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Serum miRNAs as Potential Predictive Biomarkers in Rheumatoid Arthritis

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INTRODUCTION

MicroRNAs (miRNAs) are tissue-specific, small non-coding RNAs which have been implicated in cancer, viral, neurodegenerative and autoimmune diseases due to their important role in numerous biological processes such as cell differentiation and homeostasis, through the regulation of gene expression. A number of studies have reported that dysregulated miRNA expression influences immune regulation, enhances pro-inflammatory signalling pathways and leads to the overproduction of pro-inflammatory cytokines in Rheumatoid Arthritis (RA). Early diagnosis is crucial in halting the development of RA in order to prevent disability and maintain quality of life and recent studies have suggested that the presence or absence of circulating miRNAs may pre-empt the development of arthritis or predict an individual's response to a particular treatment.

AIMS

- To compare expression levels of circulatory miRNAs in serum from healthy control (HC) and RA patients
- To assess the effect of methotrexate (MTX) on miRNA expression
- To compare expression levels of circulatory miRNAs in serum from HC and arthralgia or 'at-risk individuals'
- To determine whether miRNA levels can be predictive of disease development or response to MTX treatment.

METHODS

Serum samples were collected from HC (n=20), RA (n=50) and arthralgia (n=10) patients and a second serum sample was collected from a cohort of the RA patients, prescribed MTX (n=20), 3 months post-treatment.

The FirePlex miRNA Immunology V2 panel was selected for multiplex analysis of 68 miRNAs in each serum sample, using the FirePlex Analysis Workbench software.

SUMMARY

- miR-126-3p, let-7d-5p, miR-431-3p, miR-221-3p, miR-24-3p, miR-130a-3p, miR-339-5p, let-7i-5p were significantly elevated in RA serum compared to HC serum.
- miR-17-5p was significantly lower in RA serum than HC serum.
- miR-339-5p and let-7i-5p were significantly reduced following MTX treatment
- Finally, miR-126-3p, let-7d-5p, miR-431-3p, miR-221-3p, miR-24-3p, miR-130a-3p which are elevated in RA are also significantly elevated in arthralgia/'at-risk individuals' compared to HC.

REFERENCES

1. Cuppen, B.V., et al., *Can baseline serum microRNAs predict response to TNF-alpha inhibitors in rheumatoid arthritis?* Arthritis Res Ther, 2016. **18**: p. 189.
2. Murata, K., et al., *Plasma and synovial fluid microRNAs as potential biomarkers of rheumatoid arthritis and osteoarthritis.* Arthritis Res Ther, 2010. **12**(3): p. R86.
3. Wade, S.M., et al., *Serum microRNA signature as a diagnostic and therapeutic marker in patients with psoriatic arthritis.* J Rheumatol, 2020.

CONCLUSION

This study demonstrates that serum miRNAs are altered in RA and suggests that specific miRNAs may have potential as predictive biomarkers both for disease development in 'at-risk individuals' and for response to MTX treatment.

RESULTS

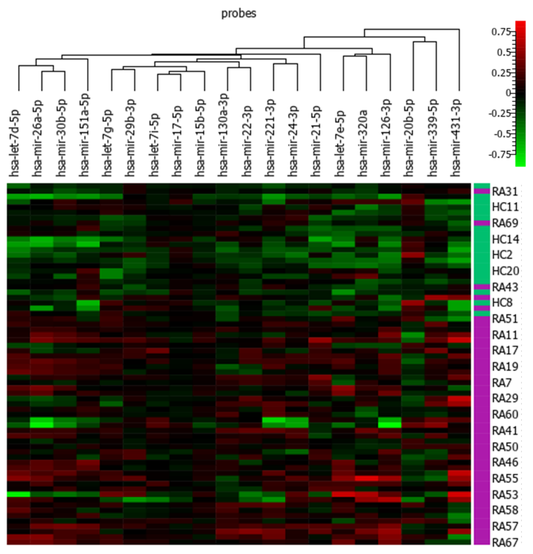

Fig 1. Top 20 differentially expressed miRs between RA & HC. Heatmap generated using FirePlex Analysis Workbench software with unsupervised two-way hierarchical miRNA (weighted linkage) and sample (Ward's linkage) clustering.
Purple: RA, Green: HC.

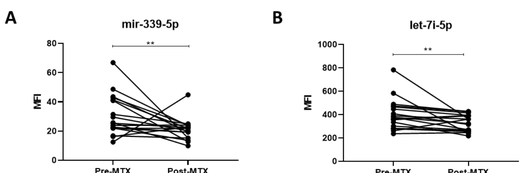

Fig 4. Effect of MTX on miRNA expression. Analysis of miRNA expression pre-MTX and 3 months post-MTX showed that 2 miRNAs were significantly reduced by MTX treatment.

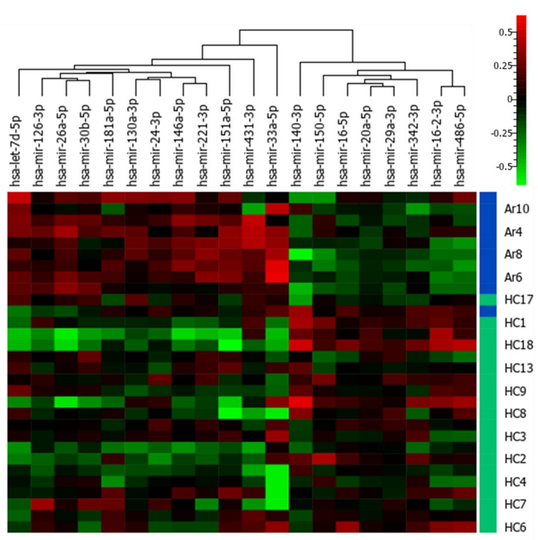

Fig 5. Top 20 differentially expressed miRs between arthralgia & HC. Heatmap generated using FirePlex Analysis Workbench software with unsupervised two-way hierarchical miRNA (weighted linkage) and sample (Ward's linkage) clustering.
Blue: Arthralgia (Ar), Green: HC

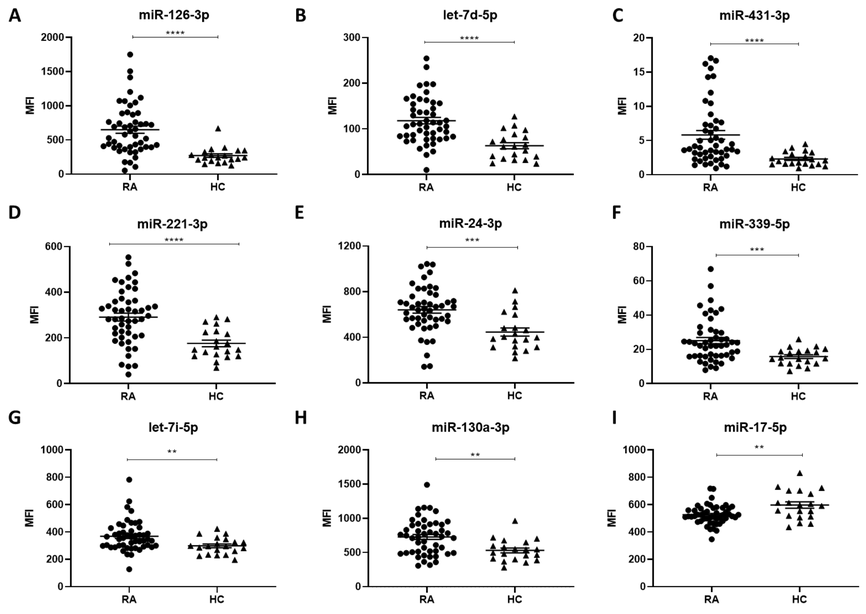

Fig 2. Expression of miRNAs between RA and HC. Analysis of the top 20 differentially expressed miRNAs identified 9 miRNAs of interest that were significantly different in RA compared to HC. Of the 9 miRNAs, 8 were significantly elevated in RA and 1 was significantly lower in RA. Results shown are Mean ±SEM for RA (N=50) and HC (N=20). ****p ≤ 0.0001, *** p ≤ 0.001, ** p ≤ 0.01 as determined by Mann-Whitney *U* test.

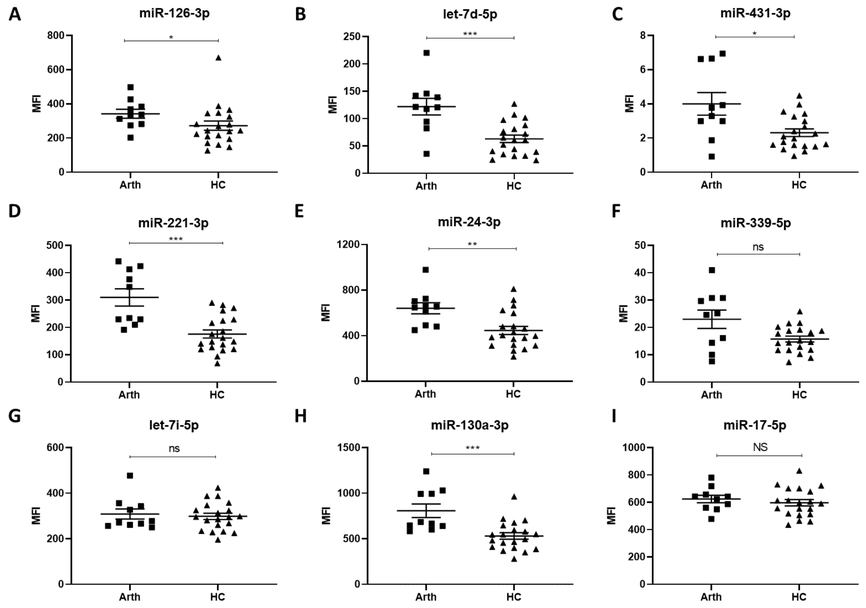

Fig 6. Expression of miRNAs between arthralgia and HC. Analysis of the top 20 differentially expressed miRNAs between arthralgia (Arth) and HC revealed that 6 miRNAs which were elevated in RA compared to HC were also elevated in arthralgia compared to HC. Results shown are Mean ±SEM for Arthralgia (N=10) and HC (N=20). *** p ≤ 0.001, ** p ≤ 0.01, * p ≤ 0.05 as determined by Mann-Whitney *U* test.

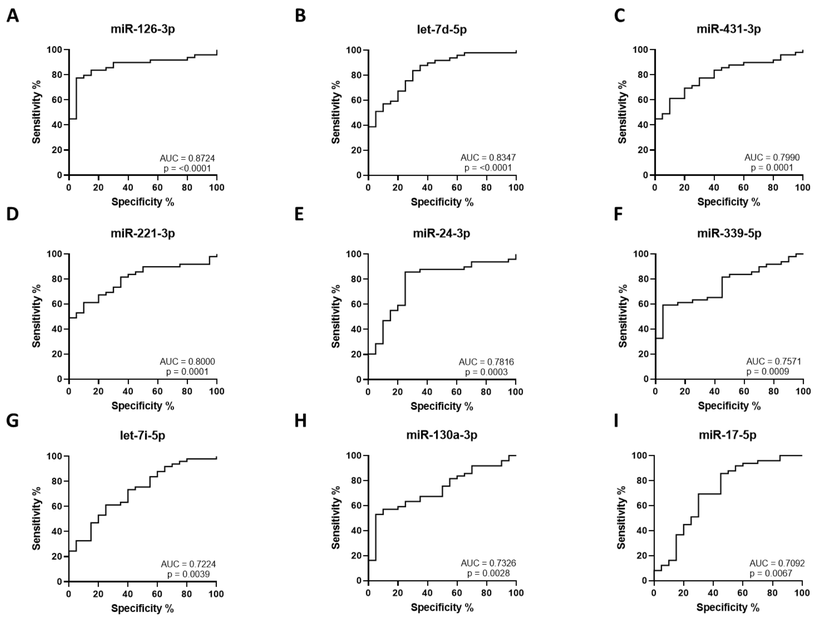

Fig 3. Receiver Operating Characteristic (ROC) curves demonstrating the diagnostic ability of miRNAs to predict RA disease. ROC curves comparing RA v HC were generated in GraphPad Prism 8. Area under the curve (AUC) was calculated with 95% confidence interval.

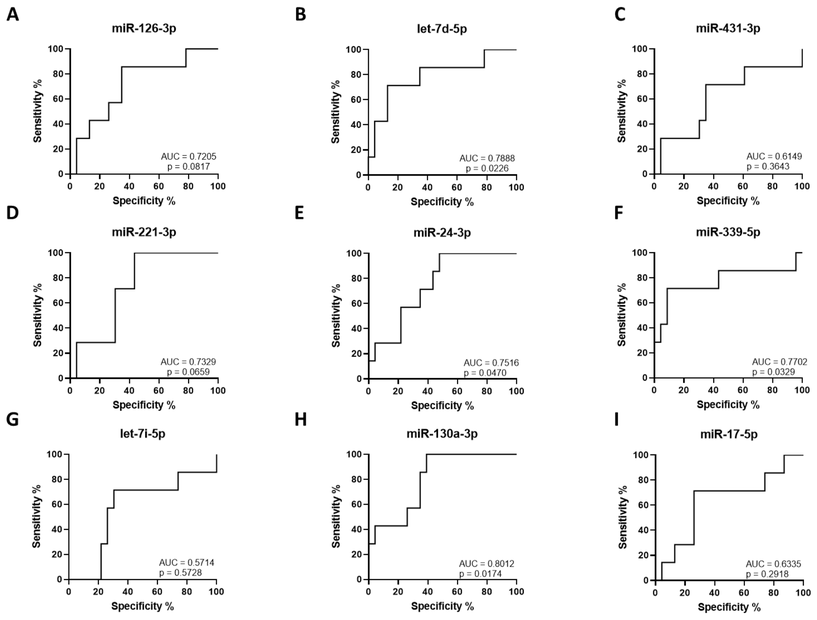

Fig 7. ROC curves demonstrating the ability of miRNA expression levels in arthralgia or 'at-risk individuals' to predict conversion to RA. ROC curves comparing Arthralgia v HC were generated in GraphPad Prism 8. Area under the curve (AUC) was calculated with 95% confidence interval.