Clinical studies and tissue analyses in the earliest phases of rheumatoid arthritis: In search of the transition from being at risk to having clinically apparent disease

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FEATURES OF THE SYNOVIUM OF INDIVIDUALS AT RISK OF DEVELOPING RHEUMATOID ARTHRITIS: IMPLICATIONS FOR UNDERSTANDING PRECLINICAL RHEUMATOID ARTHRITIS

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ABSTRACT

Objective
Previous work has suggested that subclinical inflammation of the synovium does not coincide with the appearance of rheumatoid arthritis (RA) specific autoantibodies. The objective of this study was to examine the relationship between changes in the synovium and development of arthritis over time in a markedly larger, prospective study.

Methods
Fifty-five IgM rheumatoid factor (RF) and/or anti-citrullinated protein antibody (ACPA) positive individuals, without any evidence of arthritis upon physical examination, were included in the study. All individuals underwent MRI and mini-arthroscopic synovial biopsy sampling of a knee joint at inclusion and were prospectively followed. Proportional hazard regression analysis was performed to investigate whether changes in the synovium assessed by MRI and immunohistochemistry (IHC) of synovial biopsies were associated with onset of arthritis.

Results
After a median follow-up time of 13 (IQR 6-27, range 1-47) months, 15 individuals (27%) developed arthritis. We did not observe overt synovial inflammation during the preclinical stage. However, there was non-significant association between T cell numbers and subsequent development of clinically manifest arthritis (hazard ratio: 2.8; 95% confidence interval: (0.9 to 9.1; p=0.088)). Combined with ACPA-positivity, the presence of T cells in the synovium was associated with arthritis development (double-positive vs single-positive or double-negative (HR (95%CI): 4.0 (1.4 to 11.4); p=0.010)).

Conclusion
These findings confirm and extend previous results showing the absence of clear cut synovial inflammation in individuals having systemic autoimmunity associated with RA. However, subtle infiltration by synovial T cells might precede signs and symptoms of arthritis in preclinical RA.
Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by inflammation of synovial tissue. Certain genes, such as MHC class II genes and PTPN22, increase the susceptibility of RA. In subjects with genetic susceptibility environmental factors, including smoking and perhaps periodontitis, may lead to the development of autoantibodies like rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA). These autoantibodies define individuals with systemic auto-immunity associated with RA. Whilst RA-specific autoantibodies can be found more than 10-15 years before joint inflammation becomes clinically manifest, only a minority of individuals with RA-specific autoantibodies actually develops clinically manifest RA. We have previously proposed that, whereas the initial immune response leading to the production of autoantibodies may take place at sites other than the synovium, a ‘second hit’ due to for instance a minor trauma or a viral infection may lead to citrullination of synovial proteins and subsequent epitope spreading.

Consistent with the hypothesis that the initial changes may take place at sites other than the synovium, like the lung, we found no evidence of overt synovial inflammation in the joints of 13 subjects at risk of developing RA. Because of the small sample size of that study, and in light of the importance of the implications for our understanding of the etiology of RA, we decided to validate and extend the results in a larger, prospective study.

MATERIALS AND METHODS

Study subjects
Individuals with arthralgia and/or a positive family history for RA, without any evidence of arthritis upon thorough physical examination, and who were positive for IgM-RF and/or ACPA were included in the study between June 2005 and August 2010. These individuals are at risk of developing RA and are characterized by the presence of systemic autoimmunity associated with RA (phase c according to) with or without environmental risk factors (phase b according to) and with or without symptoms without clinical arthritis (phase d according to). IgM-RF was measured using IgM-RF ELISA from Sanquin, Amsterdam, the Netherlands (upper limit of normal (ULN) 12.5 IU/mL) until December 2009 and thereafter using IgM-RF ELISA from Hycor Biomedical, Indianapolis, IN (ULN 49 IU/mL). IgM-RF levels were categorized into negative, <ULN, low positive, ≤3 times ULN, and high positive, >3 times ULN. ACPA was measured using anti-CCP2 ELISA CCPlus from Eurodiagnostica, Nijmegen, the Netherlands; ULN 25 kAU/L). These study subjects were recruited either via the outpatient clinic of the department of Clinical Immunology and Rheumatology at AMC, Amsterdam, via referral from the rheumatology outpatient clinic of Reade, Amsterdam, or via testing family members of RA patients in the outpatient clinic or at public fairs across the Netherlands. The study was performed according to the principles of the Declaration of Helsinki, approved by the institutional review board of the AMC, and all study subjects have given written informed consent.

Study design
At baseline demographic data were obtained. An MRI of an arbitrary knee joint was performed within one week before mini-arthroscopic synovial biopsy sampling of this
joint was performed. Study subjects were followed over time until arthritis onset or until January 1, 2012 (censored). There were yearly study visits. The development of arthritis, defined as a swollen joint, was the endpoint of this study. Individuals who developed arthritis underwent an interim visit, in order to confirm clinically apparent arthritis by two independent investigators (MS and DG or MH and DG).

Clinical parameters
At every study visit the following clinical and disease activity parameters were obtained: 68-joint tender joint count (68TJC) and 66-joint swollen joint count (66SJC), duration of morning stiffness (minutes), IgM-RF- (IU/mL) and ACPA-levels (kAU/L), erythrocyte sedimentation rate (ESR)(mm/hr), and serum levels of C-reactive protein (CRP)(mg/L).

MRI
Images were acquired on either a closed 1.5 Tesla (GE Signa Horizon Echospeed, LX9.0, General Electric Medical Systems, Milwaukee, WI) or open 1 Tesla (Panorama Open, Philips, Best, the Netherlands) MRI scanner, due to replacement of the former scanner. The minimum imaging protocol consisted of a sagittal STIR, an axial T2 with fat suppression and a sagittal T1 before and after contrast injection. A musculoskeletal radiologist with 15 years of experience and a research fellow with 4 years of experience in musculoskeletal radiology scored all images. Both were blind for the outcome of the study (arthritis). The presence of synovitis and hydrops were scored in 4 compartments of the knee joint (medial, lateral, central and suprapatellar; minimum and maximum score of 0 and 3, respectively, for each compartment), as well as the presence (1) or absence (0) of bone marrow edema, erosions and cartilage damage (each in 6 locations: the patella-femoral joint (2) the medial (2) and lateral (2) knee compartments).

Mini-arthroscopic synovial biopsy sampling
All study subjects underwent mini-arthroscopic synovial biopsy sampling of a knee joint at baseline. Six to 8 samples were collected for immunohistochemistry (IHC) to correct for sampling error, as described previously. The synovial biopsy samples were snap-frozen ‘en bloc’ in Tissue-Tek OCT (Miles, Elkhart, IN) immediately after collection. Sections (5 μm each) were cut and mounted on Star Frost adhesive glass slides (Knittelgläser, Braunschweig, Germany). Sealed slides were stored at -80°C until further use.

Immunohistochemistry
Synovial tissue sections were stained using mouse monoclonal antibodies for T cells (anti-CD3: clone SK7, Becton Dickinson, San Jose, CA; anti-CD4: clone SK3, Becton Dickinson; and anti-CD8: clone C8/144B; Dako, Glostrup, Denmark), B cells (anti-CD22; clone RFB4; Millipore, Billerica, MA), fibroblast-like synoviocytes (FLS) (anti-CD55; clone 67; AbD Serotec, Oxford, UK), macrophages (anti-CD68; clone EBM11; Dako), plasma cells (anti-CD138; clone B-B4; Immunotech, Marseille, France), blood vessels (anti-von Willebrand factor (vWF); clone F8/86; Dako) and citrullinated fibrinogen (anti-citrullinated fibrinogen; clone 20B2; ModiQuest Research, Nijmegen, the Netherlands).
Staining was performed using a three-step immunoperoxidase method to detect bound anti-CD55, anti-vWF, anti-CD68 and anti-CD138 antibodies, as described previously. A two-step immunoperoxidase method was used to detect bound anti-CD4, anti-CD8 and anti-citrullinated fibrinogen antibodies. For anti-CD3 and anti-CD22 we used a 2-step immunoperoxidase method with a secondary polymer-horseradish peroxidase anti-mouse antibody (Envision+ System; Dako, Glostrup, Denmark). As negative control irrelevant isotype-matched immunoglobulins were applied to the sections instead of the primary antibody. The primary antibodies were incubated for 60 minutes (or overnight for CD4, CD8, CD55, vWF and citrullinated fibrinogen). AEC was used as chromogen (Vector Laboratories; Burlingame, CA). Slides were counterstained with Gill’s hematoxylin and mounted in Kaiser’s glycerol gelatin (Merck, Darmstadt, Germany). The intensity of the staining was scored using semi-quantitative analysis by two independent observers (MH and BS or MH and NS) on a five-point scale (range 0-4), including 5 RA patients as positive control, where 0 represented no expression and 4 represented the maximum expression of all tissue sections analyzed.

Expression of CD68+ cells was performed separately for the intimal lining layer and synovial sublining. Since CD4 is not only expressed by T cells but also by macrophages (CD4dim) only bright staining was scored as positive for CD4+ T cells. Scoring of citrullinated fibrinogen was done in a dichotomous manner (presence vs absence of staining). When scores between the two observers did not match, a definite score was obtained upon mutual agreement.

Statistical analysis
Continuous, normally distributed data were presented as means (SD), and differences between study groups were analyzed using t-test for unpaired samples. Not normally distributed data were presented as medians (IQR), and differences between study groups were analyzed using Mann-Whitney U-test. Categorical data were presented as numbers (percentages), and differences between groups were analyzed using Chi-square test.

To investigate associations of synovial markers with arthritis onset, proportional hazard regression analysis (Cox) was performed. Follow-up duration was defined as the time between inclusion in the cohort and the onset of clinically manifest arthritis, or between inclusion and January 1, 2012 (censored). First, variables were tested variable-by-variable. Variables with a p-value <0.2 were arbitrarily selected for a multivariable analysis (forward and backward selection procedures). Meaningful statistical interactions were excluded upfront. Statistical analysis was performed using PASW Statistics 18 (SPSS Inc, Chicago, Il). A p-value of <0.05 was considered statistically significant.

RESULTS
Cohort description
Of the 55 individuals included in the study, 41 individuals were single positive for RF or ACPA (19 were IgM-RF-positive only, 22 were ACPA-positive only) and 14 individuals were double positive. They were followed for a median duration of 27 (IQR 14-47; range 1-75) months. Fifteen of the 55 individuals (27%) developed arthritis over time, after a median
follow-up of 13 (IQR 6-27, range 1-47) months. Clinical characteristics and fulfillment of RA criteria at the moment of arthritis onset were described previously\textsuperscript{20}. Individuals who did not develop arthritis were followed for a median duration of 37 (IQR 19-52; range 3-75) months. Figure 1 shows the cumulative hazard of arthritis development in this study.

Baseline clinical characteristics of individuals who developed arthritis during follow-up were generally comparable to those of individuals who did not develop arthritis during follow-up (see Table 1).

![Figure 1. Cumulative hazard of arthritis development. x-axis: follow-up time in months; y-axis: cumulative hazard of arthritis development.](image)

**No overt synovial inflammation, but subtle synovial infiltration by T cells might precede the development of arthritis**

Complete synovial tissue samples of 6 individuals had to be excluded for IHC because of quality standards. The synovial tissue of a range of 35 to 49 individuals could be included in the analysis for expression of the various markers by IHC. In 6 individuals MRI was not performed due to logistic reasons.

Semiquantitative scores for expression of inflammatory markers in the synovium were low compared to the 5 RA patients who were used as positive control, consistent with our previous study\textsuperscript{9} (data not shown). In the autoantibody-positive individuals the scores for the expression of CD3 and citrullinated fibrinogen were either 0 or 1. For proportional hazard regression analysis the expression levels of the other markers were dichotomized. For the expression of CD4, CD8 and CD68 scores were 0 to 3 and categorized as follows: 0: negative; and ≥1: positive. CD55 expression was scored as 1 (low positive) or as 2 (high positive). Expression of vWF was scored in a range of 0 to 3 and categorized as 1 (low) for scores ≤1 and 2 (high) for scores ≥2. Expression of CD22 and CD138 was observed in only 1 individual (2 different persons) and therefore not included in statistical analyzes.
Proportional hazard regression analysis was performed to evaluate whether clinical and MRI parameters, as well as expression of inflammatory markers in the synovium, were associated with the onset of arthritis. First, all variables were analyzed variable-by-variable, see Table 2.

ACPA-positivity showed a non-significant trend towards an association with arthritis development (hazard ratio (HR) (95%CI): 2.7 (0.8 to 9.7); p=0.119). Of note, subjects were selected based on being positive for RF and/or ACPA. Other clinical parameters were not associated with arthritis development (data not shown) as was the case for MRI parameters.

With respect to synovial tissue analysis by IHC, there was no overt synovial inflammation during preclinical RA. However, the presence of CD3+ T cells at baseline showed a trend towards an association with arthritis development after follow up (HR (95%CI): 2.8 (0.9 to 9.1); p=0.088)). There was no association between expression of CD3 and presence of arthralgia in the biopsied knee joint (p=0.210). A similar trend was seen for the expression of CD8 in the synovium (HR (95%CI): 2.8 (0.7 to10.5; p=0.133)). It appeared that in particular expression of both CD3 and CD8 (CD3+CD8+) explained the trend towards an association with...
arthritis development (double-positive vs single-positive or double-negative: HR (95%CI): 2.9 (0.9 to 9.4; p=0.086; double positive vs. double negative: HR (95%CI): 6.3 (0.8 to 53); p=0.088). This may suggest that synovial CD8+ T cells are involved in the earliest stages of RA. Expression of other synovial tissue markers for inflammatory cells and blood vessels was not associated with arthritis development, confirming and extending our previous results.

Synovial T-cell infiltration combined with ACPA-positivity is associated with arthritis development

After exclusion of potentially meaningful interactions between variables (data not shown), variables with p-value < 0.2 in the univariate proportional hazard regression analysis were tested in a multivariable analysis. See Table 3 for the results of the proportional hazard regression analysis. Combining ACPA-status with CD3 expression in the synovium in one model resulted in an increased association for CD3 with arthritis development (HR (95%CI): 3.3 (1.0 to 11.0)) compared to a univariate model with only CD3.

Combining CD8 with either ACPA and CD3 or both resulted in the absence of a significant association with development of arthritis (data not shown), which is most probably due to lack of power as a result of over stratification in this relatively small sample size. Of importance, individuals expressing CD3 in the synovial tissue were not only individuals who were ACPA positive (57% of these individuals), suggesting no direct association between CD3-positivity and ACPA-positivity. Within the subgroup of

| Table 2. Univariate proportional hazard regression analysis for the development of arthritis |
|-----------------------------------------------|------------------|------------------|
| MRI factors                                   | Hazard ratio     | P-value          |
| Synovitis (per unit)                          | 0.9 (0.6 to 1.4) | 0.649            |
| Hydrops (per unit)                            | 1.1 (0.9 to 1.5) | 0.277            |
| Cartilage degeneration (per unit)             | 0.1 (0.0 to 9.4) | 0.324            |
| Bone marrow edema (per unit)                  | 0.6 (0.2 to 2.2) | 0.487            |
| Erosions (per unit)                           | 1.9 (0.2 to 15.2) | 0.529          |
| Synovial tissue factors                       |                  |                  |
| CD3 (pos (n=21) vs neg (n=28))                | 2.8 (0.9 to 9.1) | 0.088            |
| CD4 (pos (n=23) vs neg (n=12))                | 2.9 (0.4 to 23.6) | 0.311            |
| CD8 (pos (n=19) vs neg (n=17))                | 2.8 (0.7 to 10.5) | 0.133            |
| CD55 (low (n=15) vs high (n=31))              | 2.2 (0.5 to 10.5) | 0.275            |
| CD68 Sublining (pos (n=1) vs neg (n=38))      | 0.0 (0.0 to infinite) | 0.628          |
| CD68 Lining (pos (n=5) vs neg (n=34))         | 0.5 (0.1 to 4.1) | 0.549            |
| von Willebrand Factor (low (n=17) vs high (n=21)) | 1.7 (0.5 to 5.7) | 0.373            |
| Citrullinated fibrinogen (pos (n=28) vs neg (n=14)) | 1.4 (0.4 to 4.3) | 0.591            |

pos: positive; neg: negative; CD3, CD4, CD8: markers for T cells; CD55: marker for fibroblast-like synoviocytes; CD68: marker for macrophages. Variables with p-values < 0.2 (in bold) included in multivariate analysis.
Table 3. Multivariate proportional hazard regression analysis for the development of arthritis

<table>
<thead>
<tr>
<th>Variables in model</th>
<th>Hazard ratio (95% confidence interval)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1: ACPA (pos vs neg)</td>
<td>2.7 (0.8 to 9.7)</td>
<td>0.119</td>
</tr>
<tr>
<td>Model 2: CD3 (pos vs neg)</td>
<td>2.8 (0.9 to 9.1)</td>
<td>0.088</td>
</tr>
<tr>
<td>Model 3: CD8 (pos vs neg)</td>
<td>2.8 (0.7 to 10.5)</td>
<td>0.133</td>
</tr>
<tr>
<td>Model 4: ACPA (pos vs neg)</td>
<td>2.8 (0.8 to 10.5)</td>
<td>0.118</td>
</tr>
<tr>
<td>CD3 (pos vs neg)</td>
<td>3.3 (1.0 to 11.0)</td>
<td>0.048</td>
</tr>
</tbody>
</table>

ACPA: anti-citrullinated protein antibodies; pos: positive; neg: negative; CD3, CD8: markers for T cells.

Table 4. Frequency table for development of arthritis within subgroups of ACPA-status and CD3-expression in the synovium

<table>
<thead>
<tr>
<th>ACPA+CD3+ *</th>
<th>ACPA+CD3- (N=20)</th>
<th>ACPA-CD3+ (N=9)</th>
<th>ACPA-CD3- (N=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follow up time in months, median (IQR)</td>
<td>22 (13-37)</td>
<td>25 (6-53)</td>
<td>40 (27-71)</td>
</tr>
<tr>
<td>Arthritis developed, n (%)</td>
<td>7 (58%)</td>
<td>3 (15%)</td>
<td>2 (22%)</td>
</tr>
</tbody>
</table>

ACPA: anti-citrullinated protein antibodies; CD3: marker for T cells; * CD3-expression missing from 2 ACPA-positive individuals who developed arthritis, 2 ACPA-positive individuals who did not develop arthritis and 2 ACPA-negative individuals who did not develop arthritis.

ACPA+CD3+ individuals, however, significantly more individuals developed arthritis than in the other subgroups (p=0.037) (Table 4).

To investigate whether there is an association for subgroups of ACPA- and CD3-positivity with onset of arthritis we performed proportional hazard regression analysis for these subgroups as well. Positivity for both ACPA and CD3 was associated with arthritis development (double-positive vs single-positive or double-negative (HR (95%CI): 4.0 (1.4 to 11.4); p=0.010)), as depicted in Figure 2.

Synovial expression of citrullinated fibrinogen in individuals at risk of developing arthritis

The role of synovial citrullinated peptides in the pathogenesis of RA is unknown and has never been studied in individuals at risk of developing the disease21. Therefore, we examined the synovial expression of citrullinated fibrinogen, one of the major candidate autoantigens in RA. Expression of citrullinated fibrinogen in the synovium was not only observed in individuals who developed arthritis after follow-up (42%), but also in those who did not (30%) (p=0.462), and was not predictive of arthritis onset. Moreover, expression of citrullinated fibrinogen was not dependent on ACPA status, being observed in 39% of ACPA positive and 21% of ACPA negative individuals (p=0.247).
Taken together, we confirm that there is no evident synovitis in individuals at risk of developing RA during phases c and d (according to 5). However, we found an indication for subtle T-cell infiltration preceding the development of clinically manifest arthritis.

DISCUSSION

We evaluated the synovium by MRI and IHC before the onset of clinical signs and symptoms of arthritis in autoantibody-positive individuals who are at risk of developing RA. After adjustment for differences in follow-up duration we observed that the presence of inflammatory cells or blood vessels in the synovial tissue was not associated with the development of arthritis. Consistent with these findings, MRI showed no indication of synovitis, confirming and extending our previous work 9. However, there was a trend towards increased synovial T cell numbers in subjects who subsequently developed arthritis compared to those who did not (yet); this effect was stronger when combined with ACPA-status.

Thus, there is generally no overt subclinical synovitis present more than 1 month before the onset of arthritis in individuals who are at risk of developing RA. This suggests that infiltration of the synovial tissue by inflammatory cells would be a process relatively late in RA pathogenesis, which occurs close to the onset of clinically manifest disease. In contrast, systemic changes may be observed years before the onset of arthritis, as shown by the detection of RA-specific auto-antibodies (IgM-RF and ACPA) 6-8, increasing CRP levels towards arthritis onset 22, and increased monocyte chemotactic protein-1 (MCP-1) 23 in individuals who developed RA later on. To gain new insights into the earliest phase of RA pathogenesis it will therefore be important to study compartments of the immune system other than the synovium as well. For this reason, the first studies analyzing lymph node tissue in different phases of RA are underway 24.

However, our results suggest that subtle infiltration of the synovium by T cells might precede the onset of clinically manifest arthritis. The exact role of T cells in the
pathogenesis of RA is still not completely clear\textsuperscript{25, 26}. The abundant presence of synovial 
T cells in established RA and the association with certain MHC class II genes clearly 
support the involvement of T cells in the disease pathogenesis. We recently found highly 
expanded T-cell clones in the synovium of early RA patients compared to longstanding 
RA patients\textsuperscript{27}, suggesting a role for T-cell involvement in the early phase of disease. In 
the current study, it appeared that in particular expression of both CD3 and CD8 was 
associated with arthritis development. Activated CD8\textsuperscript{+} T cells may have cytotoxic activity 
by the production of granzymes and perforin, leading to cell death of (pathogenic) cells, 
and can produce proinflammatory cytokines like interferon (IFN)\textgamma and TNF. We previously 
found that soluble granzyme B levels are an independent predictor of erosive disease in 
RF positive RA\textsuperscript{28}. The possible role of synovial CD8\textsuperscript{+} T cells during the preclinical stage of 
RA pathogenesis is however still unclear and our findings will first need to be confirmed 
in an independent cohort. Interestingly, our observations were done in the synovial tissue 
of knee joints which were not amongst the joints that initially showed clinical signs of 
arthritis in any of the individuals who developed arthritis after follow-up. This suggests 
a time lag between what is observed in the synovial tissue and clinical signs of arthritis. 
Another interesting observation is that expression of citrullinated fibrinogen in the 
synovial tissue was not associated with development of arthritis or ACPA status. Previous 
work has shown that the presence of citrullinated proteins is not specific for RA synovial 
tissue\textsuperscript{29, 30}, since citrullinated peptides can also be found in inflamed or cancer tissue 
outside the synovium\textsuperscript{29, 31}. Our data suggest that initial ACPA formation is not necessarily 
directed against joint specific peptides, but rather against citrullinated peptides in other 
compartments of the body. Citrullination of peptides in the lung, possibly as a result 
of smoking, suggests that the lung may be an early site of RA-related auto-immunity\textsuperscript{3}. 
Interestingly, in a comparable cohort of autoantibody-positive individuals at risk for 
RA, airway abnormalities were observed comparable to those found in RA patients, but 
significantly more than in autoantibody-negative controls\textsuperscript{10}.

A possible limitation of our study is that synovial inflammation was examined in knee 
joints only whereas the disease usually presents in the small joints of the hands or feet. 
Obviously, it is difficult to obtain sufficient synovial tissue from non-arthritic small joints 
to allow reliable analysis. However, in 44\% of the cases synovial biopsy sampling was 
performed in a symptomatic, painful knee joint (without swelling) and it can therefore be 
expected that if the pain was due to synovial inflammation in that joint, we would have 
detected this by MRI and synovial biopsy. Moreover, 25\% of the individuals in the cohort 
underwent ultrasonography or MRI of the hands as well in the context of regular patient 
care, and there was also no sign of synovitis in the small joints in these subjects.

In conclusion, in this prospective cohort study of autoantibody-positive individuals 
at risk of developing RA, we confirm in a relatively large study that there is no clear 
cut synovial inflammation before the development of clinically apparent arthritis. It is 
possible however that subtle infiltration of the synovium by T cells might precede the 
onset of arthritis, but this needs further validation. We propose a model where systemic 
autoimmunity may exist years before the onset of RA. Apparently, a second hit (for
instance a trauma or an infection leading to expression of citrullinated antigens) in the synovium is needed for arthritis development. This could subsequently lead to expansion of the ACPA repertoire and progression towards synovial inflammation.

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