

A Novel Blood-Based Assay Differentiates Seropositive and Seronegative Rheumatoid Arthritis with High Sensitivity and Specificity from Healthy Individuals and Those with Other Inflammatory Diseases or Osteoarthritis

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Background

- Rheumatoid arthritis (RA) is a systemic disease characterized by synovial involvement. Inflamed synovium harbors critical pathological signals central to disease progression and clinical manifestations.
- Synovial biopsies have been the primary method for gaining insights into the pathobiology of inflamed synovial tissue, however the use of biopsies in routine clinical practice remains limited.
- Blood-based assays, while prevalent in clinical diagnostics, have not sufficiently elucidated the complex molecular and cellular narratives inherent to the synovium.

Objective

- We aimed to develop a novel non-invasive assay to detect synovial signal in the blood and evaluate its ability to differentiate seropositive and seronegative RA from healthy controls, osteoarthritis, and non-RA inflammatory conditions.

Methods

Overview of Platform

- This novel platform provides a non-invasive, real-time assessment of organ- and disease-specific epigenomic and transcriptomic signals.
- Cell-free DNA (cfDNA) was extracted from 1 mL of plasma using a proprietary chromatin capture workflow, followed by library preparation and sequencing.



- Plasma samples from 89 individuals meeting 2010 ACR/EULAR classification criteria for RA, 29 healthy controls, 12 individuals with osteoarthritis and 61 individuals with an inflammatory condition were processed.

Table 1: Cohort used to develop classifier

	Number of Samples	Number of Patients	White, % (Race Unknown ^a)	Female, %	Age, Median (Q1, Q3)
Overall, including:	229	191 ^b	63.3 (n=188)	67.9	56.0 (40.0–66.0)
Rheumatoid Arthritis ^c	89	89	58.4 (n=89)	86.5	58.0 (48.0–68.0)
Non-RA:	140	102	67.7 (n=99)	51.8	51.0 (34.0–65.0)
Healthy controls	66	29 ^d	42.3 (n=26)	53.6	41.0 (34.2–51.8)
Ankylosing spondylitis	13	13	84.6 (n=13)	30.8	59.0 (36.0–63.0)
Crohn's disease	11	10	80.0 (n=10)	30.0	59.0 (37.2–70.0)
Psoriasis	18	18	66.7 (n=18)	50.0	44.5 (33.0–56.8)
Psoriatic arthritis	10	10	70.0 (n=10)	50.0	65.5 (63.0–71.8)
Ulcerative colitis	10	10	70.0 (n=10)	50.0	27.0 (23.2–68.0)
Osteoarthritis	12	12	91.7 (n=12)	91.7	75.5 (23.2–41.0)

^a Race was known for some patients, the number of which is indicated in parentheses.

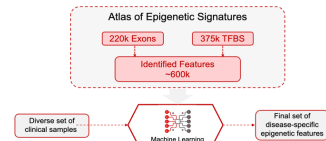
^b One patient did not have demographic characteristics recorded but was included in the sample because plasma and healthy status were known.

^c Among RA patients, 70% were seropositive, 30% were seronegative and 96% were biologic-naïve.

^d RA, rheumatoid arthritis.

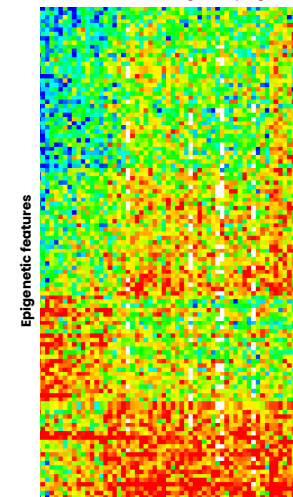
Results

Figure 1. Machine Learning (ML) Framework



- The ML model identified disease-specific signatures.

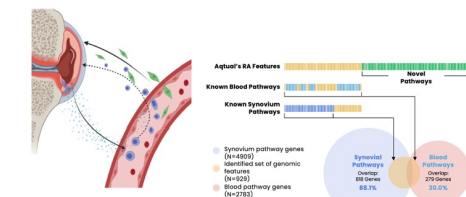
Figure 2. ML model identified epigenetic markers indicative of distinctive regulatory signals



RA patients

- The ML model identified 3,425 epigenetic features with statistically significant discrimination between patients with and without RA.
- 1,120 markers were selected based on the highest silhouette scores for distinctiveness.

Figure 3. The identified set of genomic features overlapped with known synovial and blood pathway genes



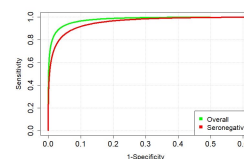
Nyström U, et al. *Front Immunol*. 2021 Jun 8;12:638066. doi: 10.3389/fimmu.2021.638066. RA, rheumatoid arthritis.

- Comparison of the gene set derived from genomic features with previously published lists, which integrated and cross-referenced publicly available microarray gene expression data from both synovial tissue and blood, showed significant overlap.

Figure 4. The RA specific classifier demonstrated a sensitivity of 90.8% and a specificity of 96.1%

	OVERALL	SERONEGATIVE
	Mean (95% CI)	Mean (95% CI)
AUC	0.991 (0.989,0.993)	0.971 (0.969,0.973)
Sensitivity, %	90.8 (88.9,92.7)	83.7 (79.6,87.8)
Specificity, %	96.1 (94.8,97.4)	95.4 (94.0,96.8)
Positive Likelihood Ratio	24.13 (15.14,33.12)	21.46 (13.48,29.44)
Negative Likelihood Ratio	0.10 (0.098,0.102)	0.17 (0.166,0.174)

Normal/Healthy	100% (NA)
Ankylosing spondylitis	93.8% (0.87,99)
Crohn's Disease	100% (NA)
Psoriasis	91.1% (0.85,97)
Psoriatic arthritis	76.0% (0.65,0.87)
Ulcerative colitis	100% (NA)
Osteoarthritis	86.7% (0.77,0.96)



- Sensitivity remained high in seronegative RA patients.
- The assay demonstrated high specificity for all groups, including healthy controls, osteoarthritis, and other inflammatory conditions.

- Validation of the RA classifier was evaluated on two independent sets of samples: 1) patients enrolled in The Brigham and Women's Hospital RA Sequential Study (BRASS) registry (n=19) and 2) samples purchased from biobanks (n=17 for RA, n=46 for non-RA).

Table 2. Validation of the classifier on independent cohorts: BRASS cohort and Biobank samples

	BRASS Cohort	Biobank
Rheumatoid Arthritis (#)	19	17
Non-RA (#)	--	46
Sensitivity: Mean (95% CI)	0.95 (0.74, 0.99)	0.89 (0.49, 0.95)
Specificity: Mean (95% CI)	--	0.92 (0.67, 0.99)

- The classifier achieved a sensitivity of 89% and a specificity of 92% with the biobank samples, and a sensitivity of 95% with the BRASS registry samples.

Conclusions

- We have developed a novel blood-based assay that detects both organ-specific and systemic biological processes in patients with RA.
- This non-invasive assay has shown potential to differentiate RA patients from healthy controls, osteoarthritis patients, and those with other non-RA inflammatory diseases.
- The RA classifier demonstrated high sensitivity and specificity, while maintaining sensitivity in seronegative patients.
- Despite the small sample size, the RA classifier showed high specificity against neighboring inflammatory and autoimmune diseases
- Validation in two independent cohorts confirmed the performance of the classifier.
- This research highlights one application of this novel chromatin capture assay to detect synovial signal in the blood
- Studies are underway to use this technology to develop drug class-specific therapy response.