Detection of Synovial Signatures in Peripheral Blood of Patients With Rheumatoid Arthritis via a Novel Blood-Based DNA Capture Assay

Peter C. Taylor[1], Jenya Antonova [2], Jennifer Geis[3], Katharine Dilger[3], David Chernoff[3], Diana Abdueva[3], Nancy Shadick*[4], Michael E. Weinblatt*[4]

[1]Botnar Research Centre, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Old Rd, Headington, Oxford OX3 7LD, UK. [2]Compass Strategy and Research, Inc. [3]Aqtual Inc., Hayward, California. [4]Brigham and Women's Hospital, Harvard Medical School; *Both Dr. Shadick and Dr. Weinblatt are last authors.

Background

- In rheumatoid arthritis (RA), biomarkers derived from synovial biopsies have shown an association with responsiveness to conventional synthetic disease-modifying antirheumatic drugs (csDMARD) therapy selection[1]
- However, biopsies require special training for clinicians and are semi-invasive for patients
- Blood-based tests are non-invasive and are a common procedure in clinical practice
- Prior research has reported synovium pathway transcriptomics based on synovial fluid samples[2]
- However, prior efforts to capture synovial signals in patients with RA via blood have failed because of the limited circulation of synovial-specific biomarkers and the dilution of molecular signals within the bloodstream

Objectives

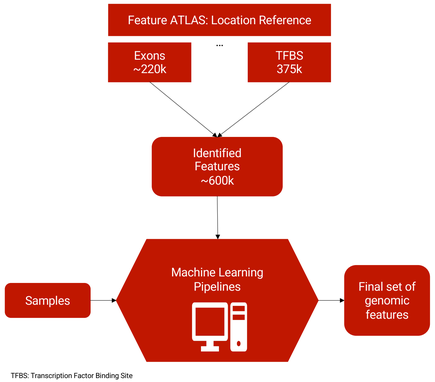
- This study aimed to investigate synovium-specific transcriptomic signals in blood plasma by employing a novel DNA capture platform to comprehensively analyze and characterize the molecular signatures in RA which differentiate from control samples derived from healthy individuals, patients with degenerative joint disease, or a range of inflammatory conditions other than RA

Methods

- The research process is summarized in **Figure 1**
- **RA specimen collection and plasma separation:**
  - Plasma samples from patients with RA and inflammatory conditions were obtained from Bio-Options (Brea, CA) Biorepository. Blood specimens were collected in EDTA blood collection tubes following the manufacturer's instructions and centrifuged for 10 minutes at 1500 x g. The plasma layer was then transferred to 2 mL cryogenic storage tubes and stored at -80°C.
- **cfDNA Isolation**
  - A proprietary chromatin capture workflow was used for DNA isolation and library preparation. Cell-free DNA (cfDNA) was extracted from a median of 1 mL of plasma using a proprietary protocol. Following isolation, cfDNA yield and quality were determined using the Qubit dsDNA High Sensitivity Assay (Thermo Scientific) and Tapestation High Sensitivity D5000 Assay (Agilent).
- **Library Preparation & Sequencing**
  - Libraries were built using reagents from the KAPA Hyper Prep Kit and a modified library preparation workflow that depletes nucleosomal (canonical) fragments. Libraries were quantified using the Qubit dsDNA High Sensitivity assay (Thermo Scientific) and Tapestation High Sensitivity D5000 assay (Agilent). Libraries were pooled and Whole Genome Sequencing was performed on NovaSeq 6000 instruments.
- **Feature Extraction**
  - Genome Alignment and Read Processing
    - Reads were aligned to the human genome (hg19) using bowtie21 (v2.4.4). Duplicate fragments were marked and removed using samtools2 markdup (v1.16). BAM files were converted to fragment tables and processed using R (v4.1.3) and python (v3.9.13) scripts.
- **Feature Identification**
  - The epigenome atlas, comprised of 600,000 features, was evaluated using bootstrap methods. An extended feature matrix of coverage in Exons' and Transcription Factor Binding Sites (TFBS) was constructed. A candidate list of genomic feature locations was generated using bootstrap method that robustly differentiates RA from non-RA samples by aggressively thresholding the separation of distributions at the 5th percentile of 1000 replicates.
- **Model Training**
  - Gene and Pathway Comparison
    - Candidate feature locations were mapped to genes. The genes were compared to the reported pathways[2] identified in whole blood and synovial tissue of patients with RA.
  - Machine Learning Model (**Figure 2**)
    - A machine-learning model was trained using 5 times repeated 10-fold cross validation. The final list of genomic features was compared to the published list of synovium pathway transcriptomics based on synovial fluid samples.[2]

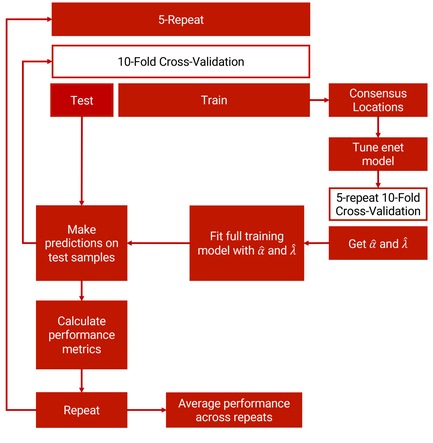
Methods

Figure 1. Summary of Research Process



TFBS: Transcription Factor Binding Site

Figure 2. Machine Learning Model Schema



Results

Table 1. Patient Demographics and Clinical Characteristics

	# of patients	# of samples	White, %	Female, %	Age	
					Range	Mean
Overall, including:	191[a]	229	63.3	67.9	19–88	53.6 (16.63)
Rheumatoid Arthritis[b]	89	89	58.4	86.5	28–66	57.0 (14.20)
Non-RA:	102	140	67.7	51.8	19–88	50.6 (18.06)
Healthy controls	29	66	42.3[c]	53.6	20–63	42.6 (12.15)
Ankylosing spondylitis	13	13	84.6	30.8	28–66	50.5 (14.80)
Crohn's Disease	10	11	80.0	30.0	23–87	55.7 (21.97)
Psoriasis	18	18	66.7	50.0	27–70	46.1 (13.74)
Psoriatic arthritis	10	10	70.0	50.0	46–88	65.8 (11.62)
Ulcerative colitis	10	10	70.0	50.0	19–67	33.9 (16.03
Osteoarthritis	12	12	91.7	91.7	47–87	73.3 (10.46)

[a] One patient did not have demographic characteristics recorded but was included in the sample because plasma and healthy status were known.
[b] Among patients with RA, 70% were seropositive and 96% were bDMARD-naive.
[c] Race was not known for three patients in the healthy control group.
bDMARD, biologic disease modifying anti-rheumatic drug.

Figure 3. The Identified Set of Genomic Features Overlapped With the Known Synovial and Blood Pathway Genes

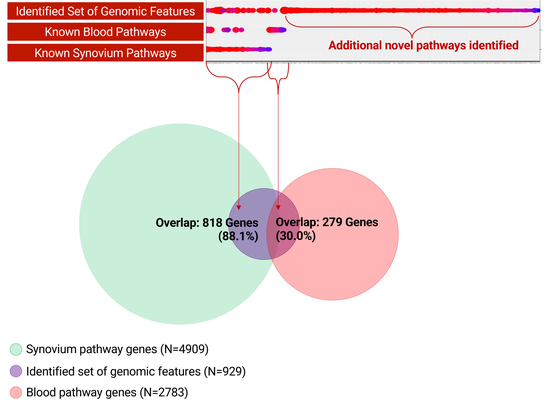
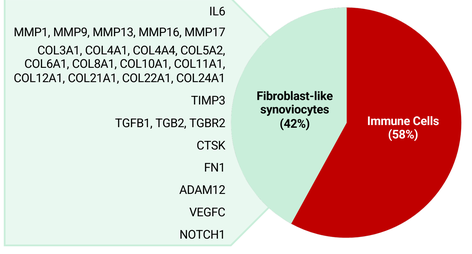


Table 2. FLS-Associated Genes Identified Within Synovial Signatures

Gene Symbol	Gene Name
IL6	Interleukin 6
MMP1	Matrix Metalloproteinase 1
MMP9	Matrix Metalloproteinase 9
MMP13	Matrix Metalloproteinase 13
MMP16	Matrix Metalloproteinase 16
MMP17	Matrix Metalloproteinase 17
COL3A1	Collagen Type III Alpha 1 Chain
COL4A1	Collagen Type IV Alpha 1 Chain
COL4A4	Collagen Type IV Alpha 4 Chain
COL5A2	Collagen Type V Alpha 2 Chain
COL6A1	Collagen Type VI Alpha 1 Chain
COL8A1	Collagen Type VIII Alpha 1 Chain
COL10A1	Collagen Type X Alpha 1 Chain
COL11A1	Collagen Type XI Alpha 1 Chain
COL12A1	Collagen Type XII Alpha 1 Chain
COL21A1	Collagen Type XXI Alpha 1 Chain
COL22A1	Collagen Type XXII Alpha 1 Chain
COL24A1	Collagen Type XXIV Alpha 1 Chain
TIMP3	Tissue Inhibitor Of Metalloproteinases 3
TGFB1	Transforming Growth Factor Beta 1
TGFB2	Transforming Growth Factor Beta 2
TGFBR2	Transforming Growth Factor Beta Receptor 2
CTSK	Cathepsin K
FN1	Fibronectin 1
ADAM12	A Disintegrin And Metalloproteinase Domain 12
VEGFC	Vascular Endothelial Growth Factor C
NOTCH1	Notch 1

FLS, fibroblast-like synoviocytes.

Figure 4. Synovial Signature Distribution



Results

- **Sample**
  - Patients had a mean (standard deviation, SD) age of 53.6 (16.63) (95% CI: [21.0, 86.2]) years and were mostly female (67.9%) and white (63.3%; **Table 1**)
  - Patients with RA were mostly seropositive (70%) and biologic-naïve (93%)
- **Gene Mapping and Pathway Analysis**
  - Pathway and functional analysis of the candidate feature set was studied via traditional gene set enrichment analysis by associating *cis* regulatory regions of the classifier with 929 genes. The genes were compared to the reported pathways[2] identified in whole blood and synovial tissue of patients with RA and further mapped onto cell types found within the synovium
  - In the list of identified genomic features, 88.1% overlapped with synovium pathway genes[2] and 30% with blood pathway genes (**Figure 3**)
  - Within the identified synovial signatures, 42% represent fibroblast-like synoviocyte (FLS)-associated genes (**Table 2; Figure 4**)
  - The remaining 58% of the features showed significant enrichment of immune cell types, including CD4+ and CD8+ T-cells, B-cells, and macrophages (**Figure 4**)

Conclusions

- The developed non-invasive DNA capture assay identified synovium-specific gene expression signatures in blood plasma of patients with RA
- Further clinical research is needed to validate these synovial signatures and confirm the clinical utility of the developed classification system
- A reliable method of identifying synovial signals is a promising finding towards the development of diagnostic, predictive, and prognostic tests aimed toward individualized precision-medicine-based care

References

1. Humby F, et al. (2019); PMID: 30878974. 2. Rychkov D, et al. (2021); PMID: 34177888

Disclosures

PT: Consulting role for AbbVie, Aqtual, Inc., Biogen, Fresenius, Galapagos, Gilead, GlaxoSmithKline, Janssen, Lilly, Nordic Pharma, Pfizer, Sanofi, and UCB. Grant support from Galapagos; **JA**: Consulting role for Aqtual, Inc.; **JG**: Employment with Aqtual, Inc.; **KD**: Employment, intellectual property/patents, stock options or bonding holdings in a for-profit corporation or self-directed pension plan with Aqtual, Inc.; **DC**: Consulting role for Aqtual, Inc. and Reflexion Pharma. Employment and stock options or bonding holdings in a for-profit corporation or self-directed pension plan with SetPoint Medical.; **DA**: Employment, intellectual property/patents, officer or board member, ownership interest, stock options or bonding holdings in a for-profit corporation or self-directed pension plan with Aqtual, Inc.; **NS**: Grant/Research support from AbbVie, Aqtual, Briston-Myers Squibb, and Janssen. **MW**: Consulting role for AbbVie, Aclaris, Amgen, Bristol-Myers Squibb, Corevitas, Eli Lilly, Gilead, Glaxo Smith Kline, Horizon, Johnson & Johnson, Pfizer, Prometheus Laboratories, Rani, Revolo, Sanofi, Sci Rhom, Scipher, Set Point, UCB. Grant/Research support from AbbVie, Aqtual, Bristol-Myers Squibb, and Janssen. Stock options or bond holdings in a for-profit corporation or self-directed pension plan with Canfite, Scipher, and Inmedix.

Acknowledgements

We are grateful for the contributions of Maggie Loui, PhD, who provided invaluable recommendations on how to optimize data reporting for clinical audiences.

Medical writing assistance was provided by Sarah Franco, PhD.

Presented at American College of Rheumatology (ACR) Convergence Conference on 12 November 2023 in San Diego, CA.