Rheumatoid arthritis - aetiology, pathogenesis and animal models

**POS0377** FIBROCYTES IN EARLY AND LONGSTANDING RHEUMATOID ARTHRITIS: A 6-MONTH TRIAL WITH REPEATED SYNOVIAL BIOPSY, IMAGING, AND LUNG FUNCTION TEST

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**Background:** Fibrocytes are bone marrow-derived cells, that express both hematopoietic and stromal markers. In murine collagen-induced arthritis models circulating fibrocytes have been found to home to inflamed joints and enhance arthritis activity. The cell has, therefore, been proposed to be precursor cells for the fibroblast-like synoviocytes (FLS), which are central in the Rheumatoid Arthritis (RA) pathogenesis. Fibrocytes levels are further correlated with disease progression and mortality in interstitial lung disease (ILD) and identified as a precancerous potential of bone erosions (253±139 vs. 202±7 pg/mL, p=0.002) (Figure 1D). However, serum CCN1 concentrations were significantly higher in the serum of RA patients compared to controls (233±118 vs. 279±75 pg/mL, p=0.045) (Figure 1C).

**Methods:** Twenty patients with early RA (ERA) and 20 patients with longstanding RA (LRA) were enrolled in a six months prospective study. Sixteen patients undergoing wrist arthroscopy were healthy controls. RA patients underwent pulmonary function tests, ultrasound, and synovial ultrasound-guided needle biopsy of the same wrist, at baseline and six months. Wrist magnetic resonance imaging of the same wrist, at baseline and six months. Wrist magnetic resonance imaging

**Results:** Fibrocyte levels did not decline during the trial despite effective RA treatment. Fibrocytes were primarily located around vessels and in the subintimal area in the synovium (Figure 1A and 1B, fibrocytes marked with arrows). In the ERA group, increased synovitis assessed by ultrasound was moderate and strongly correlated to respectively increased in circulating and synovial fibrocyte levels. Increased synovitis assessed by MRI during the trial was not correlated to both increased numbers of circulating and cultured fibrocytes. Absolute diffusion capacity level was overall weakly negatively correlated to the level of circulating and synovial fibrocytes. The decline in forced ventilatory capacity and diffusion capacity during the trial was moderately correlated to increased levels of synovial fibrocytes (Figure 1C and 1D).

**Conclusion:** Our findings point to fibrocytes as key mediators of RA pathogenesis, and as a possible pathogenic link between the disease process in the synovium and RA lung affection. Studies are needed to investigate if the new therapies targeting fibrocyte differentiation/migration could be a path forward in RA/RA-ILD treatment.

**Disclosure of Interests:** None declared

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**POS0378** CCN1: AN ANGIOGENIC ACTOR IMPLICATED IN THE STRUCTURAL DAMAGES OF RHEUMATOID ARTHRITIS

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**Background:** We have previously shown that decreased expression of the deacetylase sirtuin-1 (SIRT1) contributes to the proliferative, activated and proangiogenic profile of endothelial cells (EC) in rheumatoid arthritis (RA) (1). The matricellular protein CCN1, characterized by proangiogenic and immuno-modulatory properties, may be directly implicated in these processes, since its expression is negatively regulated by SIRT1 (1).

**Objectives:** To study the implication of CCN1 in RA pathogenesis.

**Methods:** CCN1 expression was assessed in ECs (25 RA and 10 controls) by quantitative RT-PCR, western blot and ELISA, in the synovial tissue (5 RA and 5 controls) by immunohistochemistry and immunofluorescence, and in the serum (205 RA and 20 controls) by ELISA. Inhibition of CCN1 in RA ECs was achieved through the use of shRNA and neutralizing monoclonal antibodies. The functional consequences of CCN1 inactivation in RA ECs were studied i) in vitro by the analysis of proliferation (cell impedance), tube formation in Matrigel and migration in Boyden chambers; and ii) in vivo in the murine model of tumor necangiogenesis.

**Results:** CCN1 mRNA and protein expression were increased by 1.72- (p = 0.012) and 7.2-fold (p=0.008) in RA ECs compared to controls, respectively. CCN1 concentrations were significantly increased in RA EC culture supernatants (890±153 vs. 359±199 pg/mL, p=0.007). CCN1 was overexpressed in the synovial tissue of RA patients (Figure 1A) and confocal microscopy analyses revealed a prominent CCN1 expression in the vascular endothelium (CD31 +) and T cells (CD3 +) (Figure 1B).

**In vitro**, recombinant TNF-α and IL-17 induced the mRNA and protein expression of CCN1 in RA ECs. CCN1 inactivation was associated with reduced proliferative capacities, delayed capillary tube formation and decreased migration of RA ECs (Figure 1E). In vivo, subcutaneous transplantation of CT26 tumor cells combined with RA ECs transplanted with CCN1 shRNA to CB17 SCID mice was associated with a 51% reduction in tumor volume (p=0.008) and a 27% reduction in tumoral vascular density (p=0.032) compared with mice transplanted with MOCK transplanted RA-ECs (Figure 1F).

Serum concentrations of CCN1 were significantly reduced in the serum of RA patients compared to controls (233±118 vs. 279±75 pg/mL, p=0.045) (Figure 1C).

**Conclusion:** CCN1 is overexpressed in ECs and the synovial tissue of patients with RA. CCN1 can regulate the functional properties of RA ECs and their angiogenic potential in vivo. CCN1 could represent a new therapeutic target, which is being evaluated in experimental models of erosive arthritis.

CCN1 may be a reliable biomarker of structural damages given the association between its serum concentrations and the extent of radiographic lesions. The performance of CCN1 serum levels to predict structural progression is under investigation.

**REFERENCES:**

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Figure 1. Implication of CCN1 in the pathogenesis of rheumatoid arthritis (RA). A, Representative immunohistochemistry staining for CCN1. B, Representative confocal microcopy analyses. C-D, CCN1 serum concentrations; statistical test: Student t test. **" p<0.01. E, Representative images of RA endothelial cell (EC) migration; Y-axis shows the number of migrated cells, statistical test: Wilcoxon test, *" p<0.05. F, Representative subcutaneous tumors. X-axis shows the fluorescence area in %, statistical test: Wilcoxon test, *" p<0.05.