort 1)	374	35	12	32	Developing of a prediction model	The AUC value of a model with 9 variables was 0.82 (95% CI 0.75-0.89).
Preventive trials							
Bos et al, 2010 [57]	Amsterdam, the Netherlands (cohort 1)	83	20-21	NP	26	Two intramuscular injections with dexamethasone or placebo at baseline and 6 weeks	No differences in arthritis development; lower levels of ACPA and IgM-RF up to 6 months.

* All results are based on a single time point value at the time of inclusion in the cohort. †Two different cohorts (cohort 1 and cohort 2) from Amsterdam were examined in these studies and included IgM-RF and/or ACPA-positive individuals with nontraumatic arthralgia, unless indicated otherwise. ‡IgM-RF and/or ACPA-positive individuals with arthralgia (n=51) or without arthralgia with a family history of RA (n=4). §All ACPA positive. NP=not provided.

matrix protein, but this was only present in a subgroup of autoantibody-negative patients during the period closest to diagnosis [21]. The other study reported an increase in N-terminal type I procollagen propeptide and osteoprotegerin; this latter finding is not straightforward to explain [35].

Lipid profile and cardiovascular disease - Whether the lipid profile is changed in the preclinical phase is also unknown. Although a more atherogenic lipid profile was found in preclinical serum samples from RA patients compared to controls in one study [36], in a North American study increased levels of proatherogenic factors were less prevalent in pre-RA cases compared to controls [37]. Large studies were performed on the risk of cardiovascular disease, and contradictory results were also observed in those studies. Both increased and similar prevalences of myocardial infarction and coronary heart disease in patients in the preclinical phase of RA compared to controls were reported [38–40].

From autoimmunity with symptoms to clinical arthritis

In the nested case-control studies described above, the phases F (RA) and C (systemic autoimmunity) were studied, without addressing the phase in between (phase D, symptoms without clinical arthritis). A combination of phase C (systemic autoimmunity, with or without phase A and B) and phase D, however, was the starting point of prospective studies that examined progression from autoantibody-positive arthralgia to the onset of arthritis. The large majority of the published prospective data originate from the same cohort of patients with arthralgia in Amsterdam, the Netherlands. Patients with a combination of any kind of nontraumatic arthralgia and IgM-RF or ACPA positivity were recruited and followed up [41]. After a median duration of 7–12 months, 18–35% of the autoantibody-positive arthralgia patients developed clinically detectable arthritis [41–48]. Interestingly, as described above, in patients without symptoms (phase C) the chance of RA development in those who were ACPA-positive was estimated to be 5.3% [11] and 16% [10] and thus was seemingly lower than the chance observed in patients with autoantibodies and arthralgia included in the Amsterdam study. In this cohort study there were no requirements regarding the type of symptoms, which is consistent with the description of phase D by the EULAR study group [12] where the type of symptoms that predispose to RA were also not explicated. Intriguingly, however, in a subanalysis of patients with so-called inflammatory arthralgia (defined as symmetric arthralgia of small joints), 6 of 10 patients developed arthritis [41]. The risk factors for progression to clinical arthritis that were identified are described below (see also Table 2). For most factors, identification was based on one cohort study and replication in other longitudinal studies is still lacking.

Autoantibodies - The presence and level of ACPA [41] and the number of recognized epitopes [45] were associated with arthritis development (90% of the arthralgia patients who developed arthritis were ACPA-positive, compared to 58% of the patients who did not develop

arthritis). In this cohort, the presence of IgM-RF (but not its level) was only associated with arthritis development in the concomitant presence of ACPA [41].

Markers of systemic inflammation - Although some markers showed a trend toward higher levels in the patients who developed arthritis, no acute-phase reactant or cytokine was significantly associated with an increased risk of arthritis [41,47].

Lipid profile and cardiovascular risk - Slight differences were seen in lipid profile between the patients whose symptoms did and those whose symptoms did not progress to arthritis. After adjustment for ACPA status, a lower apolipoprotein A-I level was associated with progression to arthritis [48]. Another observational study of the Amsterdam cohort examining IgM-RFM- and/or ACPA-positive individuals with arthralgia or individuals with a family history of RA showed that smoking and being overweight were associated with arthritis development [49].

Imaging of local joint inflammation in the preclinical phase - Thus far, we have not yet discussed whether there is local inflammation in the joints in the preclinical phase. Practical, and perhaps also ethical, hurdles hamper performing synovial biopsies in patients with symptoms without clinical arthritis. Nonetheless, inflammation was demonstrated in synovial tissue from clinically uninvolved knees in RA patients who later developed clinically detectable arthritis in the biopsied knee, indicating that a phase of asymptomatic arthritis precedes clinical arthritis [50].

Recently, three different imaging modalities (ultrasonography, magnetic resonance imaging [MRI], and positron emission tomography [PET]) were used in cross-sectional or prospective studies of autoantibody-positive patients with arthralgia, assessing the presence of local subclinical inflammation. The study that used ultrasonography demonstrated abnormalities that were associated with progression to arthritis at the level of the joint but not at the level of the patient [44]. More studies of ultrasonography are required to evaluate whether it is valuable for identifying preclinical inflammation.

Another study from Amsterdam used PET and observed increased uptake on the hand or wrist in 4 of the 9 ACPA-positive arthralgia patients who later developed arthritis [46]. A comparable study evaluating MRI of the knees of autoantibody-positive arthralgia patients did not find differences between patients who did and those who did not progress to clinical arthritis. It was not reported what proportion of the scanned patients had symptoms in the scanned knee [43]. A recent cross-sectional MRI study was performed on a different set of ACPA-positive arthralgia patients than those included in the prospective cohort described above and evaluated inflammation using MRI of the hand and foot joints [51]. ACPA-positive patients with arthralgia of small joints had higher MRI inflammation scores than healthy controls, but lower scores than ACPA-positive RA patients. Furthermore, the MRI inflammation levels were significantly associated with the CRP levels in the ACPA-positive

arthralgia patients [51]. These data support the notion that there is also local inflammation in the preclinical phase of RA. Despite these positive initial findings, the numbers of patients included in these studies were small, and further imaging studies are needed to increase insight into processes occurring locally in the joint in the preclinical phase.

Summary of what is acknowledged

In summary, multiple studies have evaluated various biomarkers in the preclinical phases of RA. There is convincing evidence that autoantibody development and maturation occurs before clinically detectable arthritis develops. The time course between autoantibody and symptom development is still indefinite, and multiple large prospective studies starting in the symptom-free period have not been published. Furthermore, based on the data available, there is suggestive evidence that inflammation occurs in the preclinical phase of RA, both locally in the joint and measurable in the systemic circulation. Some of the results on this subject are contradictory, however, as increased levels of acute-phase reactants and cytokines were observed in some of the nested case-control studies, but not in the prospective cohort study. The time course between the appearance of autoantibodies and inflammation also remains to be elucidated.

There are factors that should be taken into consideration when interpreting the results of the studies reviewed here. The number of RA cases in most studies was not large, and in the prospective studies depended on the duration of follow-up. In addition, the available findings obtained in prospectively followed up autoantibody-positive arthralgia patients are largely based on a single Dutch cohort. Hence, these data are not yet replicated and the generalizability of the findings has not yet been assessed. Future studies in large and different cohorts are required for validation.

What subsequently needs to be assessed?

Although evidence has emerged that autoimmune deregulation starts in the preclinical phase, several major issues are yet unexplored. Table 3 provides a research agenda. The biologic basis for the development of RA-related autoimmunity is not yet elucidated. It has been suggested that RA-related autoantibodies are generated on extraarticular sites and are associated with mucosal inflammation [52–54]. Associations with periodontitis, antibodies to Porphyromonas gingivalis [52,53], and airway abnormalities [54] have been described, but the causality is unclear.

Observations that autoantibodies against citrullinated vimentin can activate osteoclasts [55] and that the presence of RF is a significant predictor of cardiovascular events and mortality in individuals without RA [56] suggest that the presence of these autoantibodies is harmful. Given the prevalence of ACPA in the general population of 1%, a relevant question is to consider when the presence of autoantibodies is benign or detrimental.

For individuals with abnormal test results, e.g., the presence of RA-related

autoantibodies or abnormalities on imaging, the main question is what the absolute risk is for progression to arthritis. These risks may differ in the presence of several other abnormal test results and also depend on the presence or absence of certain symptoms. Furthermore, in order to explore the preclinical phase at the individual level, the persons who will progress to developing arthritis and RA need to be identified with high accuracy (for instance with a greater than 80% chance of arthritis/RA development if test results are positive). The development of such risk stratification is basic to the design of preventive trials. To date, one preventive trial has been performed in a total population of autoantibody-positive arthralgia patients; dexamethasone was not effective in the median study period of 26 months [57].

Longitudinal cohort studies

Large and multiple prospective follow-up studies are needed to answer these questions and to validate the answers. Fortunately, several initiatives have been formed to establish such cohorts, starting with either the general population or high-risk populations (e.g., family members of RA patients, arthralgia patients, or ACPA-positive individuals). One of the approaches of the Studies of the Etiology of RA (SERA) group in the US and of El-Gabalawy et al in studying North American Native populations in Canada is to start with persons who are at increased risk because of family history [58]. These first-degree relatives of RA patients who do not themselves have clinical arthritis are being followed up. The Canadian North American Native population forms a unique population to identify individuals who may be in the pre-RA period because of the high prevalence of ACPA (4-19%) and RA (±3%) [59]. Cross-sectional baseline results of these studies have already shown higher levels of multiple inflammation markers [60] and an association of some inflammation markers with autoantibodies in these first-degree relatives without clinical arthritis [61].

The two available cohorts of autoantibody-positive individuals from Amsterdam, the Netherlands, are extensively described in this review [41,43]. The Leiden approach is to longitudinally study patients with "clinically suspect arthralgia." This indicates arthralgia that, because of the character of the symptoms, the rheumatologist suspects will progress to arthritis over time; IgM-RF or ACPA-positivity is not required. The presence of local inflammation in all of these persons will be studied in detail with dedicated extremity MRI of the wrist, hand, and foot joints.

Hopefully, the results of future studies will increase our understanding of the processes occurring during the preclinical phase of RA and enable targeted intervention in these processes before the clinical picture that is characteristic for RA has evolved.

Table 3. Research agenda for examining the preclinical phase of RA

1. Where (in what tissue or organ) does inflammation in the light of RA start?
2. What is the timing between genetic factors, environmental exposures, and the development of autoimmunity and arthritis? Similarly, what is the temporal relationship between the onset of inflammation, autoantibodies and symptoms in the pre-RA phase?
3. In which persons or circumstances is the presence of RA-related autoantibodies not harmful and not associated with progression towards disease and in which persons or circumstances are RA-related autoantibodies detrimental, indicating a very early phase of RA?
4. What is the predictive value of the variables mentioned below in the pre-RA stages A, B, C and D for the development of RA?
    - Genetic and epigenetic variants
    - Environmental factors
    - RA-related autoantibodies
    - Serologic inflammation markers
    - Presence of certain symptoms
    - Patient characteristics
    - Imaging abnormalities
    - Histological abnormalities
5. Does preclinical inflammation occur in ACPA- and/or RF-negative RA?
6. Can we, with the help of prospective studies, adequately identify the individuals who will progress to RA in each of the pre-RA phases? (This may be challenging given the low prior chance on RA in the earliest pre-RA phases) Such risk stratification is basic to the development of dedicated preventive trials in the pre-RA phases.
7. Does treatment in the pre-RA phase prevent disease chronicity?

REFERENCES

1. Finckh A, Liang MH, van Herckenrode CM, et al. Long-term impact of early treatment on radiographic progression in rheumatoid arthritis: A meta-analysis. Arthritis Care Res 2006;55:864–72.

2. Van der Linden MP, le Cessie S, Raza K, et al. Long-term impact of delay in assessment of patients with early arthritis. Arthritis Rheum 2010;62:3537–46.

3. Aho K, Palosuo T, Raunio V, et al. When does rheumatoid disease start? Arthritis Rheum 1985;28:485–9.

4. Jónsson T, Thorsteinsson J, Kolbeinsson A, et al. Population study of the importance of rheumatoid factor isotypes in adults. Ann Rheum Dis 1992;51:863–8.

5. Aho K, von Essen R, Kurki P, et al. Antikeratin antibody and antiperinuclear factor as markers for subclinical rheumatoid disease process. J Rheumatol 1993;20:1278–81.

6. Kurki P, Aho K, Palosuo T, et al. Immunopathology of rheumatoid arthritis. Antikeratin antibodies precede the clinical disease. Arthritis Rheum 1992;35:914–7.

7. Del Puente A, Knowler WC, Pettitt DJ, et al. The incidence of rheumatoid arthritis is predicted by rheumatoid factor titer in a longitudinal population study. Arthritis Rheum 1988;31:1239–44.

8. Walker DJ, Pound JD, Griffiths ID, et al. Rheumatoid factor tests in the diagnosis and prediction of rheumatoid arthritis. Ann Rheum Dis 1986;45:684–90.

9. Silman AJ, Hennessy E, Ollier B. Incidence of Rheumatoid Arthritis in a Genetically Predisposed Population. Rheumatology 1992;31:365–8.

10. Rantapää-Dahlqvist S, de Jong BA, Berglin E, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum 2003;48:2741–9.

11. Nielen MM, van Schaardenburg D, Reesink HW, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: A study of serial measurements in blood donors. Arthritis Rheum 2004;50:380–6.

12. Gerlag DM, Raza K, van Baarsen LG, et al. EULAR recommendations for terminology and research in individuals at risk of rheumatoid arthritis: report from the Study Group for Risk Factors for Rheumatoid Arthritis. Ann Rheum Dis 2012;71:638–41.

13. Van der Woude D, Houwing-Duistermaat JJ, Toes RE, et al. Quantitative heritability of anti-citrullinated protein antibody-positive and anti-citrullinated protein antibody-negative rheumatoid arthritis. Arthritis Rheum 2009;60:916–23.

14. Hoovestol RA, Mikuls TR. Environmental Exposures and Rheumatoid Arthritis Risk. Curr Rheumatol Rep 2011;13:431–9.

15. Lahiri M, Morgan C, Symmons DP, et al. Modifiable risk factors for RA: prevention, better than cure? Rheumatology 2012;51:499–512.

16. Klareskog L, Stolt P, Lundberg K, et al. A new model for an etiology of rheumatoid arthritis: Smoking may trigger HLA–DR (shared epitope)–restricted immune reactions to autoantigens modified by citrullination. Arthritis Rheum 2006;54:38–46.

17. Keenan BT, Chibnik LB, Cui J, et al. Effect of interactions of glutathione S-transferase T1, M1, and P1 and HMOX1 gene promoter polymorphisms with heavy smoking on the risk of rheumatoid arthritis. Arthritis Rheum 2010;62:3196–210.

18. Jørgensen KT, Wiik A, Pedersen M, et al. Cytokines, autoantibodies and viral antibodies in premorbid and postdiagnostic sera from patients with rheumatoid arthritis: case–control study nested in a cohort of Norwegian blood donors. Ann Rheum Dis 2008;67:860–6.

19. Kokkonen H, Söderström I, Rocklöv J, et al. Up-regulation of cytokines and chemokines predates the onset of rheumatoid arthritis. Arthritis Rheum 2010;62:383–91.

20. Shadick NA, Cook NR, Karlson EW, et al. C-reactive protein in the prediction of rheumatoid arthritis in women. Arch Intern Med 2006;166:2490–4.

21. Turesson C, Bergström U, Jacobsson LT, et al.

Increased cartilage turnover and circulating autoantibodies in different subsets before the clinical onset of rheumatoid arthritis. Ann Rheum Dis 2011;70:520–2.

22. Majka DS, Deane KD, Parrish LA, et al. Duration of preclinical rheumatoid arthritis-related autoantibody positivity increases in subjects with older age at time of disease diagnosis. Ann Rheum Dis 2008;67:801–7.

23. Chibnik LB, Mandl LA, Costenbader KH, et al. Comparison of Threshold Cutpoints and Continuous Measures of Anti-Cyclic Citrullinated Peptide Antibodies in Predicting Future Rheumatoid Arthritis. J Rheumatol 2009;36:706–11.

24. Sokolove J, Bromberg R, Deane KD, et al. Autoantibody Epitope Spreading in the Pre-Clinical Phase Predicts Progression to Rheumatoid Arthritis. PLoS ONE 2012;7:e35296.

25. Van der Woude D, Rantapää-Dahlqvist S, Ioan-Facsinay A, et al. Epitope spreading of the anti-citrullinated protein antibody response occurs before disease onset and is associated with the disease course of early arthritis. Ann Rheum Dis 2010;69:1554–61.

26. Van de Stadt LA, de Koning MH, van de Stadt RJ. Development of the anti-citrullinated protein antibodies repertoire prior to the onset of rheumatoid arthritis. Arthritis Rheum 2011;:3226–33.

27. Masi AT, Aldag JC, Sipes J. Do elevated levels of serum C-reactive protein predict rheumatoid arthritis in men: correlations with pre-RA status and baseline positive rheumatoid factors. J Rheumatol 2001;28:2359–61.

28. Aho K, Palosuo T, Knekt P, et al. Serum C-reactive protein does not predict rheumatoid arthritis. J Rheumatol 2000;27:1136–8.

29. Rantapää-Dahlqvist S, Boman K, Tarkowski A, et al. Up regulation of monocyte chemoattractant protein-1 expression in anti-citrulline antibody and immunoglobulin M rheumatoid factor positive subjects precedes onset of inflammatory response and development of overt rheumatoid arthritis. Ann Rheum Dis 2007;66:121–3.

30. Karlson EW, Chibnik LB, Tworoger SS, et al. Biomarkers of inflammation and development of rheumatoid arthritis in women from two prospective cohort studies. Arthritis Rheum 2009;60:641–52.

31. Deane KD, O'Donnell CI, Hueber W, et al. The number of elevated cytokines and chemokines in preclinical seropositive rheumatoid arthritis predicts time to diagnosis in an age-dependent manner. Arthritis Rheum 2010;62:3161–72.

32. Nielen MM, van Schaardenburg D, Reesink HW, et al. Increased levels of C-reactive protein in serum from blood donors before the onset of rheumatoid arthritis. Arthritis Rheum 2004;50:2423–7.

33. Kokkonen H, Mullazehi M, Berglin E, et al. Antibodies of IgG, IgA and IgM isotypes against cyclic citrullinated peptide precede the development of rheumatoid arthritis. Arthritis Res Ther 2011;13:R13.

34. Nielen MM, van Schaardenburg D, Reesink HW, et al. Simultaneous development of acute phase response and autoantibodies in preclinical rheumatoid arthritis. Ann Rheum Dis 2006;65:535–7.

35. Van Schaardenburg D, Nielen MM, Lems WF, et al. Bone metabolism is altered in preclinical rheumatoid arthritis. Ann Rheum Dis 2011;70:1173–4.

36. Van Halm VP, Nielen MM, Nurmohamed MT, et al. Lipids and inflammation: serial measurements of the lipid profile of blood donors who later developed rheumatoid arthritis. Ann Rheum Dis 2007;66:184–8.

37. Myasoedova E, Crowson CS, Kremers HM, et al. Total cholesterol and LDL levels decrease before rheumatoid arthritis. Ann Rheum Dis 2010;69:1310–4.

38. Maradit-Kremers H, Crowson CS, Nicola PJ, et al. Increased unrecognized coronary heart disease and sudden deaths in rheumatoid arthritis: A population-based cohort study. Arthritis Rheum 2005;52:402–11.

39. Holmqvist ME, Wedrén S, Jacobsson LTH, et al. No increased occurrence of ischemic heart disease prior to the onset of rheumatoid arthritis: Results from two Swedish population-based rheumatoid arthritis cohorts. Arthritis Rheum 2009;60:2861–9.

40. Kerola AM, Kerola T, Kauppi MJ, et al. Cardiovascular comorbidities antedating the diagnosis of rheumatoid arthritis. Ann Rheum Dis 2013;72:1826–9.

41. Bos WH, Wolbink GJ, Boers M, et al. Arthritis development in patients with arthralgia is strongly associated with anti-citrullinated protein antibody status: a prospective cohort study. Ann Rheum Dis 2010;69:490–4.

42. Van Baarsen LG, Bos WH, Rustenburg F, et al. Gene expression profiling in autoantibody-positive patients with arthralgia predicts development of arthritis. Arthritis Rheum 2010;62:694–704.

43. Van Sande MG, de Hair MJ, van der Leij C, et al. Different stages of rheumatoid arthritis: features of the synovium in the preclinical phase. Ann Rheum Dis 2011;70:772–7.

44. Van de Stadt LA, Bos WH, Meursinge Reynders M, et al. The value of ultrasonography in predicting arthritis in auto-antibody positive arthralgia patients: a prospective cohort study. Arthritis Res Ther 2010;12:R98.

45. Van de Stadt LA, van der Horst AR, de Koning MH, et al. The extent of the anti-citrullinated protein antibody repertoire is associated with arthritis development in patients with seropositive arthralgia. Ann Rheum Dis 2011;70:128–33.

46. Gent YY, Voskuyl AE, Kloet RW, et al. Macrophage positron emission tomography imaging as a biomarker for preclinical rheumatoid arthritis: Findings of a prospective pilot study. Arthritis Rheum 2012;64:62–6.

47. Limper M, van de Stadt L, Bos W, et al. The Acute-phase Response Is Not Predictive for the Development of Arthritis in Seropositive Arthralgia – A Prospective Cohort Study. J Rheumatol 2012;39:1914–7.

48. Van de Stadt LA, van Sijl AM, van Schaardenburg D, et al. Dyslipidaemia in patients with seropositive arthralgia predicts the development of arthritis. Ann Rheum Dis 2012;71:1915–6.

49. De Hair MJ, Landewé RB, van de Sande MG, et al. Smoking and overweight determine the likelihood of developing rheumatoid arthritis. Ann Rheum Dis 2013;72:1654–8.

50. Kraan MC, Versendaal H, Jonker M, et al. Asymptomatic synovitis precedes clinically manifest arthritis. Arthritis Rheum 1998;41:1481–8.

51. Krabben A, Stomp W, van der Heijde DM, et al. MRI of hand and foot joints of patients with anticitrullinated peptide antibody positive arthralgia without clinical arthritis. Ann Rheum Dis 2013;72:1540–4.

52. Hitchon CA, Chandad F, Ferucci ED, et al. Antibodies to Porphyromonas gingivalis Are Associated with Anticitrullinated Protein Antibodies in Patients with Rheumatoid Arthritis and Their Relatives. J Rheumatol 2010;37:1105–12.

53. Mikuls TR, Thiele GM, Deane KD, et al. Porphyromonas gingivalis and disease-related autoantibodies in individuals at increased risk of rheumatoid arthritis. Arthritis Rheum 2012;64:3522–30.

54. Demoruelle MK, Weisman MH, Simonian PL, et al. Brief Report: Airways abnormalities and rheumatoid arthritis–related autoantibodies in subjects without arthritis: Early injury or initiating site of autoimmunity? Arthritis Rheum 2012;64:1756–61.

55. Harre U, Georgess D, Bang H, et al. Induction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin. J Clin Invest 2012;122:1791–802.

56. Liang KP, Maradit-Kremers H, Crowson CS, et al. Autoantibodies and the Risk of Cardiovascular Events. J Rheumatol 2009;36:2462–9.

57. Bos WH, Dijkmans BA, Boers M, et al. Effect of dexamethasone on autoantibody levels and arthritis development in patients with arthralgia: a randomised trial. Ann Rheum Dis 2010;69:571–4.

58. Kolfenbach JR, Deane KD, Derber LA, et al. A prospective approach to investigating the natural history of preclinical rheumatoid arthritis (RA) using first-degree relatives of probands with RA. Arthritis Care Res 2009;61:1735–42.

59. El-Gabalawy HS, Robinson DB, Hart D, et al. Immunogenetic Risks of Anti-Cyclical Citrullinated Peptide Antibodies in a North American Native Population with Rheumatoid Arthritis and Their First-degree Relatives. J Rheumatol 2009;36:1130–5.

2

60. El-Gabalawy HS, Robinson DB, Smolik I, et al. Familial clustering of the serum cytokine profile in the relatives of rheumatoid arthritis patients. Arthritis Rheum 2012;64:1720–9.

61. Hughes-Austin JM, Deane KD, Derber LA, et al. Multiple cytokines and chemokines are associated with rheumatoid arthritis-related autoimmunity in first-degree relatives without rheumatoid arthritis: Studies of the Aetiology of Rheumatoid Arthritis (SERA). Ann Rheum Dis 2013;72:901–7.

62. Kolfenbach JR, Deane KD, Derber LA, et al. Autoimmunity to peptidyl arginine deiminase type 4 precedes clinical onset of rheumatoid arthritis. Arthritis Rheum 2010;62:2633–9.

63. Van de Stadt LA, Witte BI, Bos WH, et al. A prediction rule for the development of arthritis in seropositive arthralgia patients. Ann Rheum Dis 2013;72:1920–6.

2