

For classical monocytes:

Multiple Comparisons								
Dependent Variable		(I)SpA	(J)SpA	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
PC3 Score	LSD	0	1	-.67304*	0.319531	0.039	-1.31242	-0.03366
			2	-0.07008	0.300298	0.816	-0.67097	0.53082
		1	0	.67304*	0.319531	0.039	0.03366	1.31242
			2	.60297*	0.295668	0.046	0.01134	1.19460
		2	0	0.07008	0.300298	0.816	-0.53082	0.67097
			1	-.60297*	0.295668	0.046	-1.19460	-0.01134

No significant results for intermediate and non-classical monocytes were acquired.

As shown above expression patterns of miRNAs correlating the most with the PC3 in classical monocyte subset could potentially discriminate between perSpA vs axSpA and HC.

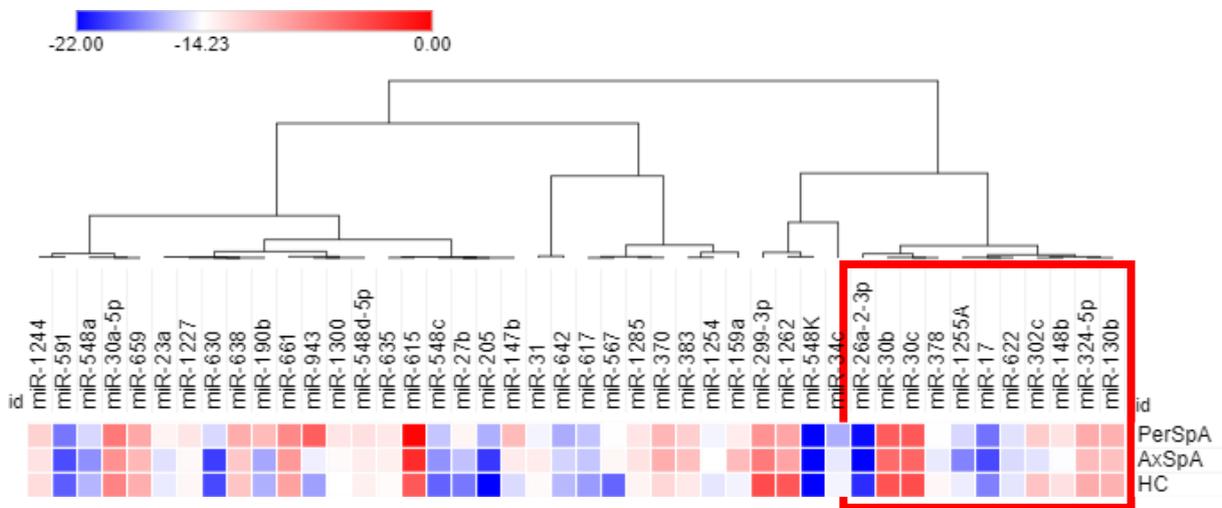
Based on the component loading values for PC3 (table below), these miRNAs would be:

miR-148b, miR-324-5p, miR-130b, miR-17, miR-30b, miR-1255, miR-302c, miR-26a-3p, miR-548a-5p

Rotated Component Matrix^a			
	Component		
	1	2	3
miR-23a	0.989	0.020	0.075
miR 34c	0.994	-0.060	-0.046
miR 591	0.037	0.446	0.225
miR 567	0.962	-0.063	-0.056
miR 661	-0.053	0.847	-0.063
miR 615	0.049	0.777	-0.143
miR 630	-0.002	0.201	0.027
miR 943	-0.026	-0.073	-0.070
miR 617	-0.050	0.586	0.246
miR 30b	0.432	-0.067	0.771
miR 31	-0.076	-0.142	0.114
miR 27b	-0.028	-0.029	0.011
miR 1262	-0.032	-0.046	0.213
miR 302c	-0.022	-0.013	0.461
miR 17	0.040	0.015	0.880
miR 159a	-0.025	0.312	-0.032

<i>miR 635</i>	-0.076	0.463	0.151
<i>miR 548d-5p</i>	-0.127	0.344	0.442
<i>miR 1300</i>	-0.024	0.830	-0.011
<i>miR 1227</i>	-0.075	0.351	0.118
<i>miR 383</i>	-0.042	0.098	-0.032
<i>miR 30c</i>	0.983	-0.081	0.116
<i>miR 370</i>	-0.028	0.612	-0.117
<i>miR 1285</i>	-0.101	0.500	-0.010
<i>miR 147b</i>	0.043	0.776	-0.146
<i>miR 205</i>	0.047	0.750	-0.045
<i>miR 642</i>	-0.058	-0.081	0.038
<i>miR 299-3p</i>	-0.035	-0.072	-0.039
<i>miR 1254</i>	-0.079	0.402	-0.129
<i>miR 26a-2-3p</i>	0.264	-0.080	0.457
<i>miR 548K</i>	0.878	-0.061	0.086
<i>miR 30a-5p</i>	0.994	-0.051	0.003
<i>miR 1255A</i>	0.020	-0.046	0.708
<i>miR 378</i>	0.994	-0.060	-0.046
<i>miR 190b</i>	-0.007	0.093	0.044
<i>miR 148b</i>	-0.016	0.091	0.945
<i>miR 1244</i>	-0.091	0.533	0.529
<i>miR 324-5p</i>	-0.029	0.078	0.944
<i>miR 130b</i>	-0.077	0.067	0.921
<i>miR 548a</i>	0.994	-0.057	-0.047
<i>miR 659</i>	-0.053	0.618	-0.135
<i>miR 638</i>	0.994	-0.048	-0.048
<i>miR 622</i>	-0.037	-0.041	0.130
<i>miR 548c</i>	0.994	-0.057	-0.046
<i>Extraction Method: Principal Component Analysis.</i>			
<i>Rotation Method: Varimax with Kaiser Normalization.^a</i>			
<i>a. Rotation converged in 5 iterations.</i>			

Performing the hierarchical clustering of miRNA expression data one might realize that expression profiles of 8 out of 9 depicted miRNAs in classical monocyte subset are closely related (picture below)

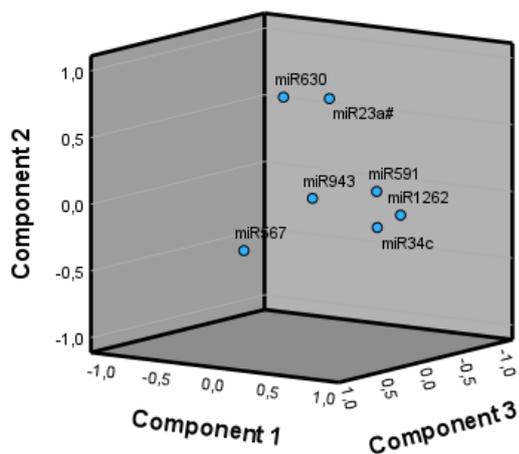


Conclusion: combined expression patterns of miR-148b, miR-324-5p, miR-130b, miR-17, miR-30b, miR-1255, miR-302c, miR-26a-3p in classical monocytes could potentially discriminate between perSpA vs. axSpA and HC

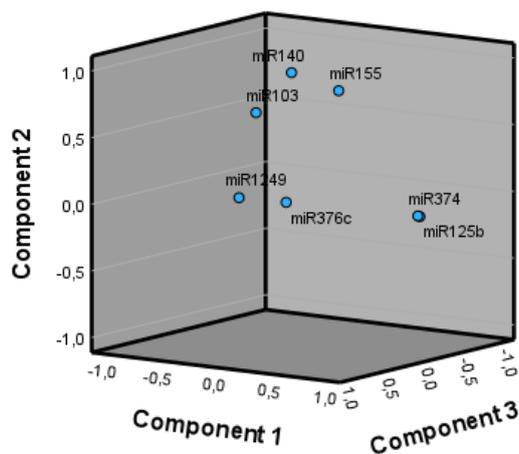
B. Including differentially expressed miRNAs fulfilling inclusion criteria described in the M&M section of the manuscript:

The analysis was performed as described above. PCA was performed and 3 major PC were extracted:

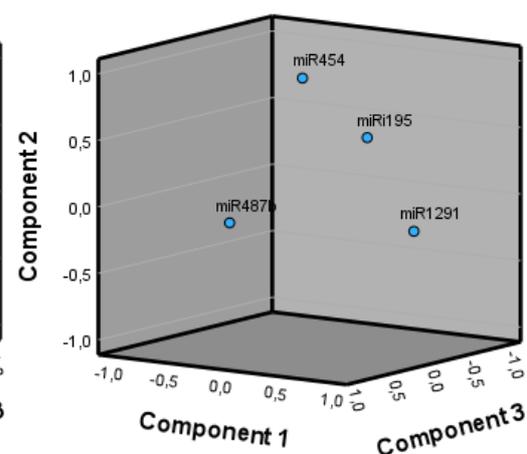
Classical monocytes



Intermediate monocytes



Non-classical monocytes

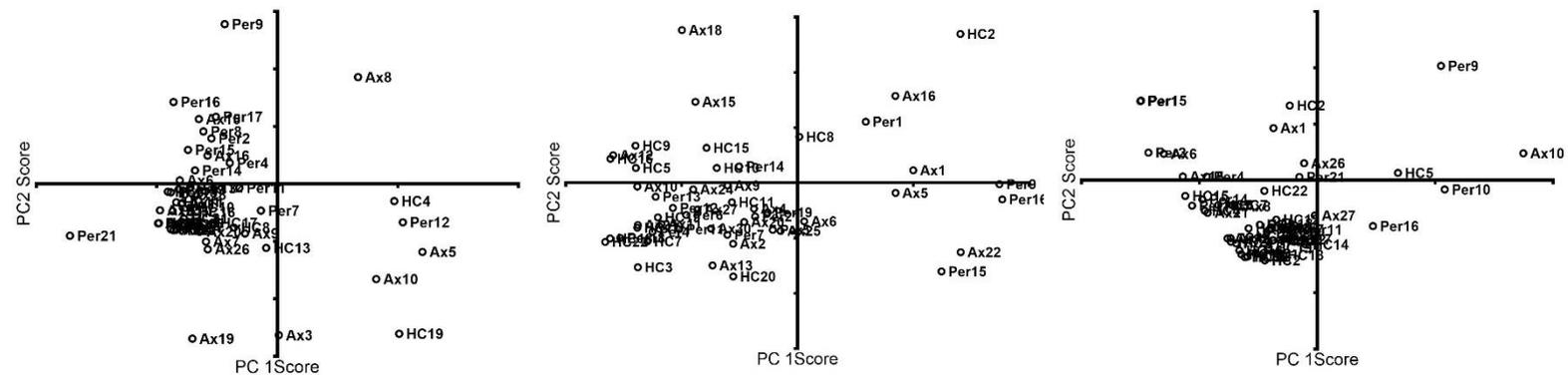


Next, the PC scores were extracted and analyzed for all the major PCs. PC scores were plotted to see whether there are any clear distribution patterns among AxSpA, perSpA and HC samples:

Classical monocytes

Intermediate monocytes

Non-classical monocytes



Although no clear grouping for AxSpA, perSpA and HC was observed, PC scores were further processed using multivariate analysis. Below, the results of multivariate analysis are presented (only the statistically significant results are shown). For this analysis samples were assigned as follows: perSpA = 1, AxSpA = 2, HC = 0.

For classical monocytes:

Multiple Comparisons								
Dependent Variable		(I)SpA	(J)SpA	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
PC2 Score	LSD	0	1	-.85610*	0.303223	0.006	-1.46285	-0.24936
			2	0.08356	0.284972	0.770	-0.48666	0.65379
	1	0	.85610*	0.303223	0.006	0.24936	1.46285	
		2	.93967*	0.280578	0.001	0.37823	1.50110	
	2	0	-0.08356	0.284972	0.770	-0.65379	0.48666	
		1	-.93967*	0.280578	0.001	-1.50110	-0.37823	

For Intermediate monocytes:

				Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
Dependent Variable		(I)SpA	(J)SpA				Lower Bound	Upper Bound
PC3 Score	LSD	0	1	-.81212*	0.3143	0.0124	-1.4418	-0.1825
			2	-.63031*	0.2993	0.0397	-1.2299	-0.0308
		1	0	.81212*	0.3143	0.0124	0.1825	1.4418
			2	0.1818	0.3037	0.5518	-0.4266	0.7902
		2	0	.63031*	0.2993	0.0397	0.0308	1.2299
			1	-0.1818	0.3037	0.5518	-0.7902	0.4266

For Non-Classical monocytes:

				Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
Dependent Variable		(I)SpA	(J)SpA				Lower Bound	Upper Bound
PC1 Score	LSD	0	1	-0.0202	0.3273	0.9510	-0.6771	0.6367
			2	-,79861*	0.2935	0.0088	-1.3876	-0.2096
		1	0	0.0202	0.3273	0.9510	-0.6367	0.6771
			2	-,77842*	0.3241	0.0199	-1.4288	-0.1280
		2	0	,79861*	0.2935	0.0088	0.2096	1.3876
			1	,77842*	0.3241	0.0199	0.1280	1.4288
PC3 Score	LSD	0	1	-0.2796	0.3350	0.4078	-0.9519	0.3926
			2	-,75370*	0.3004	0.0153	-1.3565	-0.1509
		1	0	0.2796	0.3350	0.4078	-0.3926	0.9519
			2	-0.4741	0.3317	0.1589	-1.1397	0.1915
		2	0	,75370*	0.3004	0.0153	0.1509	1.3565
			1	0.4741	0.3317	0.1589	-0.1915	1.1397

As shown above:

- For Classical monocytes expression patterns of miRNAs correlating the most with the PC2 could potentially discriminate between perSpA vs axSpA and HC.
- For Intermediate monocytes expression patterns of miRNAs correlating the most with the PC3 could potentially discriminate between HC vs axSpA and perSpA.

- For non-Classical monocytes expression patterns of miRNAs correlating the most with the PC1 could potentially discriminate between axSpA vs perSpA and HC. Also PC3 could discriminate between axSpA vs HC.

Based on the component loading values for corresponding PCs these miRNAs would be:

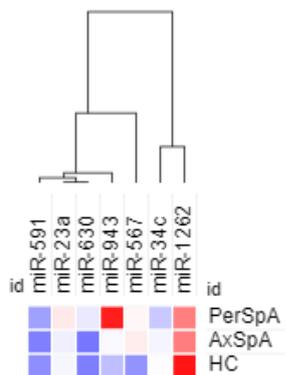
miR-23a and miR-630 – for classical monocytes

miR-1249 – for intermediate monocytes

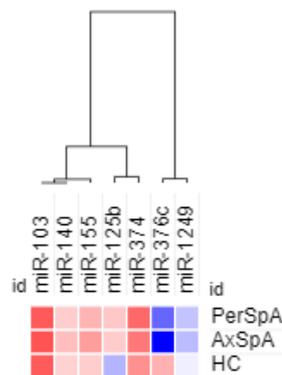
miR-195 and miR-1291 for PC1 and **miR-487b** for PC3 – for non-classical monocytes

Performing the hierarchical clustering of miRNA expression data one might realize that expression profiles of **miR-23a and miR-630** in classical monocyte subset are closely related (picture below). On the other hand in non-classical monocytes, expression profiles of **miR-195 and miR-1291 cluster together, whereas miR-487b** is separated from others miRNAs.

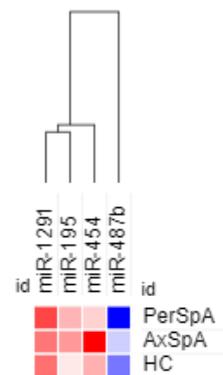
Classical monocytes



Intermediate monocytes



Non-classical monocytes



Concluding – based on Principal Component Analysis –

- expression patterns of **combined miR-23a and miR-630** in classical monocyte subset could serve as a good discriminator between perSpA vs axSpA and HC.
- Expression pattern of **miR-1249** in intermediate monocyte subset could well discriminate between HC vs axSpA and perSpA.
- Combined expression patterns of **miR-195 and miR-1291** in non-classical monocyte subset could serve as a good discriminator between axSpA vs perSpA and HC. On the other hand differential expression of **miR-487b** could be used to discriminate between axSpA vs HC.