Rheumatoid arthritis: predictors of clinical response to TNF blockade
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CHAPTER 4

Synovial synoviolin in relation to response to TNF blockade in patients with rheumatoid arthritis and psoriatic arthritis

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ABSTRACT

Introduction: The E3 ubiquitin ligase synoviolin functions as an anti-apoptotic factor and its expression is associated with arthritis development in mice. Recent research suggested that increased synoviolin levels in peripheral blood are related to non-response to infliximab treatment in patients with rheumatoid arthritis (RA). Therefore, we investigated synoviolin expression in the synovium and its relationship to later response to tumor necrosis factor (TNF) blockade in patients with RA compared with psoriatic arthritis (PsA).

Methods: In a prospective study, synovial tissue samples were obtained from 54 active RA and 21 active PsA, biological agent naïve patients before initiation of anti-TNF antibody treatment. Synoviolin expression was detected by immunohistochemistry and double immunofluorescence, and related to clinical response. We also investigated the relationship between synoviolin expression and TNF expression in the synovium of RA patients.

Results: At baseline, there was marked synoviolin expression in RA synovium, but this was not specific for this disease. In RA patients, synoviolin was expressed by fibroblast-like synoviocytes, macrophages, and few CD3+ T cells. There was no association between synoviolin expression at baseline and later clinical response to TNF blockade in either patient group. There was no correlation between synoviolin and TNF expression in RA synovium.

Conclusions: Synovial synoviolin expression was abundantly expressed in RA patients, but related to neither clinical disease activity nor clinical response to anti-TNF treatment.
Recently, synoviolin, a novel E3 ubiquitin ligase, was identified in rheumatoid arthritis (RA) fibroblast-like synoviocytes (FLS) and synovial tissue, where it may contribute to the dysregulated proliferation and apoptosis seen in RA\textsuperscript{1,2}. Synoviolin expression is also associated with arthritis development in mice\textsuperscript{1}. The exact mechanism of synoviolin regulation and expression remains to be elucidated, but, recently, a role for tumor necrosis factor alpha (TNF) alpha and interleukin (IL) 1b was suggested, as these cytokines increased the expression of synoviolin in RA FLS\textsuperscript{3}. Another interesting observation is that high-level sustained expression of synoviolin in whole peripheral blood (PB) was related to decreased clinical response in RA patients treated with TNF blockade\textsuperscript{4}. Therefore, we investigated synoviolin expression in synovium and its relationship to later response to tumor necrosis factor (TNF) blockade in patients with RA compared with psoriatic arthritis (PsA) and osteoarthritis (OA).

Synovial tissue samples were obtained from 54 active RA and 21 active PsA, biological agent naïve patients before initiation of anti-TNF antibody treatment and of 9 OA patients. Patients were selected from our larger clinical studies described before\textsuperscript{5,6}. Specific monoclonal antibodies were used to detect synoviolin [Life Span Biosiences, Seattle, WA].

Synoviolin expression was detected by immunohistochemistry and double immuno-fluorescence, and related to clinical response in the RA and PsA patients\textsuperscript{7,8}. We also examined the relationship between synoviolin and TNF expression in RA synovium. The MWU test and Spearmans’ Rank Correlation test were used when appropriate.

**Figure 1.** Synovial synoviolin expression. Representative photographs showing synoviolin expression (reddish-brown staining) in rheumatoid arthritis (A), psoriatic arthritis (B) and osteoarthritis synovial tissue (C). Magnification 100x.

**Figure 2.** Expression of synovial synoviolin (IOD/mm\textsuperscript{2}). A. Synovial synoviolin expression in rheumatoid arthritis (RA), psoriatic arthritis (PsA) and osteoarthritis (OA). B. and C. Synovial synoviolin expression in responders and non-responders in RA and PsA patient groups, respectively. In Figure 2A, B and C data are presented as median with IQR and whiskers (5\textsuperscript{th} and 95\textsuperscript{th} percentile). *p < 0.05. (Mann Whitney U test)
Synoviolin was expressed in RA, PsA and OA patients (Figure 1). Baseline expression of synoviolin (median [IQR]) was higher in RA compared with PsA patients ($p = 0.04$) (Figure 2A). The median expression in the OA group tended to be lower compared with both groups, but this difference was not statistically significant (Figure 2A). There was no correlation between baseline synoviolin and TNF expression in the synovial tissue of the RA patients (data not shown). In RA patients, in the intimal lining layer synoviolin was expressed by 50-60% of CD68+ intimal macrophages and 40-50% of CD55+ FLS in RA synovium. In the synovial sublining synoviolin was mainly expressed by CD68+ macrophages (80%), but also by CD3+ T cells (<10%). Synoviolin expression at baseline was not related to later clinical response to TNF blockade in either group (Figure 2B and 2C).

A significant group of RA patients does not respond well to TNF blockade. Recently, we have shown that synovial TNF expression, the presence of synovial lymphocyte aggregates and increased expression of genes related to inflammation partly predict later response to TNF blockade in RA patients$^{6,9,10}$ and it is conceivable that combining different biomarkers could enhance treatment outcome. The results presented here confirm previous work showing overexpression of synoviolin in the synovium of RA patients$^1$. However, its expression was not specific for RA. In contrast to the previously reported association between synoviolin levels in whole peripheral blood and response to anti-TNF treatment$^4$, synovial synoviolin expression is not predictive of later response to TNF blockade, indicating that measurement of synovial synoviolin is not a good predictive biomarker in this setting. This apparently contradictory observation could theoretically be explained by retention of different inflammatory cells in inflamed synovial tissue compared to peripheral blood. Previous work suggested that TNF could induce synoviolin expression$^3$. However, we did not find a correlation between synovial synoviolin and TNF expression. Thus, the role of synoviolin in the pathogenesis of RA is more complex than previously anticipated.

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