Supplementary Material

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Tables and Scatterplots

Table 1: Treatment at the visits

ERA group	**Baseline**	**3 months**	**6 months**	**Missings / N**
n	20	19	20	
Untreated, n (%)	20 (100)	2 (11)	3 (16)	
Methotrexate, n (%)	0 (0.0)	16 (84)	15 (79)	
MTX and HCQ, n (%)	0 (0.0)	1 (5)	1 (5)	1
Peroral steroid, n (%)	0	2	1	1
LRA group	**Baseline**	**3 months**	**6 months**	**Missings / N**
n	20	19	20	
Untreated, n (%)	2 (10.0)	1 (5)	1 (5)	
Methotrexate, n (%)	9 (45)	8 (42)	7 (37)	
MTX and HCQ, n (%)	1 (5)	1 (5)	1 (5)	
MTX+SZ+HCQ, n (%)	6 (30)	4 (21)	4 (21)	
MTX and Biological, n (%)	1 (5)	3 (16)	3 (16)	
Biological Alone, n (%)	1 (5)	2 (10)	2 (10)	1
Peroral steroid, n (%)	1	1	1	0

MTX: Methotrexate, HCQ: Hydroxychloroquine; SZ: Salazopyrine

Two patients in the ERA group and one in the LRA group did not want to receive treatment. All other patients in ERA group received Methotrexate therapy. Four patients in the LRA group was during the study started in Biological therapy.

Table 2 : Synovial biopsy inflammation at baseline and 6 months

	ERA			HC	
	Baseline	**6 months**	**p**	**Baseline**	**p¤**
n	20	20		16	
Krenn score					
Linning Cell layer, mean (sd)	2.1 (0.8)	1.8 (0.7)	0.19	1.6 (0.9)	0.09
Stromal activation, mean (sd)	2.1 (0.6)	1.6 (0.6)	**0.02**	1.4 (0.6)	**0.002**
Infiltrates, mean (sd)	1.9 (0.7)	1.6 (0.8)	0.27	1.3 (0.6)	**0.02**
Total score, mean (sd)	6.0 (1.5)	5.0 (1.5)	**0.01**	4.2 (1.7)	**0.002**
Specific Markers					
CD20, mean (sd)	1.2 (1.1)	0.8 (09)	0.06	0.3 (0.5)	**0.001**
CD3, mean (sd)	2.0 (1.2)	1.9 (0.9)	0.70	0.7 (0.7)	**<0.001**
CD68, mean (sd)	2.8 (0.9)	2.4 (0.8)	0.19	1.6 (0.8)	**<0.001**
CD138, mean (sd)	1.3 (1.2)	1.1 (0.9)	0.45	0.3 (0.6)	**0.002**
	LRA				
Krenn score					
Lining Cell layer, mean (sd)	2.3 (0.8)	2.1 (0.8)	0.24	1.6 (0.9)	**0.01**
Stromal activation, mean (sd)	2.2 (0.8)	1.9 (0.7)	0.27	1.4 (0.6)	**0.003**
Infiltrates, mean (sd)	2.2 (0.7)	2.2 (0.5)	0.77	1.3 (0.6)	**<0.001**
Total score, mean (sd)	6.8 (1.9)	6.1 (1.6)	0.17	4.2 (1.7)	**<0.001**
Specific Markers					
CD20, mean (sd)	1.8 (1.5)	1.5 (1.2)	0.40	0.3 (0.5)	**<0.001**
CD3, mean (sd)	2.5 (1.2)	2.2 (1.0)	0.48	0.8 (0.7)	**<0.001**
CD68, mean (sd)	3.0 (0.7)	2.8 (0.8)	0.39	1.6 (0.8)	**<0.001**
CD138, mean (sd)	2.1 (1.2)	2.0 (1.3)	0.74	0.3 (0.6)	**<0.001**

¤ p-value The same control group values versus respectively ERA and LRA Baseline Significant p values in bold.

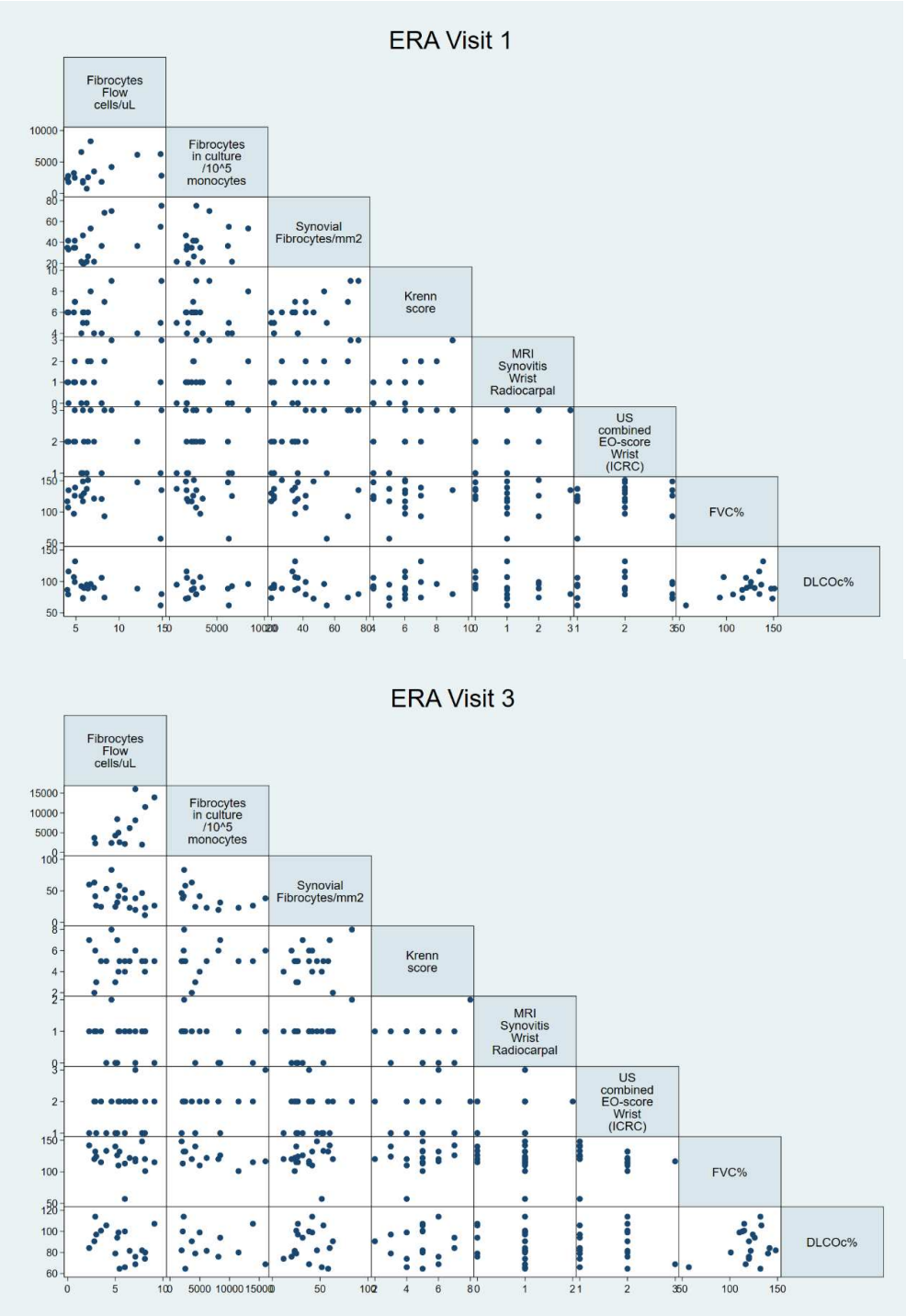
Synovial fibrocytes were moderately correlated to degree of infiltration in synovium of specific cell types as $CD68^+$ macrophages (r=0.57, p<0.001), $CD3^+$ T-cells (r=0.56 p<0.001), $CD20^+$ B-cells (r=0.61, p<0.001) and $CD138^+$ plasma cells (r=0.62, p<0.001) .

Table 3: Pairwise correlation between DAS28CRP, Synovial inflammation, imaging scores, fibrocytes and lungfunction. Baseline correlations (over the diagonal) and 6 months data (below the diagonal).

ERA	DAS28-CRP	Krenn-score	Hand Larsen score*	MRI RAMRIS Synovitis score	US EO Combined score	FVC (% pred.)	DLCOc (% pred.)	Circulating fibrocytes	Cultured fibrocytes	Synovial Fibrocytes
DAS28CRP	-	r=0.32 p=0.09	**r=0.28 p=0.03**	r=0.23 p=0.25	r=0.13 p=0.35	r=-0.01 p=0.95	r=0.02 p=0.94	r= 0.02 p=0.91	r=0.01 p=0.96	r=0.30 p=0.09
Krenn-score	r=0.32 p=0.10	-	**r=0.31 p=0.02**	**r=0.85 p<0.001**	**r=0.77 p<0.001**	r=0.06 p=0.70	r=0.06 p=0.73	r=0.12 p=0.69	r=0.04 p=0.89	**r=0.72 p<0.001**
Hand Larsen score*	r=0.14 p=0.48	r=0.27 p=0.21	-	r=0.36 p=0.18	r=0.36 p=0.19	r=-0.17 p=0.61	r=0.16 p=0.37	r=-0.04 p=0.67	r=0.45 p=0.29	r=0.28 p=0.31
MRI RAMRIS Synovitis score	r=0.10 p=0.72	r=0.13 p=0.66	**r=0.44 p=0.04**	-	**r=0.73 p<0.001**	r=-0.07 p=0.68	r=-0.28 p=0.14	r=0.29 p=0.29	r=0.10 p=0.68	**r=0.70 p<0.001**
US EO Combined score	r=0.15 p=0.57	r=0.18 p=0.89	**r=0.46 p=0.007**	r=0.27 p=0.18	-	r=0.24 p=0.28	r=0.03 p=0.89	r=0.04 p=0.87	r=0.08 p=0.79	**r=0.65 p<0.001**
FVC (% pred.)	r=-0.36 p=0.07	r=0.19 p=0.18	r=-0.03 p=0.88	r=-0.21 p=0.19	r=-0.15 p=0.65	-	r=0.37 p=0.18	r=-0.26 p=0.51	r=-0.61 p=0.11	r=-0.32 p=0.27
DLCOc (% pred.)	r=-0.27 p=0.25	r=-0.04 p=0.79	r=-0.02 p=0.91	r=-0.20 p=0.39	r=0.00 p=1.0	r=0.22 p=0.44	-	**r=-0.46 p=0.02**	r=-0.14 p=0.60	r=-0.32 p=0.06
Circulating fibrocytes	r=0.19 p=0.35	r=0.03 p=0.88	r=0.21 p=0.28	r=0.19 p=0.29	r=0.18 p=0.41	r=-0.24 p=0.14	r=-0.36 p=0.16	-	r=0.17 p=0.51	**r=0.57 p=0.002**
Cultured fibrocytes	r=0.17 p=0.49	r=0.14 p=0.72	r=-0.06 p=0.84	r=0.38 p=0.20	r=0.43 p=0.13	**r=-0.53 p=0.048**	r=-0.16 p=0.66	**r=0.62 p=0.02**	-	r=0.26 p=0.22
Synovial Fibrocytes	r=0.08 p=0.71	r=0.27 p=0.42	r=0.05 p=0.83	r=0.47 p=0.06	r=0.06 p=0.84	r=0.06 p=0.79	r=-0.01 p=0.95	**r=-0.46 p=0.02**	r=-0.51 p=0.05	-
LRA										
DAS28CRP	-	r=0.03 p=0.87	r=0.04 p=0.82	**r=0.42 p=0.04**	r=0.37 p=0.14	r=-0.03 p=0.86	r=-0.16 p=0.52	**r=-0.54 p=0.006**	r=-0.35 p=0.26	r=0.27 p=0.11
Krenn-score	r=0.32 p=0.10	-	r=0.35 p=0.11	**r=0.61 p=0.002**	**r=0.83 p<0.001**	r=-0.28 p=0.17	r=-0.20 p=0.44	r=-0.03 p=0.89	r=0.05 p=0.88	**r=0.58 p=0.002**
Hand Larsen score*	**r=0.49 p=0.02**	r=0.21 p=0.42	-	**r=0.53 p=0.006**	r=0.23 p=0.20	r=0.01 p=0.95	r=0.07 p=0.82	r=0.10 p=0.63	r=0.35 p=0.13	r=-0.13 p=0.50
MRI RAMRIS Synovitis score	NA	NA	NA	-	**r=0.72 p<0.001**	r=0.03 p=0.87	r=0.06 p=0.81	r=0.16 p=0.48	r=-0.20 p=0.37	r=0.20 p=0.24
US EO Combined score	**r=0.57 p=0.01**	**r=0.81 p<0.001**	r=0.36 p=0.05	NA	-	r=-0.21 p=0.26	r=-0.16 p=0.53	r=--0.26 p=0.25	r=-0.20 p=0.53	**r=0.51 p=0.004**
FVC (% pred.)	r=0.03 p=0.90	r=-0.36 p=0.10	r=0.03 p=0.92	NA	r=-0.38 p=0.09	-	r=0.28 p=0.16	r=-0.02 p=0.91	r=-0.21 p=0.48	**r=-0.46 p=0.01**
DLCOc (% pred.)	r=-0.37 p=0.09	**r=-0.51 p=0.004**	r=0.00 p=0.99	NA	r=-0.32 p=0.10	r=0.17 p=0.50	-	r=-0.18 p=0.44	r=0.05 p=0.85	**r=-0.41 p=0.02**
Circulating fibrocytes	r=0.08 p=0.76	r=0.16 p=0.16	**r=0.46 p=0.01**	NA	r=0.19 p=0.38	r=-0.02 p=0.96	r=-0.30 p=0.13	-	r=0.46 p=0.08	r=0.21 p=0.27
Cultured fibrocytes	r=-0.12 p=0.67	r=0.21 p=0.33	r=-0.19 p=0.39	NA	r=0.05 p=0.83	r=-0.42 p=0.08	**r=-0.46 p=0.02**	**r=0.51 p=0.009**	-	r=0.28 p=0.43
Synovial Fibrocytes	r=0.04 p=0.85	r=0.27 p=0.12	r=-0.02 p=0.93	NA	r=0.17 p=0.37	r=-0.18 p=0.47	r=-0.38 p=0.10	r=0.13 p=0.51	r=-0.21 p=0.27	-

*Total Larsen of the hand undergoing biopsy: EO: EULAR-OMERACT combined score of radio-carpal inter-carpal wrist joint, MRI of radio carpal joint of biopsied wrist. NA: Not available, as the LRA did not have 6-month MRI. Significant correlations in bold. Correlations by linear regression with robust cluster estimation. Results were not corrected for multiple testing.

Selected scatterplots for Table 3 correlations



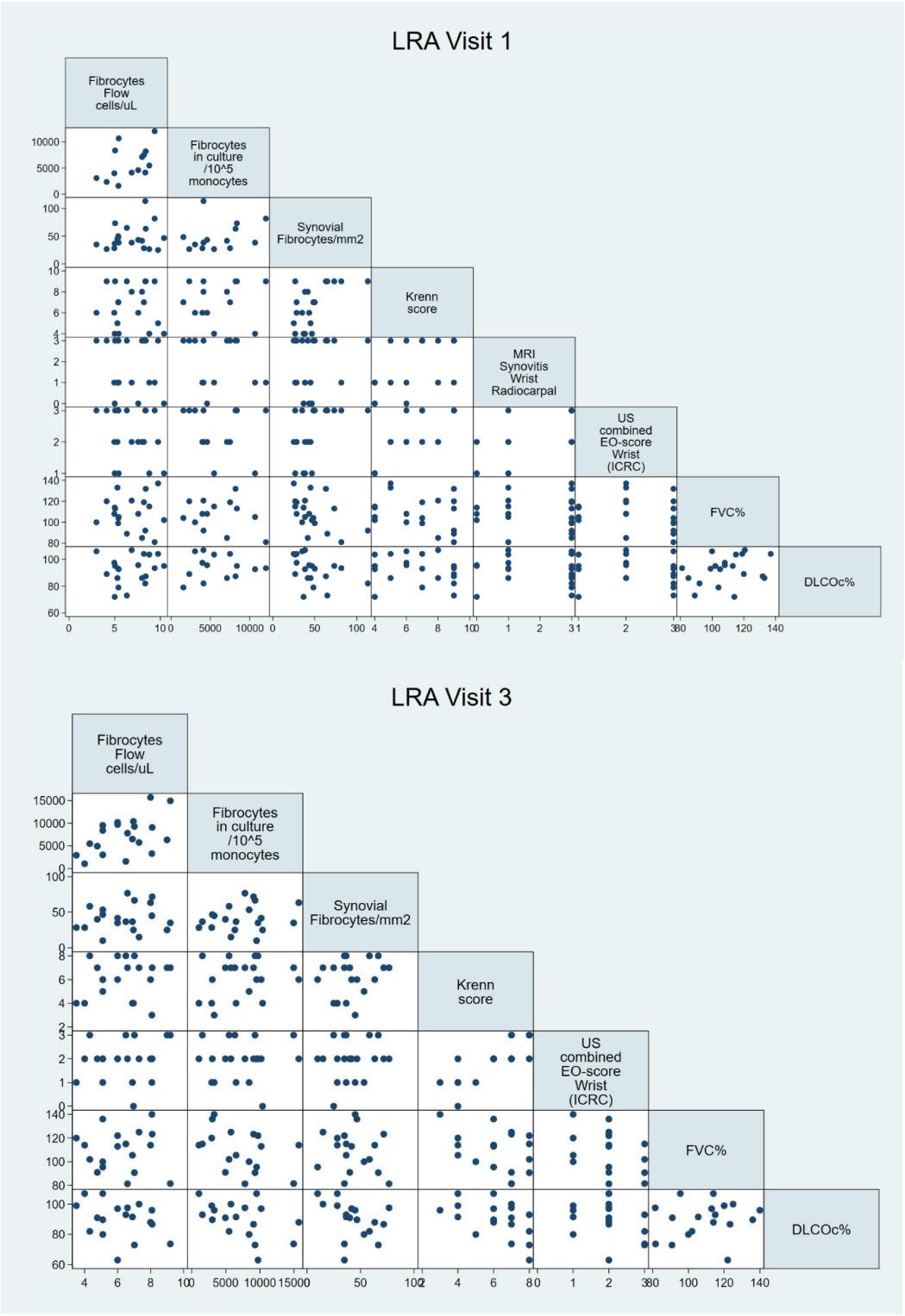

LRA Visit 1
Fibrocytes Flow cells/uL
Fibrocytes in culture /10^5 monocytes
Synovial Fibrocytes/mm2
Krenn score
MRI Synovitis Wrist Radiocarpal
US combined EO-score Wrist (ICRC)
FVC%
DLCOc%
LRA Visit 3
Fibrocytes Flow cells/uL
Fibrocytes in culture /10^5 monocytes
Synovial Fibrocytes/mm2
Krenn score
US combined EO-score Wrist (ICRC)
FVC%
DLCOc%

Table 4

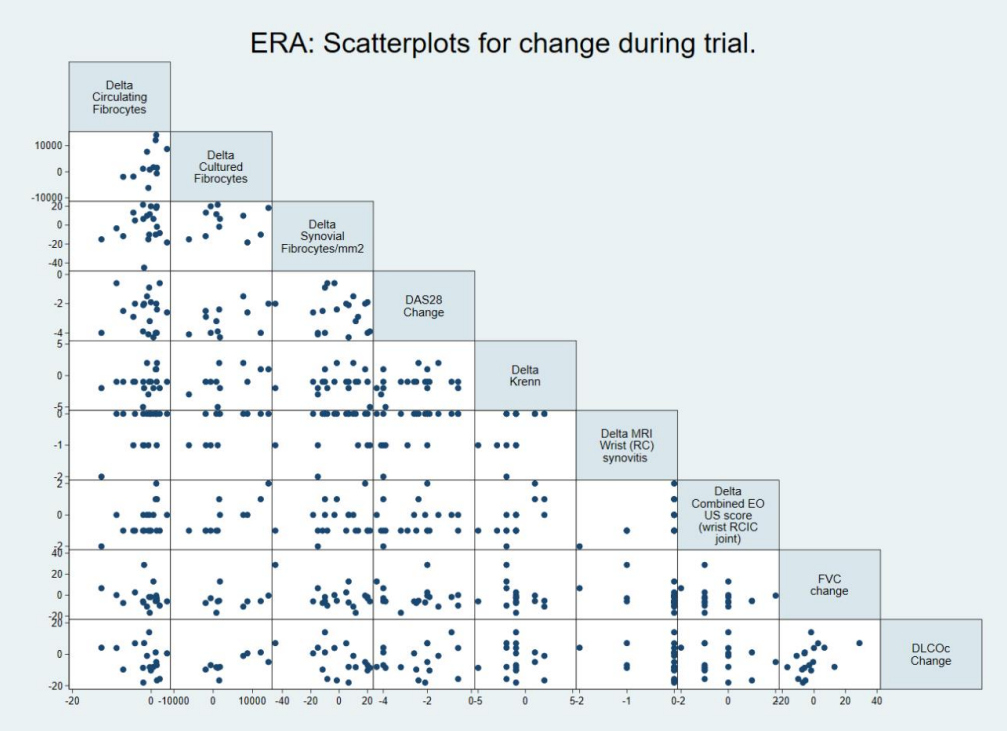
Pairwise correlation between change in DAS28CRP, synovial biopsy inflammation, inflammation on US and MRI imaging , lungfunction and fibrocyte levels.

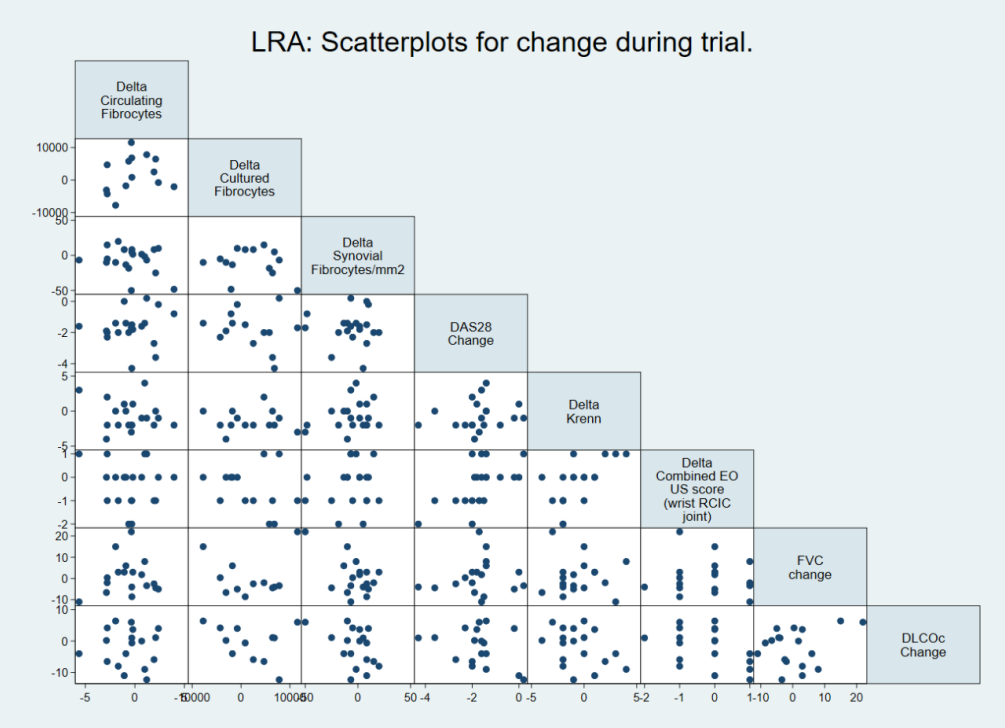
ERA correlations (over the diagonal) and LRA data (below the diagonal).

LRA/ERA	ΔDAS28	ΔKrenn-score	ΔMRI Synovitis	ΔUS score	ΔFVC	ΔDLCOc	ΔCirculating fibrocytes	ΔCultured fibrocytes	ΔSynovial Fibrocytes
ΔDAS28CRP	-	r=0.30 p=0.20	**r=0.52** **p=0.02**	r=0.19 p=0.42	r=0.04 p=0.81	r=0.13 p=0.59	r=-0.00 p= 0.99	r=0.46 p=0.13	r=0.12 p=0.60
ΔKrenn-score	r=0.17 p=0.46	-	**r=0.50** **p=0.03**	**r=0.65** **p=0.002**	r=-0.11 p=0.53	r=-0.02 p=0.93	r=0.22 p=0.96	r=0.56 p=0.05	r=0.09 p=0.71
ΔMRI Synovitis	NA	NA	-	**r=0.59** **p=0.006**	r=-0.42 p=0.08	r=-0.22 p=0.38	**r=0.47** **p=0.04**	**r=0.60** **p=0.04**	r=0.18 p=0.45
ΔUS score	**r=0.60** **p=0.005**	**r=0.65** **p=0.002**	NA	-	r=-0.13 p=0.60	r=-0.07 p=0.76	**r=0.47** **p=0.03**	**r=0.81** **p=0.001**	r=0.14 p=0.56
ΔFVC	r=0.12 p=0.62	r=0.00 p=0.99	NA	r=0.06 p=0.82	-	r=0.44 p=0.07	r=-0.21 p=0.39	r=0.01 p=0.97	**r=-0.52** **p=0.03**
ΔDLCOc	r=-0.36 p=0.14	r=-0.25 p=0.32	NA	r=-0.44 p=0.07	**r=0.37** **p=0.02**	-	r=-0.30 p=0.22	**r=0.69** **p=0.03**	r=-0.44 p=0.06
ΔCirculating fibrocytes	r=0.11 p=0.63	r=-0.20 p=0.94	NA	r=-0.14 p=0.53	r=0.09 p=0.90	r=-0.03 p=0.90	-	r=0.36 p=0.25	r=0.14 p=0.54
ΔCultured fibrocytes	r=-0.24 p=0.42	r=-0.02 p=0.94	NA	r=-0.24 p=0.40	r=0.04 p=0.91	r=-0.32 p=0.30	r=0.23 p=0.43	-	r=0.03 p=0.91
ΔSynovial Fibrocytes	r=-0.02 p=0.94	r=0.25 p=0.28	NA	r=0.10 p=0.65	**r=-0.50** **p=0.03**	r=-0.42 p=0.08	r=-0.26 p=0.26	r=-0.17 p=0.55	-

Δ: Change between 6 months visit and baseline visit data; Significant correlations in bold. US score: Ultrasound EULAR OMERACT combined score of radiocarpal joint of biopsied wrist, MRI RAMRIS synovitis score of radiocarpal joint of biopsied wrist. NA: Not available, as LRA did not have 6-month MRI. Correlations by linear regression. Results were not corrected for multiple testing.

Selected scatterplots for Table 4 correlations





Methods

Synovial Biopsy Procedure

Briefly, local anesthetic was injected into the soft tissue up to the joint capsule and into the joint space and a Quick-Core biopsy needle (16-gauge; Cook Medical) was then guided by US and placed within the joint capsule to retrieve synovium. The goal was a total of 20 biopsies obtained per biopsy procedure (10 stored in Formalin and 10 in RNALater). Biopsies in formalin were paraffin embedded and then sequential 3 µm sections underwent immune-histochemical staining for T cells (CD3), B cells (CD20), macrophages (CD68), and plasma cells (CD138) as previously described[1]. The degree of synovial inflammation was also assessed on hematoxylin and eosin (H&E) stained sections utilizing the Krenn score[1].

Ultrasound

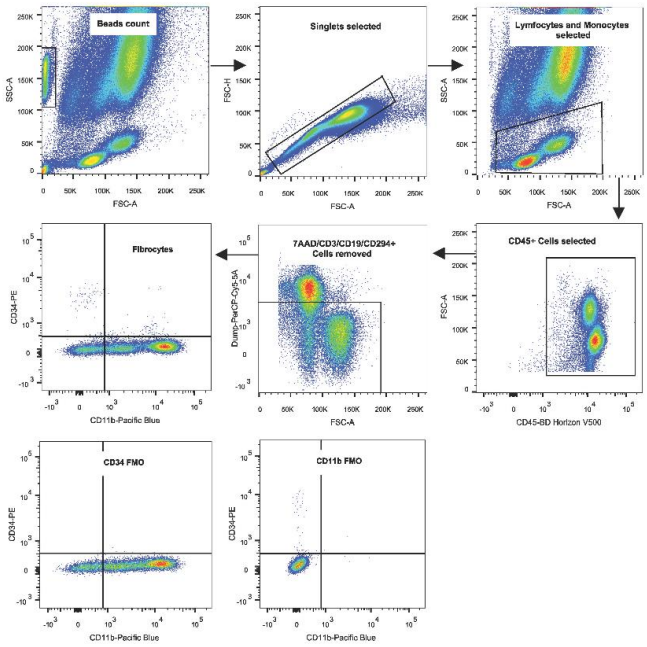
Ultrasound was performed on a GE Logiq E9, with a ML-6-15 probe as previously described[2]. The probe was placed and images scored according to the EULAR OMERACT (EO) ultrasound scoring system, with a score for grey-scale score (0-3), Doppler (0-3) and a combined score (0-3)[3]. Images were scored by experienced rheumatologist (SAJ).

Circulating fibrocytes measured by Flowcytometry

100 µL of whole blood were transferred to a Truecount absolute counting tube (BD Bioscience, San Jose, USA) and labelled with the following monoclonal antibodies: CD45-V500C (dose 5 µL Clone 2D1), CD34-PE (dose 5 µL Clone 581), CD11b-Pacific Blue (dose 5 µL Clone ICRF44), Via-Probe (7-AAD) (dose 2 µL), CD3-PE-Cy5 (dose 2.5 µL Clone HIT3a), CD19-PerCP-Cy5.5(dose 2.5 µL Clone HIB19), CD294-PerCP-Cy5.5 (dose 2.5 µL Clone BM16) (All BD Bioscience, San Jose, USA), all titrated for no-wash staining. After 20 minutes of dark incubation at room temperature, 2

ml of High Yield Lyse Solution (Thermo Fisher Scientific, Waltham, USA) were added for red cell lysis and tubes were stored for 10 minutes in the dark. Immediately hereafter, the sample was analyzed using a BD FACSCanto II (4 laser) flow cytometer, which was adjusted using BD Cytometer Setup and Tracking Beads. Compensation settings were performed using BD CompBead antibody-capturing particles (BD Bioscience, San Jose, USA) and the automatic compensation setup tool in the BD FACS Diva software. Fluorescence-minus-one controls were used for accurate definition of cells that had fluorescence above background levels. Relevant isotype controls for CD45-V500C (dose 5 µL Clone X40), CD34-PE (dose 5 µL Clone MOPC-21) and CD11b-Pacific Blue (dose 5 µL Clone MOPC-21) (all BD Bioscience, San Jose, USA) were used in the initial setup and frequently between tests. Data was acquired using BD FACSDiva software (BD Bioscience, San Jose, USA) and analyzed on FlowJo version 10 (Treestar, Ashland, USA).

The following figure and text describes the flow cytometry gating strategy used (image and text from Just et al. [4], with permission as it is open access and distributed under the terms of the Creative Commons CC BY license, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.)



"Gating strategy for quantification of fibrocytes using lysed peripheral blood. Representative flow cytometry analysis showing the gating strategy. Population was initially gated on the basis of the forward side scatter characteristics and debris were eliminated, then lymphocyte and monocytes were selected, the $CD45^+$ cells were gated in the plot "CD45-V500" versus forward scatter. Hereafter unwanted cells as B-Cells ($CD19^+$), T-Cells ($CD3^+$), basophils and eosinophils ($CD294^+$) and dead cells ($7AAD^+$) were removed using a PerCP-Cy5-5A dump channel. Finally, the $CD45^+$ $CD34^+$ $Cd11b^+$ fibrocyte population was measured in the "CD11b-Pacific blue versus CD34-PE" plot. Fluoroscence minus one (FMO) controls are shown at the bottom" from [4].

Culture of fibrocytes

Briefly, PBMCs were isolated from 20 ml of peripheral EDTA blood using Lymphoprep (StemCell, Grenoble, France) according to manufacturer's instructions. The culture media consisted of the following products for the production of a total of 50 ml: 47 ml of FibroLife (LifeLine Cell

Technology, Frederick, USA), 0.5 ml of HEPES (10 mM), 0.5 ml Non-essential amino acids (NEAA), 0.5 ml of Sodium pyruvate (1 mM), 0.5 ml of Glutamine (2mM), 0.5 ml of Pen-Strep (Penicillin (100 U/ml) and Streptomycin (100 µg/ml)), and 0.5 ml of ITFS-3 (All Sigma-Aldrich, St. Louis, USA) and 2.5 µL of (at 100 µg/ml) IL-4 (CellSystems, Troisdorf, Germany). A monocyte, lymphocyte, granulocyte and eosinophil count were performed using PBMCs (Sysmex, Kobe, Japan). Hereafter cells were adjusted to 1×10^6 cells/ml culture media. Flat bottom 24-well plates (Nunc, Thermo Fisher Scientific) were used in combination with glass coverslips (Thermo Fisher Scientific) placed at the bottom of each well or by culturing directly in flat bottom 96-well plates (Nunc, Thermo Fisher Scientific). Ten wells were each filled with either 200 µl of cell suspension and supplemented with a further 500 µl of culture media for 24-well plates and 50 µl of cell suspension and supplemented with a further 150 µl of culture media for 96-well plates. The plate was placed in a humidified incubator (37°C, 5% CO_2) for 5 days. Non-adherent cells (e.g. T and B-cells) were discarded and the remaining cells were harvested, washing twice in PBS and drying it and hereafter staining in the well as previously described.[5]

The initial cultures were also stained for Pro-collagen type 1, which was confirmed in the spindle shaped cells, as previously shown[6]. Fibrocytes were counted in four randomly selected fields of each of three separate culture wells. Cultures were classified as failed if there were no fibrocytes, extensive cell lysis or any signs of contamination.

Staining synovial tissue for fibrocytes

Immunohistochemical staining was performed on 2µm thick sections of paraffin-embedded tissue. Sequential staining with rabbit anti- CD45 (1:100, EP68, Cell Marque) and mouse anti- α-SMA (1:1000, BS66, Nordic BioSite) was performed on a Ventana Discovery Ultra platform. For CD45 visualization a peroxidase based detection system with a purple chromogen was used and for α-

SMA a yellow phosphatase based detection (Discovery teal HRP purple and Discovery Teal phosphatase yellow, Ventana) was used. In this setup, double positive cells had an orange color. All stains performed on synovial tissue also had control stains on a range of other tissues, controlling for correct CD45 and α-SMA staining. In addition, a section of each synovial tissue was stained with both detection systems but with the second primary antibody omitted to prove that the first step did not induce staining in the second step.

Synovial fibrocytes were quantified counting in 4 areas of each 0.15 mm^2, positioned to best represent biopsy cellularity. Two were placed to include synovial lining and two in the synovial stroma. Cells in vessel walls were not counted as it was too difficult to distinguish cell types. Patient identity was blinded during cell counting.

References

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