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The Duration of Pre-Clinical Rheumatoid Arthritis-Related Autoantibody Positivity Increases in Subjects with Older Age at Time of Disease Diagnosis

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Abstract

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COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

DM participated in the design and analysis of this study and the writing of this manuscript.

KD participated in the analysis of data and preparation of manuscript.

LP participated in the analysis of samples and data and preparation of manuscript.

AB and AL contributed statistical expertise in the analysis of data for this manuscript.

CW participated in subject evaluation and determination of eligibility for this study

MR participated in the design of this study and procurement of the serum samples for analysis

WG participated in the design of this study and procurement of the serum samples for analysis, as well as subject evaluation and determination of eligibility for this study.

JN participated in the design and analysis of this study and preparation of manuscript.

VMH participated in the design and analysis of this study, antibody testing, and manuscript preparation.

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Objectives—This study investigated factors that may influence the prevalence and timing of appearance of rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) antibodies during the pre-clinical phase of rheumatoid arthritis (RA) development.

Methods—243 serial pre-diagnosis serum samples from 83 subjects with RA were examined for the presence of RF and anti-CCP antibodies.

Results—57% and 61% of subjects had at least one pre-diagnosis sample positive for RF or anti-CCP, respectively. Gender and race were not significantly associated with the prevalence or timing of pre-clinical antibody appearance. Pre-clinical anti-CCP positivity was strongly associated with the development of erosive RA (OR 4.64; 95% CI 1.71–12.63; $p=0.003$), but RF was not ($p=0.11$). Additionally, as age at the time of diagnosis of RA increased the duration of pre-diagnosis antibody positivity for RF and anti-CCP increased, with the longest duration of pre-clinical antibody positivity seen in patients diagnosed with RA over the age of 40. In no subjects did symptom onset precede the appearance of RF or anti-CCP antibodies.

Conclusions—The period of time that RF and anti-CCP are present prior to diagnosis lengthens as the age at the time of diagnosis of RA increases. This finding suggests that factors such as genetic risk or environmental exposures influencing the temporal relationship between the development of RA-related autoantibodies and clinically-apparent disease onset may differ with age.

Keywords

autoantibody; rheumatoid arthritis; rheumatoid factor; anti-cyclic citrullinated peptide antibody

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic, autoimmune, inflammatory disease affecting approximately 1% of the population. [1] It is characterized by joint pain, stiffness, and swelling, and can lead to significant morbidity and increased mortality. [2]

The diagnosis of RA is usually made when an individual develops joint symptoms meeting the American Rheumatism Association's 1987 revised classification criteria. [3] Therefore, studies investigating the pathogenesis of disease have largely focused on individuals with definable symptomatic disease. However, over the past several years, it has become clear that the clinically-apparent symptoms of RA are likely a late manifestation of disease-specific immune dysregulation. Supporting this, multiple studies have now shown that RA-related autoantibodies including rheumatoid factor (RF) and highly disease-specific anti-cyclic citrullinated peptide (anti-CCP) antibodies can be present years prior to the onset of symptoms of RA, and in some cases, combinations of autoantibodies and genetic markers can be highly predictive of future development of RA. [4–10]

These findings have led to an increased investigative focus on the pre-clinical period of disease development in order to understand the role of genetic, demographic, and environmental factors on the development of RA-related autoimmunity. However, little is known about the relationship between important factors such as age, sex, race, and disease severity and the pre-clinical appearance and behavior of the biomarkers RF and anti-CCP antibodies.

The purpose of this current study was to utilize pre-RA diagnosis serum samples from a cohort of RA subjects selected from the United States Military to investigate the relationship between age, sex, race, and disease severity and the presence and timing of appearance of pre-clinical RA-related autoantibodies.

PATIENTS AND METHODS

Study Population

The Department of Defense Serum Repository (DoDSR) was established in 1986 for the purpose of evaluating and improving the health of the United States Armed Forces. Samples are collected from military members at enlistment, deployment, and every other year of service. Currently, the DoDSR houses over 37 million specimens.

For this analysis, the study population included members of the U.S. Military assigned to the North Atlantic Medical Region who were seen at the Walter Reed Army Medical Center (WRAMC) Rheumatology Clinic between 1989 and 2003 with a diagnosis of RA. 156 cases with RA were identified from this clinic. Of these, 83 were found to have pre-diagnosis serum samples available in the DoDSR, with 66 (80%) having two or more pre-diagnosis serum samples, and 39 (47%) having four pre-diagnosis samples (Table 1). The remaining 73 subjects had their diagnosis of RA made prior to the development of the DoDSR and did not have pre-diagnosis serum samples available.

Of the 83 cases with pre-diagnosis serum samples, 81 met at least 4 of 7 criteria for RA based on the 1987 American Rheumatism Association's revised criteria. [3] The remaining two subjects met 3 of 7 criteria and were considered to have RA by board-certified rheumatologists.

Information on gender, race, symptom onset, age at the time of RA diagnosis, sero-positivity (RF only), and the presence of radiographic erosions were obtained by chart review at the WRAMC. Based on RF testing as part of clinical management at WRAMC, 67 cases (81%) were classified as sero-positive at or after the time of diagnosis (Table 1). Information on radiographic erosions was available for 76 (92%) cases, with 42 cases (51%) identified as having erosions at or after the time of diagnosis. Race was categorized as non-Hispanic white, black, and other, with the other category including Hispanics and Asians.

Eighty-three control subjects without RA were selected from the DoDSR in order to provide reference levels of autoantibody measures. These subjects were matched to the cases on gender, age, race, and medical region of assignment. They were also matched such that they had serum samples drawn and stored close to the time of the corresponding cases' serum sample collections.

All samples were de-identified prior to autoantibody assessment. After the autoantibody testing was completed, the DoDSR provided the coding key to link the serum samples to subject data. The study protocol was approved by the respective Institutional Review Boards at the WRAMC and the University of Colorado.

Autoantibody Assays

Autoantibody assays were performed at the University of Colorado at Denver and Health Sciences Center Rheumatology Clinical Research Laboratory. RF was measured by nephelometry using polystyrene beads coated with human and sheep γ -globulins according to manufacturer's specifications (Dade Behring, Newark, Delaware). The ACR Classification Criteria for RA specifies that a RF level is considered positive if present in less than 5% of control subjects. [3] Accordingly, we determined a general RF cut-off level for positivity of greater than 15.2 IU/mL in all subjects using a 95% cut-off in single samples from each of the 83 healthy military control subjects. Additionally, to account for possible age-related changes in RF levels, we determined age-specific cut-offs for RF positivity using the same military control samples divided into the following age categories: 20–29, 30–39, 40–49, and 50+.

The anti-cyclic citrullinated peptide antibody (anti-CCP) 2 ELISA assay was used to measure IgG antibodies to CCP (Diastat, Axis-Shield Diagnostics, Ltd., Dundee, Scotland). Per the manufacturer's specifications, a positive test was defined as a level of greater than 5 units per mL (U/mL). None of the control subjects were positive for anti-CCP. For anti-CCP, an age-specific cut-off was not determined as no controls had levels greater than 5 U/mL. Moreover, previous studies of unaffected control populations did not find any differences in rates of antibodies against citrullinated antigens related to age. [11]

Statistical Analysis

The prevalence of pre-diagnosis antibody positivity was determined using data from all 83 case subjects, with a single RF cut-off of >15.2 IU/mL. The relationship between the variables gender, race, age at time of diagnosis of RA, and erosive disease on the presence of pre-diagnosis RF or anti-CCP was analyzed using logistic regression modeling. The associations of RF or anti-CCP positivity with each of these variables are reported in odds ratios (OR) with 95% confidence intervals (CI) and corresponding p-values.

For each antibody (RF and anti-CCP), the timing of appearance and duration of positivity in pre-diagnosis period was analyzed separately by a parametric Weibull survival model taking into account interval and left censorship, and using only those subjects positive for RF or anti-CCP during the pre-clinical period. The serum samples were analyzed 'backwards' in time, with the earliest appearance of antibody considered as the event. This reversal of the time axis in survival analysis is a standard method for retrospective analysis of left censored observations. [12]

A subset of cases was positive for RA-related antibodies in the earliest available serum sample (Table 2). As such, the true time of pre-diagnosis antibody positivity may have been earlier than the initial sample. We accounted for this with left censoring in the survival analysis of all subjects with pre-diagnosis antibody positivity, including those whose first available sample was antibody positive. In addition, we performed survival analyses utilizing only those subjects who converted from negative to positive antibody status during the pre-clinical period. In both of these analyses (first sample-positive subjects and converters), survival models with interval censoring were used as it is assumed that subjects become positive for the antibody during the time interval between antibody negative and positive samples.

Survival analysis was also performed to evaluate the influence of the cases' characteristics on the timing of pre-diagnosis antibody appearance. Variables considered *a priori* to be potential influences on the timing of pre-clinical autoantibody positivity were age at the time of diagnosis of RA (in 10-year intervals), sex, race, the presence of erosions, and the duration of pre-clinical samples available. Comparisons between survival curves are reported in hazard ratios (HR) with associated 95% confidence intervals (CI) and p-values. Additionally, an overall test for trend of increased duration of pre-diagnosis antibody positivity with increasing age was evaluated by including the age category as a continuous covariate in the Weibull survival model.

The sequence of appearance of RF and anti-CCP in the pre-diagnosis period was analyzed using a parametric interval censored survival model for clustered data. [13] All analyses were performed using SAS version 9.1.

RESULTS

Patient Population

Demographics and clinical features of the military RA cases including age at diagnosis, gender, race, presence of erosive disease, and RF positivity at diagnosis are presented in Table 1. A total of 243 pre-diagnosis serum samples were available from cases with a mean of 2.9 samples per case (range 1–4). Samples were available a mean of 6.6 years (range 0.1–13.7) prior to RA diagnosis.

Pre-Diagnosis Antibody Prevalence

Of the 83 subjects with RA, 47 (57%) were RF positive and 51 (61%) were anti-CCP positive prior to diagnosis (Tables 2 and 3). Additionally, 57 (69%) were positive for either RF or anti-CCP prior to diagnosis, and 41 (49%) had at least one pre-clinical sample positive for both antibodies. Of the RF and anti-CCP positive cases, 22/47 (47%) and 22/51 (43%), respectively, were positive for these antibodies in their earliest available pre-diagnosis serum sample.

Factors Associated with Pre-Diagnosis Antibody Positivity

Pre-diagnosis RF or anti-CCP positivity was not significantly associated with sex, race, and age at diagnosis. Pre-diagnosis RF positivity was not associated with the development of erosive RA; however, pre-diagnosis anti-CCP positivity was strongly associated with the development of erosive RA (OR 4.64; 95% CI 1.71, 12.63; $p=0.003$) (Table 4). This association persisted after controlling for RF in anti-CCP positive cases.

Sequence of Appearance of RF and Anti-CCP in the Pre-Diagnosis Period

In all subjects with pre-diagnosis antibody positivity, RF was present a median of 6.0 years prior to RA diagnosis (95% CI 4.5, 8.0). Anti-CCP was present a median of 5.4 years prior to diagnosis (95% CI 4.1, 7.1). This difference in duration of pre-clinical antibody positivity was not statistically significant ($p=0.78$). In analysis using only those subjects known to have converted to antibody positive status in the pre-clinical period, RF was present a median of 3.6 years prior to diagnosis (95% CI 2.7, 4.8), and anti-CCP was present a median of 3.4 years prior to diagnosis (95% CI 2.6, 4.4).

Factors Affecting the Timing of Appearance of Antibody Positivity in the Pre-Diagnosis Period

In univariate analysis, gender, race, and erosive disease were not significantly associated with longer duration of pre-diagnosis RF and anti-CCP positivity. However, increased age-at-diagnosis of RA (in 10-year intervals) was associated with longer duration of RF and anti-CCP positivity in the pre-diagnosis period (Table 5, Figure 1).

In survival analysis controlling for age, sex, and the duration of pre-clinical samples available, for both RF and anti-CCP, as age at diagnosis increased by 10-year intervals, the duration of pre-clinical antibody positivity increased (Table 5, Figure 1). For RF using all subjects with pre-diagnosis antibody positivity ($N=47$), the age group 50+ had a significantly longer duration of pre-clinical antibody positivity when compared with the referent age group (20–29 year-old) (HR 0.14; 95% CI 0.02–0.83; $p=0.04$) (Table 5a). There was also a longer duration of pre-clinical antibody positivity in the 30–39 year-old and 40–49 year-old age groups when compared with the youngest age group, although no HR reached statistical significance (Table 5a).

For anti-CCP, using all subjects with pre-diagnosis antibody positivity (N=51), the 40–49 age group had a significantly longer duration of pre-clinical antibody positivity compared to the referent group (HR 0.16; 95% CI 0.04–0.68; $p=0.02$) (Table 5b). There was also a longer duration of antibody positivity in the 30–39 and 50+ year-old age-at-diagnosis groups compared to the youngest age group, although no HR reached statistical significance (Table 5b).

In trend analysis, longer duration of pre-diagnosis antibody positivity with increased age-at-diagnosis of RA (in 10-year intervals) was statistically significant for RF ($p=0.03$) and marginally significant for anti-CCP ($p=0.07$) (Figure 2).

Univariate analysis was also performed using only subjects with pre-clinical antibody conversion from negative to positive. For RF converters (N=25), the age group 40–49 had significantly longer duration of pre-clinical antibody positive when compared with the age group 20–29 (HR 0.21; 95% CI 0.06, 0.72, $p<0.01$). For anti-CCP converters (N=29), the age-group 40–49 had significantly longer duration of pre-clinical antibody positivity when compared with 20–29 year-olds (HR 0.14; 95% CI 0.04, 0.51; $p<0.01$). In addition, trend analysis for RF and anti-CCP in these converters showed significant increase in the duration of pre-clinical antibody positivity with increasing age (for RF, $p=0.02$; for anti-CCP, $p=0.01$).

Antibody Appearance in Relationship to Symptom Onset

Symptoms of RA during the pre-diagnosis period were obtained by chart review and were defined as pain, stiffness, or swelling in peripheral joints. Symptoms were present a median of 0.5 years before diagnosis, regardless of pre-clinical antibody status or age at diagnosis.

Of the 57 subjects with RF or anti-CCP present prior to the diagnosis of RA, 47 (82.5%) had antibodies appear before symptom onset. In the other 10 subjects, data were insufficient to determine the median time of antibody appearance prior to symptom onset; however, none of the individuals could be classified as reporting symptoms prior to the appearance of autoantibodies.

DISCUSSION

We used the resources of the Walter Reed Army Medical Center Rheumatology Clinic and the Department of Defense Serum Repository to examine serial stored serum samples for the presence of RA-related autoimmunity in the pre-diagnosis period. Of the 83 cases of RA examined, 57% and 61% of subjects exhibited RF and anti-CCP antibody positivity, respectively, prior to diagnosis. Sex, race, and age at diagnosis did not significantly influence the prevalence of pre-clinical antibody positivity. However, the presence of pre-clinical anti-CCP positivity (but not RF) was associated with a significantly increased risk for the development of erosive disease – a finding similar to that recently published by Berglin et al. [14]

Our reported prevalence rates of pre-clinical RF and anti-CCP positivity are higher than those previously reported. Nielen *et al* found a pre-clinical prevalence of RF of 28% and anti-CCP of 41%, and Rantapaa-Dahlqvist *et al* reported the prevalence of RF of 19% and anti-CCP of 34%. [4, 5] This may reflect methodologic differences in RF or anti-CCP antibody testing, or study design issues such as ascertainment of diagnosis of RA and symptom data. It is also possible that these differences reflect immunologic differences in RA in the differing populations or differences in genetics or exposures to risk factors for autoimmunity. [15, 16] Another possible explanation for this finding is that, although the military RA cases were not preferentially selected based on RF positivity, there may have

been some selection bias in the original inclusion of these subjects in the military clinic from which they were chosen.

The association of the presence of pre-clinical anti-CCP but not RF with erosive disease may be explained by recent assertions that anti-CCP plays a direct pathogenic role in joint inflammation. This is supported by recent work which showed that the addition of antibodies to citrullinated peptides significantly enhances joint inflammation in mouse models of collagen-induced inflammatory arthritis. [17] Also, human studies have shown that anti-CCP measured after RA diagnosis may be more strongly related to erosive disease than RF. [18] Interestingly, in this military RA cohort, the duration of pre-clinical anti-CCP positivity was not associated with a higher risk for erosive disease. This will need further exploration in future studies.

Most strikingly, we found that subjects that were older at the time of diagnosis of RA had a significantly longer duration of pre-clinical autoantibody positivity. This may seem at first glance to be due to the possibility of longer pre-diagnosis symptoms or greater duration of stored serum samples available in older military RA subjects. However, symptoms of RA were not present earlier in older versus younger subjects, and the increased duration of pre-clinical antibody positivity persisted after controlling statistically for the duration of pre-clinical samples available.

There is controversy whether levels of RF may increase as a result of age, with some studies showing age-associated increases and others showing no such increases. [19–21] In this study, using a single RF cut-off (>15.2 IU/mL), there was a trend towards decreased RF positivity in older cases. In addition, we also evaluated the prevalence and duration of pre-diagnosis RF positivity using age specific cut-offs for RF determined in the military control population. Using these cut-offs, we found no significant differences in the prevalence of pre-diagnosis RF positivity, and in all RF positive cases as well as RF converters only, we found results similar to those in our original analysis, with the duration of pre-diagnosis RF positivity increasing as age-at-diagnosis increased in survival models and in trend analysis (data not shown). These findings are consistent in this study with a lack of confounding effect of increasing background rates of RF positivity studying older subjects. For anti-CCP, current literature suggests that levels of antibodies against citrullinated proteins are not non-specifically elevated in older subjects [11]. In our analysis, as none of our controls had CCP levels greater than 5 U/mL, age-specific cut-offs were not determined. Additionally, using the standardized anti-CCP cutoff, there was a non-significant trend towards decreased anti-CCP positivity in older RA subjects.

Several mechanisms might explain the increased duration of pre-clinical antibody positivity as age-at-diagnosis of RA increases. Biologic senescence may lead to alterations in the dynamics between initial immune dysregulation and the onset of clinically-apparent disease. [22] Because of this, in an older individual, even though RA-specific autoimmune dysregulation and autoantibody production may be present, the effector mechanisms necessary for the development of clinical disease may be impaired, leading to a longer period of time between antibody positivity and clinically-apparent joint disease.

Another explanation is that the relationship between genetic factors and environmental exposures may differ as age at diagnosis changes. [23, 24] For example, in a subject with younger-onset disease, the genetic contributions to RA development may be greater, requiring less environmental exposures over a shorter period of time to trigger disease than someone who develops RA later in life. In addition, environmental exposures that possibly lead to RA-related immune dysregulation may differ in younger versus older disease onset age groups. For example, in subjects with disease onset at a young age there may be a robust

environmental exposure such as infection which acts as the triggering mechanism for RA, leading to a short period of time between detectable pre-clinical autoimmunity and clinically apparent disease. Conversely, in older patients, environmental influences on disease that are more dependent on long-term cumulative exposures such as smoking or intake of dietary components may predominate disease risk. [15, 16]

Study Limitations

In this military population with RA, the high prevalence of males (59%) and the low mean age at RA diagnosis (39.9 years old) make this group less representative of RA in the general population. In addition, the severity of RA may have influenced resignation from the military, with younger subjects with severe disease leaving service early, leading to a preponderance of less-severe disease in subjects that were older at the time of diagnosis. However, in this cohort, erosive disease was not significantly more prevalent in younger individuals. Also, given the close evaluation of all military personnel with joint complaints, and that we focused our analysis on age at the time of diagnosis, we believe that differences in disease severity minimally influenced our findings of increasing duration of pre-clinical antibody positivity as age at diagnosis increased.

There is limited previous data suggesting that race may influence RA-related autoantibody positivity. [25–30] In our analysis, we did not find a significant relationship between race and pre-clinical prevalence rates of RF or anti-CCP positivity (Table 3), but it is possible that associations do exist that were not identified due to small sample sizes and limited information, and future studies with larger numbers of subjects will need to be performed. As tobacco smoke may be a major environmental influence on the development of RA-related autoimmunity, an analysis of the effect of smoking on the prevalence and timing of RA autoantibodies would be beneficial. Due to the unavailability of tobacco use data in this cohort, we were unable to examine the influence of smoking on the prevalence and duration of pre-clinical antibody positivity, and this will have to be explored in future studies. Lastly, the sample size for this study was relatively small, and results will need to be verified in larger prospective studies which are currently underway.

CONCLUSION

Based on our findings in this study, the period of time that RF and anti-CCP are present prior to diagnosis lengthens as the age at the time of diagnosis of RA increases. This novel finding adds to the knowledge of the pre-clinical period of RA development and suggests that factors such as genetic risk, environmental exposures, and immune system senescence may influence the temporal relationship between the development of pre-clinical RA-related autoantibodies and age at symptom onset. In addition, this finding has implications for the use of autoantibody biomarkers as predictive markers for risk of development of RA in asymptomatic individuals, with antibody positivity potentially having a very different meaning in terms of timing of onset of symptomatic RA in a younger versus older individual. Further prospective studies are needed in order to characterize the genetic, epidemiologic and immunologic factors that influence the development of RA.

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LIST OF ABBREVIATIONS

RF	Rheumatoid factor
ACPA	anti-citrullinated protein antibodies
RA	Rheumatoid arthritis
DoDSR	Department of Defense Serum Repository
WRAMC	Walter Reed Army Medical Center
CCP	Cyclic citrullinated peptide

References

1. Harris, E. Clinical features of rheumatoid arthritis. In: Harris, ED.; Budd, RC.; Firestein, GS., et al., editors. *Kelly's Textbook of Rheumatology*. 7. Elsevier/Sanders; Philadelphia, Pennsylvania: 2005. p. 1043-78.
2. Gabriel SE, Crowson CS, O'Fallon WM. Mortality in rheumatoid arthritis: have we made an impact in 4 decades? *J Rheumatol*. 1999 Dec; 26(12):2529-33. [PubMed: 10606358]
3. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum*. 1988 Mar; 31(3):315-24. [PubMed: 3358796]
4. Nielen MM, van Schaardenburg D, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum*. 2004 Feb; 50(2):380-6. [PubMed: 14872479]
5. Rantapaa-Dahlqvist S, de Jong BA, Berglin E, Hallmans G, Wadell G, Stenlund H, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum*. 2003 Oct; 48(10):2741-9. [PubMed: 14558078]
6. del Puente A, Knowler WC, Pettitt DJ, Bennett PH. The incidence of rheumatoid arthritis is predicted by rheumatoid factor titer in a longitudinal population study. *Arthritis Rheum*. 1988 Oct; 31(10):1239-44. [PubMed: 3178905]
7. Aho K, Palosuo T, Heliovaara M, Knekt P, Alha P, von Essen R. Antifilaggrin antibodies within "normal" range predict rheumatoid arthritis in a linear fashion. *J Rheumatol*. 2000 Dec; 27(12):2743-6. [PubMed: 11128658]
8. Aho K, Heliovaara M, Maatela J, Tuomi T, Palosuo T. Rheumatoid factors antedating clinical rheumatoid arthritis. *J Rheumatol*. 1991 Sep; 18(9):1282-4. [PubMed: 1757925]
9. Kurki P, Aho K, Palosuo T, Heliovaara M. Immunopathology of rheumatoid arthritis. Antikeratin antibodies precede the clinical disease. *Arthritis Rheum*. 1992 Aug; 35(8):914-7. [PubMed: 1379430]
10. Aho K, von Essen R, Kurki P, Palosuo T, Heliovaara M. Antikeratin antibody and antiperinuclear factor as markers for subclinical rheumatoid disease process. *J Rheumatol*. 1993 Aug; 20(8):1278-81. [PubMed: 7693940]
11. Palosuo T, Tilvis R, Strandberg T, Aho K. Filaggrin related antibodies among the aged. *Ann Rheum Dis*. 2003 Mar; 62(3):261-3. [PubMed: 12594116]
12. Klein, J.; Moeschberger, M. *Survival Analysis: Techniques for Censored and Truncated Data*. 2. Springer; 2005.
13. Leroy R, Bogaerts K, Lesaffre E, Declerck D. Multivariate survival analysis for the identification of factors associated with cavity formation in permanent first molars. *Eur J Oral Sci*. 2005 Apr; 113(2):145-52. [PubMed: 15819821]
14. Berglin E, Johansson T, Sundin U, Jidell E, Wadell G, Hallmans G, et al. Radiological outcome in rheumatoid arthritis is predicted by presence of antibodies against cyclic citrullinated peptide before and at disease onset, and by IgA-RF at disease onset. *Ann Rheum Dis*. 2006 Apr; 65(4):453-8. [PubMed: 16176994]

15. Klareskog L, Alfredsson L, Rantapaa-Dahlqvist S, Berglin E, Stolt P, Padyukov L. What precedes development of rheumatoid arthritis? *Ann Rheum Dis*. 2004 Nov; 63(Suppl 2):ii28–ii31. [PubMed: 15479868]
16. Padyukov L, Silva C, Stolt P, Alfredsson L, Klareskog L. A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of seropositive rheumatoid arthritis. *Arthritis Rheum*. 2004 Oct; 50(10):3085–92. [PubMed: 15476204]
17. Kuhn KA, Kulik L, Tomooka B, Braschler KJ, Arend WP, Robinson WH, et al. Antibodies against citrullinated proteins enhance tissue injury in experimental autoimmune arthritis. *J Clin Invest*. 2006 Apr; 116(4):961–73. [PubMed: 16585962]
18. Quinn MA, Gough AK, Green MJ, Devlin J, Hensor EM, Greenstein A, et al. Anti-CCP antibodies measured at disease onset help identify seronegative rheumatoid arthritis and predict radiological and functional outcome. *Rheumatology (Oxford)*. 2006 Apr; 45(4):478–80. [PubMed: 16287917]
19. Spector TD, Hart DJ, Powell RJ. Prevalence of rheumatoid arthritis and rheumatoid factor in women: evidence for a secular decline. *Ann Rheum Dis*. 1993 Apr; 52(4):254–7. [PubMed: 8484689]
20. Wolfe F, Cathey MA, Roberts FK. The latex test revisited. Rheumatoid factor testing in 8,287 rheumatic disease patients. *Arthritis Rheum*. 1991 Aug; 34(8):951–60. [PubMed: 1859489]
21. Aho K, Tuomi T, Heliovaara M, Palosuo T. Rheumatoid factors and rheumatoid arthritis. *Scand J Rheumatol Suppl*. 1988; 74:41–4. [PubMed: 3232033]
22. Weyand CM, Goronzy JJ. Stem cell aging and autoimmunity in rheumatoid arthritis. *Trends Mol Med*. 2004 Sep; 10(9):426–33. [PubMed: 15350894]
23. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis Rheum*. 1987 Nov; 30(11):1205–13. [PubMed: 2446635]
24. Reveille JD. The genetic contribution to the pathogenesis of rheumatoid arthritis. *Curr Opin Rheumatol*. 1998 May; 10(3):187–200. [PubMed: 9608321]
25. Del Puente A, Knowler WC, Pettitt DJ, Bennett PH. High incidence and prevalence of rheumatoid arthritis in Pima Indians. *Am J Epidemiol*. 1989 Jun; 129(6):1170–8. [PubMed: 2786329]
26. Scofield RH, Fogle M, Rhoades ER, Harley JB. Rheumatoid arthritis in a United States Public Health Service Hospital in Oklahoma: serologic manifestations in rheumatoid arthritis vary among tribal groups. *Arthritis Rheum*. 1996 Feb; 39(2):283–6. [PubMed: 8849380]
27. Ferucci ED, Templin DW, Lanier AP. Rheumatoid arthritis in American Indians and Alaska Natives: a review of the literature. *Semin Arthritis Rheum*. 2005 Feb; 34(4):662–7. [PubMed: 15692959]
28. Del Rincon I, Battafarano DF, Arroyo RA, Murphy FT, Fischbach M, Escalante A. Ethnic variation in the clinical manifestations of rheumatoid arthritis: role of HLA-DRB1 alleles. *Arthritis Rheum*. 2003 Apr 15; 49(2):200–8. [PubMed: 12687511]
29. Mikuls TR, Holers VM, Parrish L, Kuhn KA, Conn DL, Gilkeson G, et al. Anti-cyclic citrullinated peptide antibody and rheumatoid factor isotypes in African Americans with early rheumatoid arthritis. *Arthritis Rheum*. 2006 Sep; 54(9):3057–9. [PubMed: 16948136]
30. Kwok JS, Hui KH, Lee TL, Wong W, Lau YL, Wong RW, et al. Anti-cyclic citrullinated peptide: diagnostic and prognostic values in juvenile idiopathic arthritis and rheumatoid arthritis in a Chinese population. *Scand J Rheumatol*. 2005 Sep-Oct; 34(5):359–66. [PubMed: 16234183]

Figure 1a. Survival curves for the timing of the pre-clinical appearance of rheumatoid factor, stratified by age at time of diagnosis

Figure 1b. Survival curves for the timing of the pre-clinical appearance of anti-cyclic citrullinated peptide (anti-CCP) antibodies, stratified by age at time of diagnosis

Figure 1. Survival curves showing the timing of appearance of antibodies prior to diagnosis of rheumatoid arthritis, stratified by age at time of diagnosis

Survival analysis with left and interval censoring using all subjects with pre-diagnosis antibody positivity was performed in the RA cases to calculate the median time of appearance of antibodies in subjects. Subjects with positive antibodies prior to diagnosis were included (N = 47 for RF (>15.2 IU/mL) and N = 51 for anti-CCP). Time of diagnosis is at far right of figures, listed as time '0'. In each graph, the Y-axis (1-CDF) represents the proportion of cases with antibody positivity in each group based on interval censored survival analysis. **Figure 1a:** Time of appearance of RF for each age group. **Figure 1b:** Time of appearance of anti-CCP for each age group.

Figure 2a. Median times of appearance of rheumatoid factor (RF) prior to diagnosis of rheumatoid arthritis (RA)

Figure 2b. Median times of appearance of anti-cyclic citrullinated peptide (anti-CCP) prior to diagnosis of rheumatoid arthritis (RA)

Figure 2. Median duration of pre-diagnosis rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) antibody positivity by age-at-diagnosis groups
 Times of appearance are calculated using all antibody positive subjects with interval and left censored survival analysis. **Figure 2a.** Trend analysis shows significant increase in duration of pre-diagnosis RF positivity (>15.2 IU/mL) as age-at-diagnosis increases ($p=0.03$). **Figure 2b.** For anti-CCP, trend analysis for increased duration of pre-diagnosis anti-CCP positivity as age-at-diagnosis increases was marginally significant ($p=0.07$). This latter finding may be due to small sample sizes or may suggest possible non-linear trend in duration of pre-clinical antibody positivity.

Table 1

Demographic and clinical characteristics of cases with rheumatoid arthritis and serum samples available.

Cases (Total N = 83)	Mean (SD)	Range
Age at diagnosis	39.9 (10.0)	20.0–66.1
Age at diagnosis, by intervals		
20–29 (N=16)	26.1 (3.0)	20.9–29.8
30–39 (N=25)	35.2 (3.1)	30.3–39.7
40–49 (N=25)	43.8 (2.7)	40.0–48.9
50+ (N=17)	54.3 (3.6)	50.3–66.1
N (%)[*]		
Male	49 (59%)	
Race		
White	57 (69%)	
Black	21 (25%)	
Other	5 (6%)	
Erosions, N (%)		
Present	42 (51%)	
Absent	34 (41%)	
Unclassified	7 (8%)	
RF positivity at or after diagnosis ^{**}		
Sero-positive	67 (81%)	
Sero-negative	14 (17%)	
Unclassified	2 (2%)	
Pre-Clinical Serum Samples (N =243)	Mean (SD)	Range
Number of samples per case	2.9 (1.2)	(1 – 4)
Years before diagnosis of first sample	6.6 (3.7)	(0.1–13.7)

^{*} Percentage out of 83 cases;

^{**} Based on case chart review

SD = standard deviation

Table 2

Detection of autoantibodies in all cases (N=83) prior to diagnosis of rheumatoid arthritis

Autoantibody	Positive test before diagnosis N (%) [*]	Of subjects with pre-clinical antibody positivity, those with a positive test in first serum sample N (%) ^{**}
RF (>15.2 IU/mL)	47 (57%)	22 (47%)
Anti-CCP	51 (61%)	22 (43%)
RF or Anti-CCP	57 (69%)	-
RF and Anti-CCP	41 (49%)	-

* Percentages are based on data from all 83 RA cases.

** For RF, total N=47; for anti-CCP, total N=51

Table 3

Pre-clinical antibody positivity for all subjects divided into age-at-diagnosis groups for rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) *

Age at Diagnosis	N	Pre-Clinical RF Positivity (>15.2 IU/mL)	Pre-Clinical Anti-CCP Positivity (>5 U/mL)
20–29	16	11/16 (68.8%)	12/16 (75.0%)
30–39	25	16/25 (64.0%)	14/25 (56.0%)
40–49	25	14/25 (56.0%)	17/25 (68.0%)
50+	17	6/17 (35.3%)	8/17 (47.1%)

* Percentages based on number of subjects with pre-clinical antibody positivity in that age group.

Table 4

Association of erosive disease with pre-diagnosis rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) positivity

RF Positivity Pre-Diagnosis (N=47)			Anti-CCP Positivity Pre-Diagnosis (N=51)	
Variable	N (%) [*]	Odds Ratio [95% CI; p-value]	N (%) [*]	Odds Ratio [95% CI; p-value]
Erosive Disease ^{**}				
Absent	21 (44.7%)	Reference group	18 (35.3%)	Reference group
Present	26 (55.3%)	1.28 [0.51, 3.22; p=0.60]	33 (64.7%)	4.64 [1.71, 12.63; p<0.01]

^{*} Number of subjects with this variable and antibody positivity; percentage out of 47 (for RF) and 51 (for anti-CCP).

^{**} Erosive disease determined at or after time of diagnosis

Table 5

Table 5a. Duration of pre-clinical rheumatoid factor (RF) positivity for all subjects by age-at-diagnosis *				
Age at time of diagnosis of RA	N	Median duration of antibody positivity prior to diagnosis (95% CI; all values in years) **	Univariate hazard ratios (95% CI; p-value)	Adjusted hazard ratios (with 95% CI and p-values) ***
20–29	11	3.3 (2.3, 4.7)	Referent group	Referent group
30–39	16	5.5 (2.8, 11.1)	0.34 (0.12, 0.98; p=0.04)	0.40 (0.12, 1.35; p=0.14)
40–49	14	8.4 (6.2, 11.4)	0.20 (0.06, 0.61; p<0.01)	0.29 (0.08, 1.09; p=0.08)
50+	6	8.6 (3.7, 20.0)	0.18 (0.04, 0.88; p=0.03)	0.14 (0.02, 0.83; p=0.04)

Table 5b. Duration of anti-cyclic citrullinated peptide (anti-CCP) antibody positivity for all subjects by age-at-diagnosis *				
Age at time of diagnosis of RA	N	Median duration of antibody positivity prior to diagnosis (95% CI; all values in years) **	Univariate hazard ratios (95% CI; p-value)	Adjusted hazard ratios (95% CI; p-values) ***
20–29	12	2.8 (1.7, 4.5)	Referent group	Referent group
30–39	14	4.1 (2.7, 6.1)	0.55 (0.21, 1.47; p=0.23)	0.41 (0.14, 1.16; p=0.09)
40–49	17	9.2 (6.0, 14.1)	0.13 (0.04, 0.41; p<0.01)	0.16 (0.04, 0.68; p=0.02)
50+	8	6.4 (3.6, 11.2)	0.25 (0.07, 0.87; p=0.03)	0.38 (0.10, 1.40; p=0.16)

* Subjects included are those with pre-clinical RF positivity as determined by single RF cut-off (>15.2 IU/mL).

** Determined from univariate analysis. For age group 50+, the earliest 95% confidence interval (20.0 years) is outside the range of the data. This is due to small sample size and large numbers of subjects in this group with their earliest pre-clinical sample positive for rheumatoid factor.

*** Hazard ratios (HR) are reported from survival models adjusted for gender, race, and duration of pre-clinical samples available. The HR less than one indicates that the duration of antibody positivity is longer in the older age group compared to the referent group of 20–29 year-olds.

* Subjects included are those with pre-clinical anti-CCP positivity.

** Determined from univariate analysis. For the age groups 40–49 and 50+, the earliest 95% confidence intervals (14.1 and 11.2 years, respectively) are outside the range of data. This is due to the small sample size and the large numbers of subjects in these groups with their earliest pre-clinical sample positive for anti-CCP.

*** Hazard ratios (HR) are reported from survival models adjusted for gender, race, and duration of pre-clinical samples available. The HR less than one indicates that the duration of antibody positivity is longer in the older age group compared to the referent group of 20–29 year-olds.