Abstract #: 1556139

A Novel Blood-Based Assay Differentiates Seropositive and Seronegative Rheumatoid Arthritis From Healthy Individuals and Those With Other Inflammatory Diseases or Osteoarthritis

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DISCLOSURES

- **P. Taylor**: Consulting role for AbbVie, Aqtual, Inc., Biogen, Fresenius, Galapagos, Gilead, GlaxoSmithKline, Janssen, Lilly, Nordic Pharma, Pfizer, Sanofi, and UCB. Grant support from Galapoagos.
- **J. Antonova**: Consulting role for Aqtual, Inc.
- **J. Geis**: Employment with Aqtual, Inc.
- **K. Dilger**: Employment, intellectual property/patents, stock options or bonding holdings in a for-profit corporation or self-directed pension plan with Aqtual, Inc.
- **D. Chernoff**: 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.
- **N. Shadick**: Grant/Research support from AbbVie, Aqtual, Briston-Myers Squibb, and Janssen.
- **D. Abdueva**: 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.
- **M. Weinblatt**: Consulting role for AbbVie, Aclaris, Amgen, Aqtual, 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.

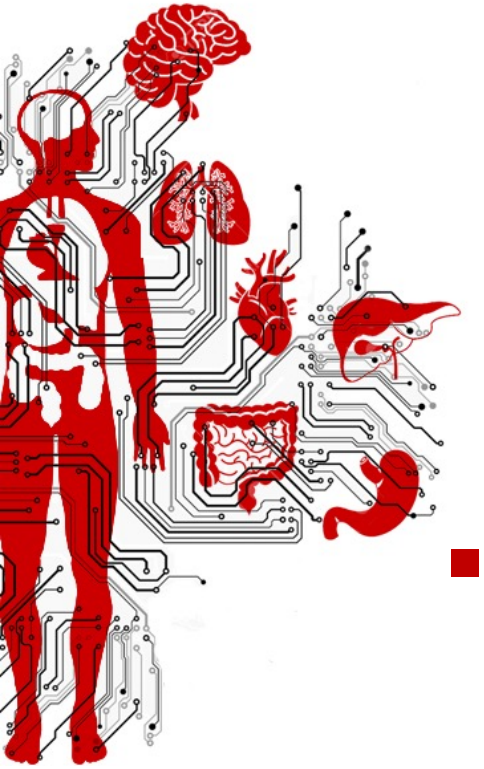
BACKGROUND

- Precision medicine has enabled significant advancements in oncology. Despite learnings from academic research in rheumatology, the integration of precision medicine into routine clinical practice remains limited.
- Rheumatoid arthritis 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.
- The developed non-invasive DNA capture assay can identify synovium-specific gene expression signatures in blood plasma of patients with RA

AIM

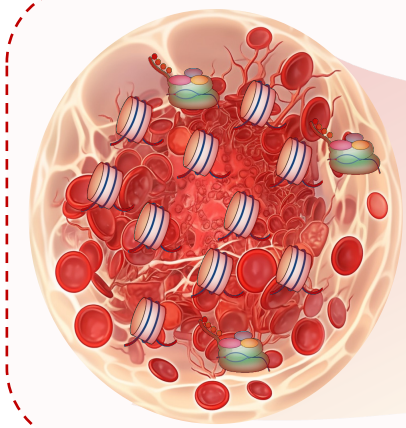
- We aimed to evaluate the clinical feasibility of the developed assay to differentiate RA patients from healthy controls and those with other conditions, including osteoarthritis and a range of inflammatory conditions

The story of cell-free DNA in blood: a window into cellular lifecycles

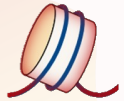


- The continuous cycle of cell turnover is a critical biological process that maintains tissue health and homeostasis.
  - Cell death releases DNA fragments into the bloodstream, these fragments carry information about a cell's regulatory mechanisms and vary depending on the originating organ and its health.
- Differences in DNA fragment patterns can signal a pathobiological state, pointing to potential organ-specific and systemic health issues.
- The unique patterns and sizes of cfDNA can convey accurate and precise holistic picture of a person's health.

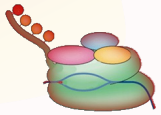
Unlocking personalized medicine: insights from long cfDNA fragments



Cell-free DNA (cfDNA) in the blood exists primarily in two forms:



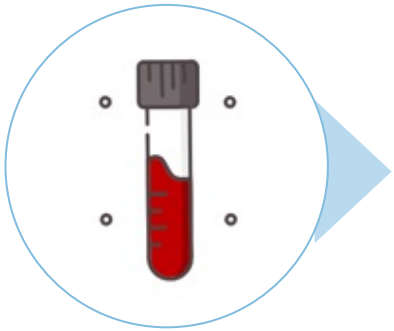
: **short fragments** associated with nucleosomes and



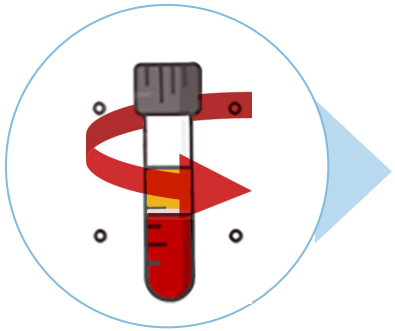
: **long fragments** from regulatory-active chromatin

- The long cfDNA fragments contain rich epigenomic and transcriptomic information, reflecting their cells of origin.
- These long fragments provide detailed insights into the epigenetic and transcriptomic landscape of their original tissue, revealing the underlying regulatory mechanisms.
- Quantification of these cfDNA fragments can yield in-depth information about tissue origin and pathobiology of the cell functions. This analysis yields critical information for understanding disease progression, customizing treatments, and evaluating the effectiveness of therapies.

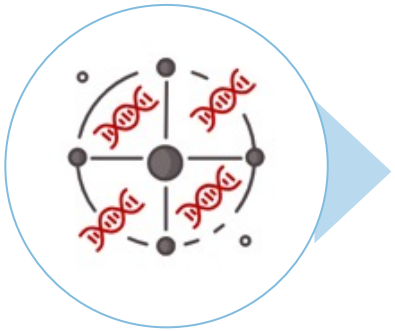
Streamlined workflow for regulatory cfDNA enrichment



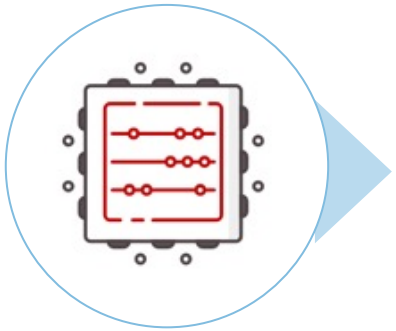
Blood draw



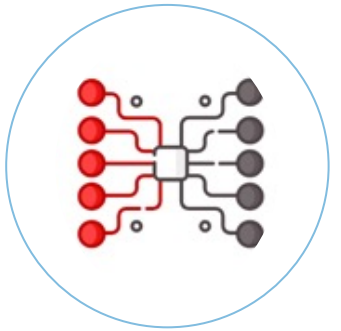
Plasma Isolation



Signature Capture

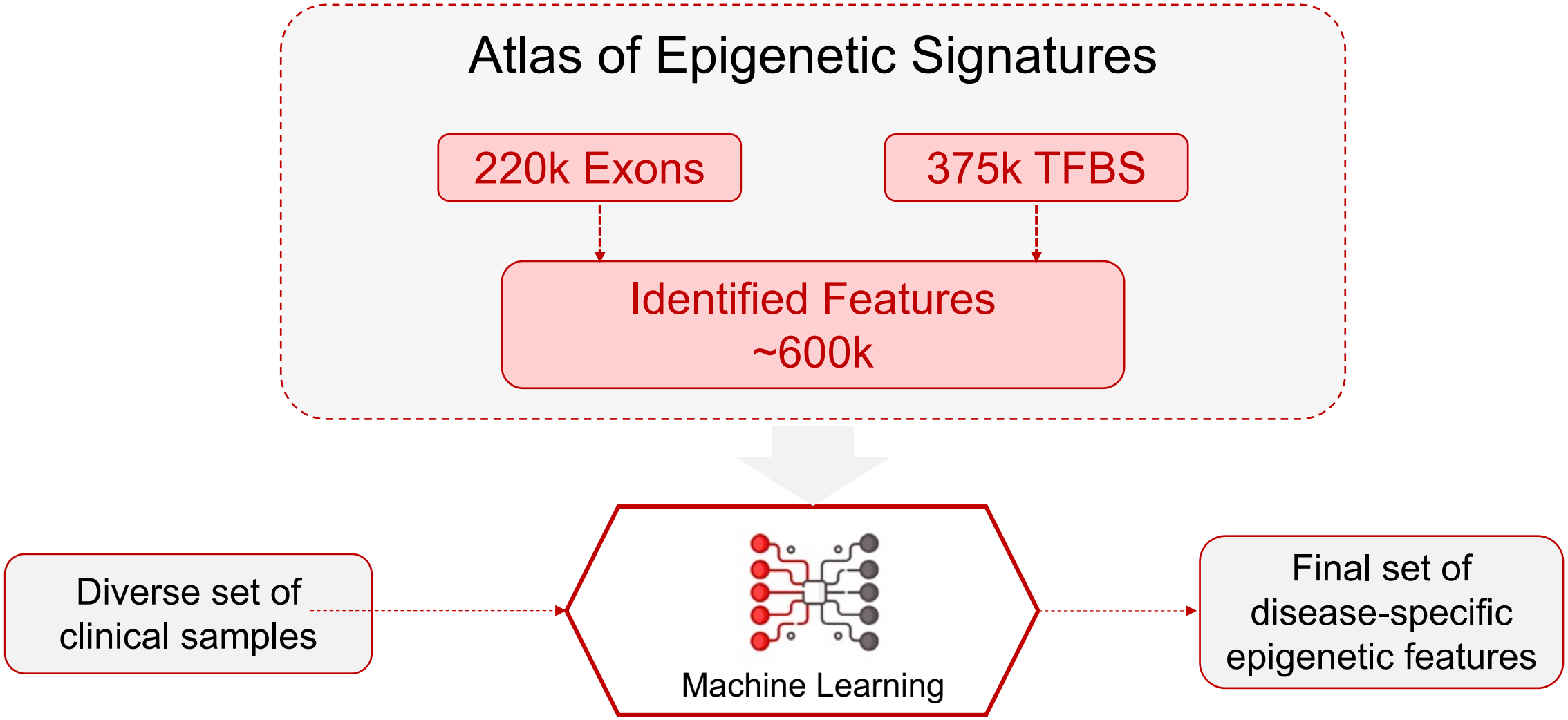


Quantification



Signal processing

Signal processing: identifying disease-specific signatures through machine learning



1. Taylor et al. ACR 2023. Abstract ID: 1556088.

AUC, area under the curve; RA, rheumatoid arthritis.

TFBS: Transcription Factor Binding Site

Methods: cohort description

	Number of Samples	Number of Patients
Overall, including:	229	191[b]
Rheumatoid Arthritis[c]	89	89
Non-RA:	140	102
Healthy controls	66	29[b]
Ankylosing spondylitis	13	13
Crohn's disease	11	10
Psoriasis	18	18
Psoriatic arthritis	10	10
Ulcerative colitis	10	10
Osteoarthritis	12	12

Data Source:

Plasma samples from patients with RA, other inflammatory conditions, OA and healthy controls were obtained from BioOptions (Brea, CA) Biorepository.

The RA patients all fulfilled the 2010 ACR/EULAR diagnostic criteria for RA.

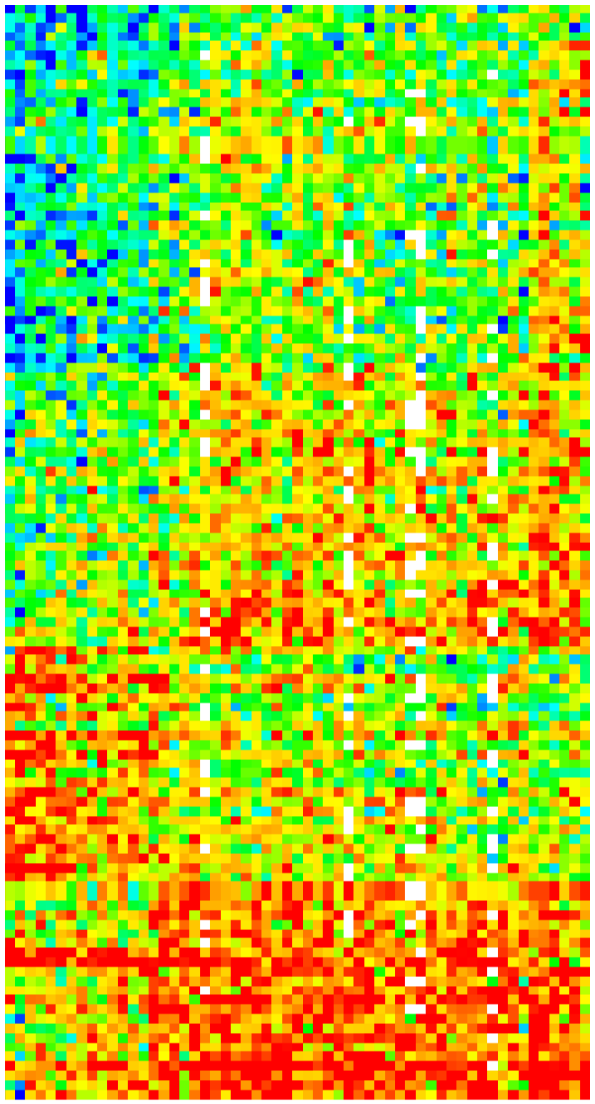
[a] One patient did not have demographic characteristics recorded but was included in the sample because plasma and healthy status were known.
OA, osteoarthritis; RA, rheumatoid arthritis.

Results: cohort description

	Number of Samples	Number of Patients	White, % (Race Known[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[b]	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.
RA, rheumatoid arthritis.

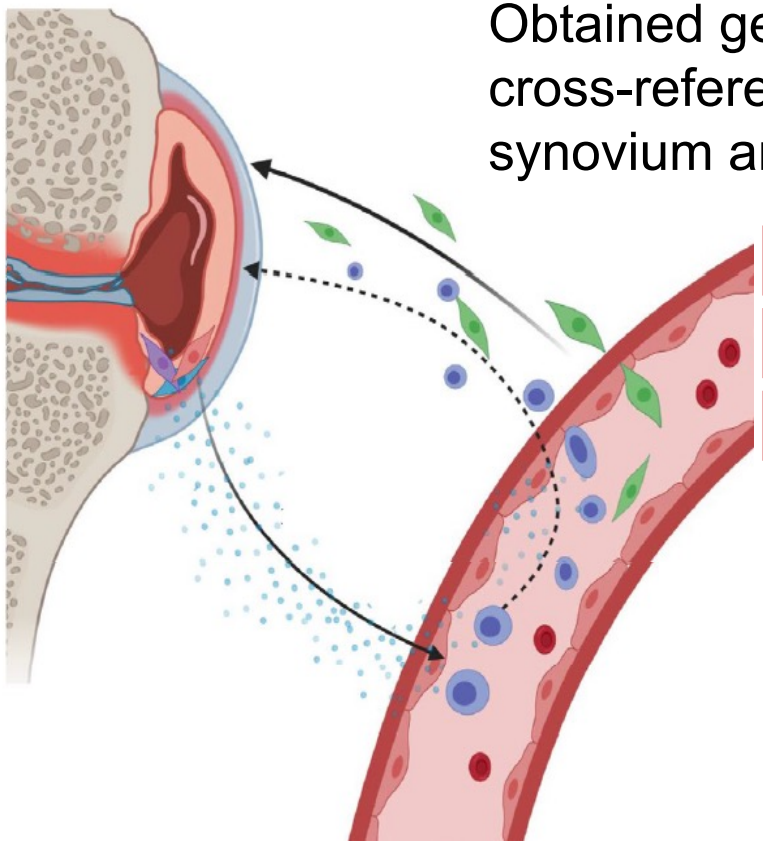
Epigenetic landscape of RA: a heatmap overview of machine learning classification



- The machine learning model identified 3,425 epigenetic features with statistically significant discrimination between patients with and without RA.
- The heatmap displays selected epigenetic markers from a broader hierarchical clustering of identified features, each with a high silhouette score indicative of distinctive regulatory signals. This visualization illustrates the diversity of regulatory signal patterns among patients with rheumatoid arthritis.
- The identified epigenetic features were mapped to 929 genes and compared to the reported pathways.

The identified set of genomic features overlapped with known synovial and blood pathways genes

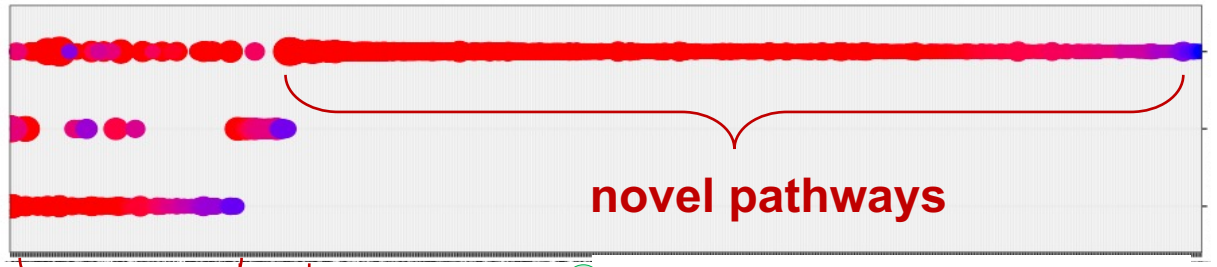
Obtained gene set was compared to previously-published lists[1] that integrated and cross-referenced publicly available microarray gene expression data from both synovium and blood tissues

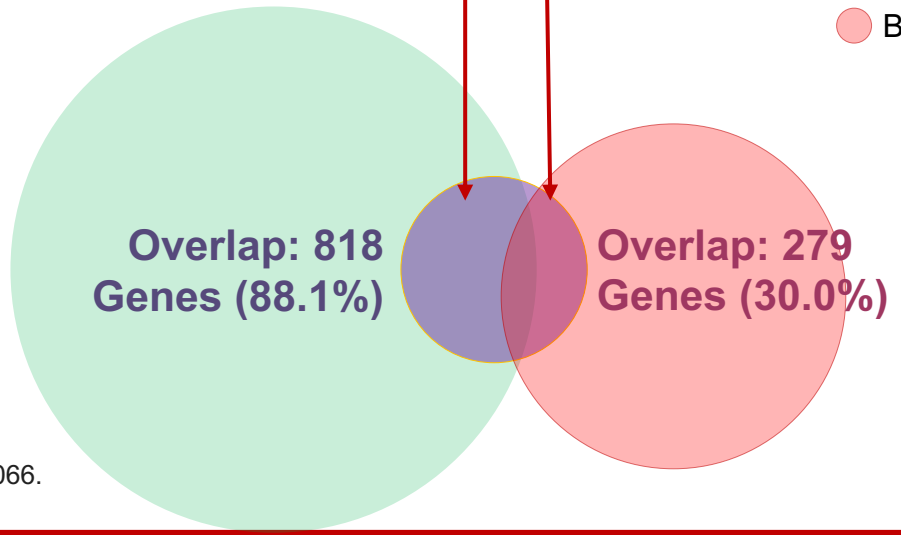


AQTUAL's RA Features

Known Blood Pathways

Known Synovium Pathways





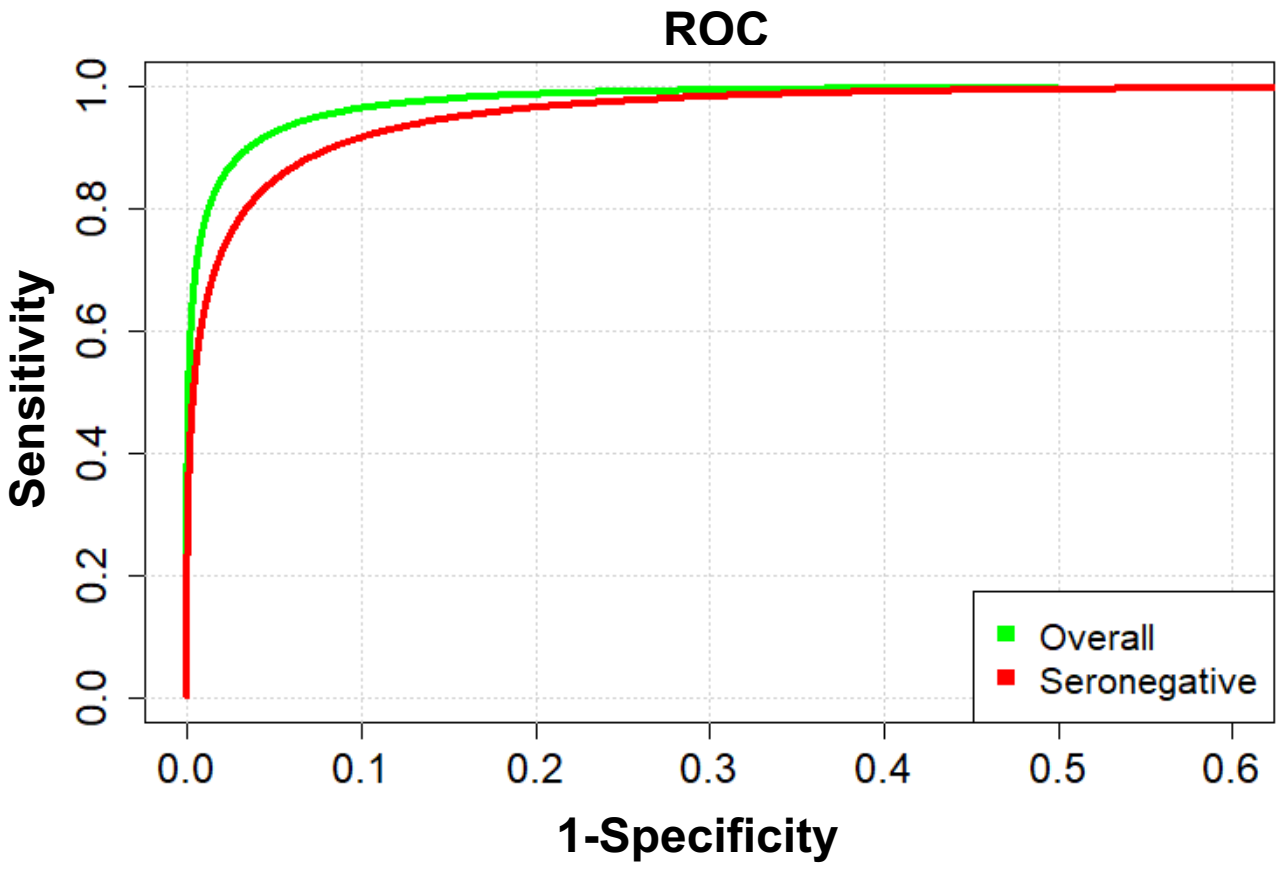
Synovium pathway genes (N=4909)

Identified set of genomic features (N=929)

Blood pathway genes (N=2783)

[1]Rychkov D, et al. *Front Immunol*. 2021 Jun 8;12:638066. doi: 10.3389/fimmu.2021.638066.
RA, rheumatoid arthritis.

Results: test performance for diagnosis of RA



	Mean (SD)	95% CI
Overall		
AUC	0.991 (0.001)	(97.2–99.6)
Sensitivity, %	90.8 (0.94)	(83.2–95.4)
Specificity, %	96.1 (0.64)	(92.7–97.9)
Positive Likelihood Ratio	24.13 (4.495)	(16.9–31.3)
Negative Likelihood Ratio	0.10 (0.001)	(0.098–0.101)
Seronegative		
AUC	0.971 (0.001)	(93.8–99.2)
Sensitivity, %	83.7 (2.03)	(63.3–91.8)
Specificity, %	95.4 (0.69)	(90.8–97.5)
Positive Likelihood Ratio	21.46 (3.99)	(15.1–27.8)
Negative Likelihood Ratio	0.17 (0.002)	(0.166–0.173)

AUC, area under the curve; RA, rheumatoid arthritis; ROC, receiver operating characteristic curve.

Results: test performance for comparator conditions

RA vs	Number of patients	Specificity, Mean (SD)	95% CI
Healthy Controls	66	100 (0)	(94.4–100.0)
Ankylosing spondylitis	13	95.4 (4.2)	(66.6–98.6)
Crohn's Disease	11	100 (0)	(74.1–100.0)
Psoriasis	18	92.2 (3.00)	(74.2–99.0)
Psoriatic arthritis	10	80.0 (0)	(49.0–94.3)
Ulcerative colitis	10	100 (0)	(72.2–100.0)
Osteoarthritis	12	88.3 (4.56)	(64.6–98.5)

RA, rheumatoid arthritis.

Conclusions

- We have developed a blood-based assay that detects both organ-specific and systemic biological processes in patients with rheumatoid arthritis
- In the current cohort, a novel non-invasive assay has shown the potential to differentiate patients with RA from healthy controls and those with osteoarthritis and non-RA inflammatory diseases
- Test performance for RA was marked with high sensitivity and overall specificity, and sensitivity was maintained in seronegative patients
- In comparator cohorts, the assay demonstrated high specificity for all groups, including healthy controls, osteoarthritis, and other inflammatory conditions; this finding was especially impressive because of the small sample size
- Further research is needed to confirm these results in an independent study
- This research exemplifies one application of the developed assay that detects synovial signal in the blood
- Future research should investigate opportunities to address unmet clinical need, for example, detection or prediction of comorbidities in order to better inform the most appropriate treatment choice for patients with various inflammatory conditions

RA, rheumatoid arthritis.

Acknowledgements & contact

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

- Future questions can be addressed to:

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