Search for new biomarkers reveals altered metabolic signature in plasma of axSpA patients

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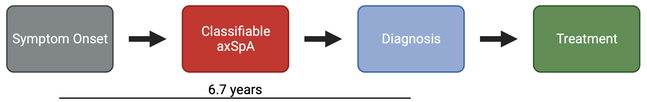
A Reliable Biomarker is Needed to Decrease Time to Diagnosis



Without a reliable biomarker of disease, the average diagnostic delay remains high at 6.7 years.[1]

Current diagnostic criteria

Imaging (mainly radiographs)
Show altered bone structure, which results from inflammation.

CRP and sedimentation rate tests
Results correlate with inflammation but are not reliable biomarkers of axSpA (AS).

Findings

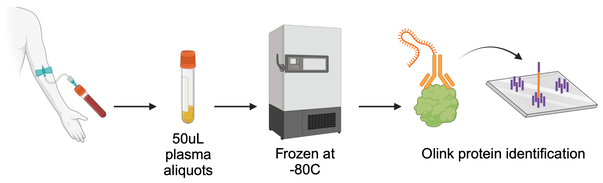
In our search for an inflammation driven biomarker that can be detected prior to radiographic disease, we found altered metabolite levels and globally lower protein levels in plasma from AS individuals.

Hypothesis

Aberrant metabolism in the immune cell compartment is driving pathogenic inflammation and the observed changes in AS plasma.

Decrease in Metabolically Relevant Proteins are Observed in AS Plasma

A)



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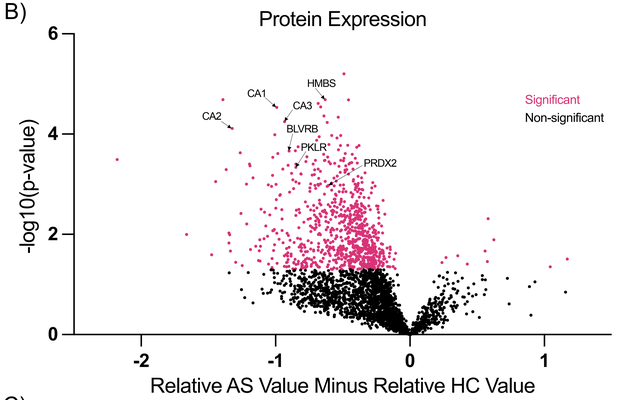


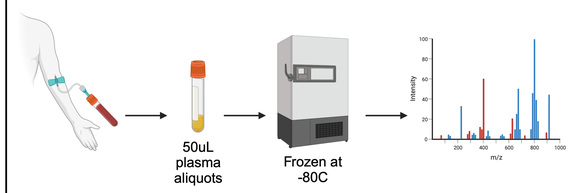
C)

Protein	P-Value	Fold Change	Function
Hydromethylbilane Synthase (HMBS)	2.06E-05	0.834	Heme production
Carbonic Anhydrase 1 (CA1)	2.95E-05	0.758	Hydration/dehydration of CO2. Promotes calcium precipitation (bone formation)
Carbonic Anhydrase 3 (CA3)	5.66E-04	0.554	Skeletal specific hydration/dehydration of CO2
Carbonic Anhydrase 2 (CA2)	7.71E-05	0.769	Hydration/dehydration of CO2. Defects associated with disease
Biliverdin Reductase B (BLVRB)	2.17E-04	0.695	Reduces biliverdin to bilirubin which acts as an antioxidant
Pyruvate Kinase L/R (PKLR)	4.59E-04	0.776	Last step of the glycolytic pathway
Peroxiredoxin-2 (PRDX2)	1.06E-03	0.702	Acts as an antioxidant by interacting with hydrogen peroxide

Figure 1: Decreased proteins are observed in AS plasma
A) Experimental design; Plasma from HC and AS individuals were collected, frozen, and run on the Olink Explore3072 platform (n = 74 biological replicates, 1 experiment). B) Volcano plot showing the relationship of differences between the AS and HC samples and corresponding p-values. C) A table showing 6 statistically altered proteins that impact metabolism. Parametric T-tests were performed for statistical analyses.

Fatty Acids and Other Metabolites are Altered in AS Plasma

A)



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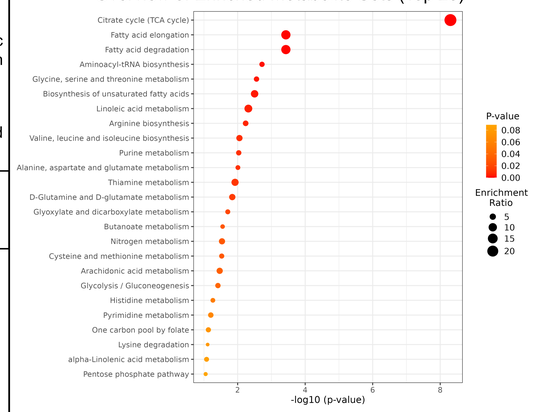

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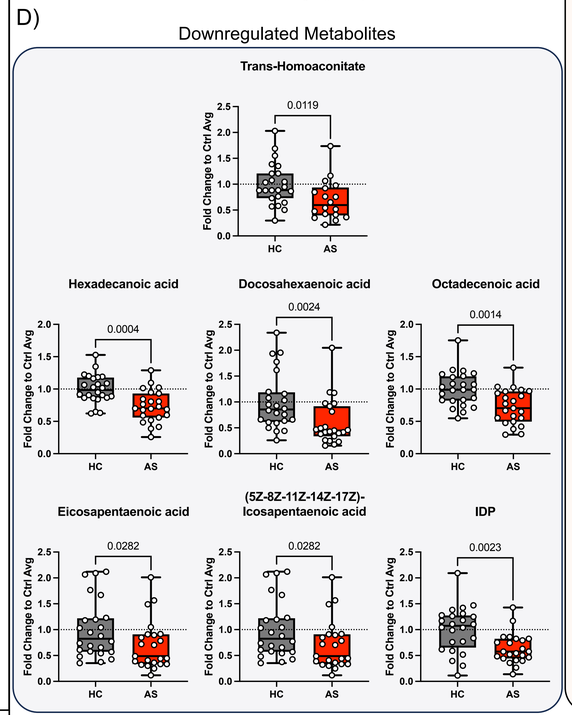

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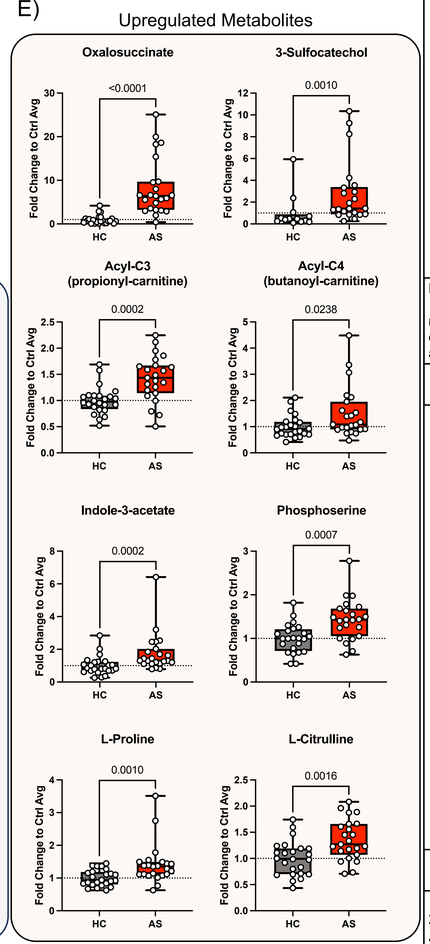
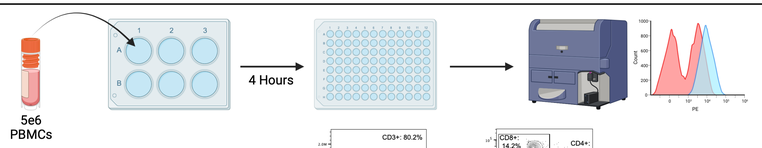


Figure 2: Alterations in metabolite levels are observed in AS plasma
A) Experimental design; plasma from HC and AS individuals were collected, frozen, and run on LCMS (n=23-24 biological replicates, 1 experiment). B) PLSDA plot and C) KEGG analysis showing the top 25 altered processes were obtained using the MetaboAnalyst platform. Graphs show fold change of D) significantly downregulated, and E) significantly upregulated metabolites. Statistical analyses for metabolite plots were performed using Mann-Whitney tests.

Increased Mitochondrial Reactive Oxygen Species are Observed in AS PBMCs

A)



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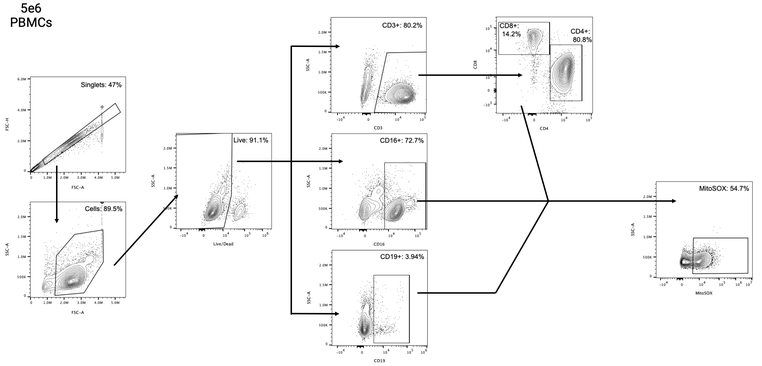
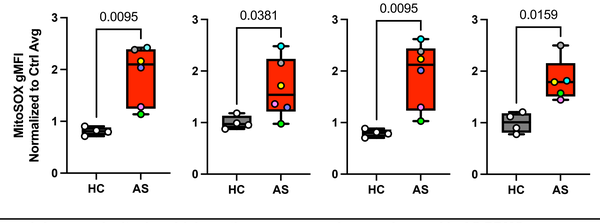


C)



D)

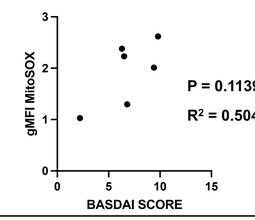


Figure 4: Increased mitochondrial reactive oxygen species are observed in AS PBMCs
A) Experimental design; frozen PBMCs from HC and AS samples were thawed and rested in RPMI for 4 hours before undergoing staining for mitochondrial reactive oxygen species (mROS) analysis (n= 4-6 biological replicates, 1 experiment). B) Flow plots showing gating strategy for collected data. C) Fold change of MitoSOX gMFI, data point colors correspond to individual AS subjects. Mann-Whitney tests were used for statistical analyses. D) Correlation analysis between BASDAI and MitoSOX gMFI. Pearson correlation was used for statistical analysis.

Conclusions and Future Directions

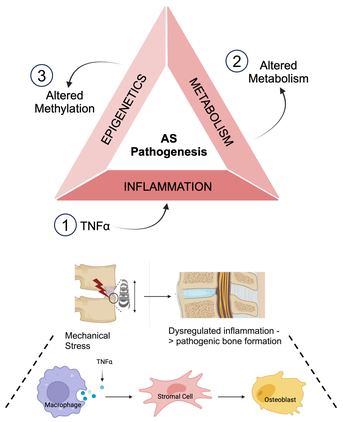
Our data suggest, in people with AS, immune cells are retaining proteins; leading to an altered metabolism that increases mROS.

We hypothesize that in AS patients, inflammation is inducing an altered metabolism and resulting in epigenetic memory that stores an inflammatory phenotype across immune cell subsets.

1) Published serum omics data in TNFα related inflammatory arthritis suggest a conserved mechanism.[2]
- Evaluate mROS and metabolic flux in PBMCs from psoriatic arthritis and IBD patients.

2) The altered fatty acid metabolomic data combined with the increases in mROS indicate alterations in mitochondrial metabolism.
- Evaluate metabolic flux of PBMCs
- Examine the characteristics of the mitochondria

3) Global decrease of proteins in the serum indicate either protein accumulation in the cells or hindered protein expression.
- Collect proteomic data on matched PBMCs from plasma cohort
- Compile published scRNA and ATAC-seq data



References

1.Zhao, S. S. *et al.* Diagnostic delay in axial spondyloarthritis: a systematic review and meta-analysis. *Rheumatology* **60**, 1620–1628 (2021).
2.Bogunia-Kubik, K. *et al.* Disease Differentiation and Monitoring of Anti-TNF Treatment in Rheumatoid Arthritis and Spondyloarthropathies. *Int. J. Mol. Sci.* **22**, 7389 (2021).

Acknowledgements

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