**Detection of antibodies to citrullinated tenascin-C in patients with early synovitis is associated with the development of rheumatoid arthritis**

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Early treatment of rheumatoid arthritis (RA) results in more effective disease suppression and can be key to a successful patient response. However, not all people who exhibit early synovitis develop RA; for example, in some, synovial inflammation resolves spontaneously (1). The factors that drive RA development remain unclear and clinical tools to predict RA development are imperfect.

Tenascin-C is a pro-inflammatory matrix molecule that is absent from healthy joints but highly expressed in the joints of RA patients (2, 3). We identified an immunodominant peptide in citrullinated tenascin-C, cTNC5, antibodies against which are detected in around half of RA patients, and can be found years before disease onset in some individuals (4). Here, we sought to determine if anti-cTNC5 antibodies can discriminate amongst people with early synovial inflammation those who develop RA and those with other outcomes.

Sera from 263 patients in the Birmingham early arthritis cohort were analysed. Patients were DMARD naïve with clinically apparent synovitis of ≥1 joint and with inflammatory joint symptoms of ≤3 months duration. Patients were followed for 18 months to ensure development of full disease phenotype and to allow any resolving arthritis time to resolve. At 18 months patients were assigned to the following outcome categories: persistent RA according to ACR 2010 criteria (5) (RA, n=101), persistent non-RA arthritis (PNRA, n= 66) and resolving arthritis (no clinically apparent joint swelling, no DMARD/steroid use in the previous 3 months, n=96). Demographic and clinical parameters were recorded, and RA patients divided into anti-CCP antibody positive and negative subsets (6, 7). Antibodies recognizing cTNC5 or a non-citrullinated peptide (rTNC5) were analysed by ELISA as described (4).

Anti-cTNC5 antibodies were found in 40.6% of people with early synovitis that went on to develop RA, but were detected in a low proportion of people who developed PNRA (6.1%), or whose disease resolved (3.1%). No significant antibody response to rTNC5 was detected (p = 0.527) (Table 1, supplementary figure 1). Anti-cTNC5 antibodies were significantly more prevalent in anti-CCP antibody positive compared to anti-CCP antibody negative RA patients (81.3 vs. 3.8%, p<0.0001)(Table 1). Anti-cTNC5 antibody levels were higher in anti-CCP antibody positive RA patients (193.1±449.8 AU) compared to patients with anti-CCP antibody negative-RA (3.56±3.30 AU), PNRA (19.42±122.7 AU) and resolving arthritis (6.60±28.02 AU) ANOVA p < 0.0001). Whilst anti-cTNC5 was not better at predicting the development of RA than anti-CCP antibody (specificity; sensitivity: 40.6%; 95.7% (cTNC5), 47.5%; 98.8% (CCP), anti-cTNC5 did detect a subset of people that developed RA who were not anti-CCP antibody positive (3.8%). Anti-cTNC5 antibody positive RA patients were more frequently anti-CCP antibody and RF positive than anti-cTNC5 antibody negative patients (Table 2).

Together these data reveal that detection of anti-cTNC5 antibodies in the sera of people with early synovitis is associated with the development of RA. Whilst similar numbers of people who developed RA were positive for anti-cTNC5 antibodies, as were positive for anti-CCP antibodies, these two groups did not entirely overlap; we identified a subset of anti-CCP antibody negative, anti-cTNC5 antibody positive patients (3.8%). This study therefore does not support replacing CCP analysis with cTNC5 analysis to accurately predict which patients presenting with early joint inflammation will go on to develop RA. However a combined analysis of CCP, cTNC5 and other citrullinated antigens may increase the number of people that can be diagnosed with RA at this early stage. Although a small proportion of the total patient number, when translated into the number of people who might otherwise be missed, this could bring significant clinical benefit.

Analysis of distinct subsets of antibodies recognizing different citrullinated peptides (ACPA) can yield information that is not possible to derive using artificial CCP peptides to detect ACPA. Arising before overt clinical symptoms, ACPA have the potential to reveal insights into disease aetiology. For example, gene/environment (MHC shared epitope and smoking) interactions are strongest in people who are dual positive for antibodies against citrullunated alpha enolase and for antibodies recognizing citrullinated vimentin (8). We previously found that anti-cTNC5 antibody positivity did associate with smoking in the EIRA cohort, however this link was weaker than that observed for APCA recognizing citrullinated enolase (4). Here we observed that the ratio of ever versus never smokers, whilst only slightly decreased in cTNC5 positive patients (52.5:47.5), was substantially decreased in anti-cTNC5 antibody negative patients (60.7:39.3%), although no significant association between anti-cTNC5 antibody status and smoking was observed. These data suggest that further studies investigating whether anti-cTNC5 antibody positivity could mark a serologically distinct subset of people who will develop RA would be of interest.

Finally, emerging evidence indicates that ACPA actively contribute to inflammation, and can directly drive tissue destruction that is the hallmark of established RA. Uncovering the identity of peptides that give rise to ACPA has started to reveal more about these mechanisms underlying disease pathogenesis. For example, immune complexes containing anti-citrullinated fibrinogen antibodies signal to induce pro-inflammatory cytokine synthesis, and antibodies to citrillinated vimentin provoke osteoclastogenesis and bone erosion (9, 10). However, little is known about the contribution of the autoantibody response to the events that drive early synovitis onto RA. Our finding that anti-cTNC5 antibodies were raised only in people whose synovitis progressed to RA opens the door for further work investigating whether these antibodies play a causal role in driving the differentiation of early joint inflammation towards persistent RA and away from disease resolution.

Table 1. Demographic, clinical and laboratory characteristics of patients in each outcome group.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Anti-CCP negative RA (n=53)** | **Anti-CCP positive RA (n=48)** | **Persistent non-RA** **(n=66)** | **Resolving arthritis****(n=96)** | **P-Value** |
| Female, n (%) | 27 (50.9) | 31 (64.6) | 37 (56.1) | 46 (47.9) | 0.274 |
| Age (years) | 55.6 ±15.7 | 55.5±14.4 | 52.1±18.9 | 45.9±16.8 | <0.0001 |
| Symptom duration (days) | 52.4±21.4 | 55.3±21.7 | 56.4±21.5 | 45.3±20.8 | 0.005 |
| CRP (mg/dl) | 10 (0-39) | 17.5 (6-43.8) | 20.5 (7.5-35.3) | 7 (0-17) | <0.0001 |
| ESR (mm/hour) | 18 (11.5-44.5) | 27.5 (18.3-51.3) | 21.5 (7.8-45.8) | 12.5 (5-27) | <0.0001 |
| DAS28 (CRP) | 4.4±1.4 | 4.4±1.4 | 3.6±1.2 | 2.8±1.3 | <0.0001 |
| DAS28 (ESR) | 4.6±1.5 | 4.7±1.6 | 3.6±1.8 | 2.9±1.5 | <0.001 |
| Smoking n (%) |  |  |  |  | 0.07 |
| Ever smoker | 28/49 (57.1) | 27/47 (57.4) | 26/64 (40.6) | 35/89 (39.3) |  |
| Never smoker | 21/49 (42.9) | 20/47 (42.6) | 38/64 (59.4) | 54/89 (60.7) |  |
| Anti-CCP positive, n (%) | 0 (0) | 48 (100) | 1 (1.5) | 1 (1.0) | <0.0001 |
| RF IgG positive, n (%) | 9 (17) | 44 (91.7) | 5 (7.6) | 10 (10.4) | <0.0001 |
| RF IgA positive, n (%) | 7 (13.2) | 26 (54.2) | 5 (7.6) | 10 (10.4) | <0.0001 |
| Anti-cTNC5 positive, n (%) | 2 (3.8) | 39 (81.3) | 4 (6.1) | 3 (3.1) | <0.0001 |
| Anti-rTNC5 positive, n (%) | 1 (1.9) | 1 (2.1)  | 3 (4.5) | 1 (1.0) | 0.527 |

Data are shown as number (percentage), mean +/- SD, or median (IQR) as appropriate.Comparisons have been performed with χ2, analysis of variance and Kruskal–Wallis tests for categorical, parametric continuous and non-parametric continuous data, respectively.

CCP, cyclic citrullinated peptide; CRP, C reactive protein; DAS, disease activity score; ESR, erythrocyte sedimentation rate; RA, rheumatoid arthritis; RF, rheumatoid factor; cTNC, citrullinated tenascin-C.

Table 2. Characteristics of RA patients with and without anti-cTNC5 antibodies.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Anti-cTNC5 negativeRA (n=60) | Anti-cTNC5 positiveRA (n=41) | P value |
| Female, n (%) | 33 (55) | 25 (60.1) | 0.682 |
| Age (years) | 55.2±16.1 | 56.1±13.3 | 0.785 |
| Symptom duration (days) | 52.3 ±21.5 | 56±21.5 | 0.400 |
| CRP (mg/dl) | 10.5 (0-43) | 18 (6-39) | 0.062 |
| ESR (mm/hour) | 18 (11-45) | 25 (19-46) | 0.372 |
| DAS28 (CRP) | 4.26±1.4 | 4.55±1.4 | 0.320 |
| DAS28 (ESR) | 4.51±1.5 | 4.82±1.6 | 0.320 |
| 28 TJC | 7.22±6.5 | 9.1±10.4 | 0.267 |
| 28 SJC | 7.6±7.2 | 6.9±5.5 | 0.595 |
| Smoking, n (%) |  |  |  |
| Ever smoker | 34/56 (60.7) | 21/40 (52.5) | 0.682 |
| Never smoker | 22/56 (39.3) | 19/40 (47.5) | 0.374 |
| Anti-CCP positive, n (%) | 9 (15) | 39(95.1) | <0.0001 |
| RF IgG positive n (%) | 16 (26.7) | 37 (90.2) | <0.0001 |
| RF IgA positive n (%) | 10 (16.6) | 23 (56.1) | <0.0001 |

Data are shown as number (percentage), mean +/- SD, or median (IQR) as appropriate. Comparisons have been performed with χ2, T test and Mann Whitney U test for categorical, parametric continuous and non-parametric continuous data, respectively.

cTNC, citrullinated tenascin-C; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; DAS, disease activity score; TJC, tender joint count; SJC, swollen joint count; CCP, cyclic citrullinated peptide; RF, rheumatoid factor.

**Figure legends**

Supplementary figure 1. Anti-cTNC5 antibody response in sera from patients with early synovitis. IgG response to cTNC5 and rTNC5 in serum samples from people with CCP negative RA (CCP- RA)(n=53), CCP positive RA (CCP+ RA)(n=48), persistent non-RA arthritis (PNRA)(n=66) and resolving RA (resolving)(n=96). The red line indicates the mean, and the black dotted lines indicate the cut-off for positivity. AU, arbitrary units; cTNC, citrullinated tenascin-C; rTNC, non-citrullinated tenascin-C.

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