Circulating microRNA expression pattern separates patients with anti-neutrophil cytoplasmic antibody associated vasculitis from healthy controls

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ABSTRACT

Objective. *Antineutrophil cytoplasmic antibody associated vasculitis (AAV) has an unpredictable course and better biomarkers are needed. Micro-RNAs in body fluids are protected from degradation and might be used as biomarkers for diagnosis and prognosis, here we explore the potential in AAV.*

Methods. *Plasma samples from two AAV cohorts (n=67 and 38) were compared with samples from healthy controls (n=27 and 45) and disease controls (n=20). A panel of 32 miRNAs was measured using a microfluidic quantitative real-time PCR system, and results were compared with clinical data.*

Results. *Seven individual miRNAs were differently expressed compared to controls in both cohorts; miR-29a, -34a, -142-3p and -383 were up-regulated and miR-20a, -92a and -221 were down-regulated. Cluster analysis as well as principal component analysis (PCA) indicated that patterns of miRNA expression differentiate AAV patients from healthy subjects as well as from renal transplant recipients. Loadings plots indicated similar contribution of the same miRNAs in both cohorts to the PCA. Renal engagement was important for miRNA expression but consistent correlations between estimated glomerular filtration rate and miRNA levels were not found. We found no significant correlation between treatment regimens and circulating miRNA levels.*

Conclusion. *In this first study ever on circulating miRNA profiles in AAV, we find clear indication of their potential as biomarkers for diagnosis and classification, but more studies are needed to identify the best markers as well as the mechanisms responsible for variations.*

Introduction

MicroRNA (miRNA) is a recently discovered class of small non-coding RNAs, which regulate gene expression by binding to the 3' untranslated region of their target mRNAs, causing translational repression and/or mRNA degradation (1). Accordingly, miRNAs have a critical role in many biological and pathological processes, such as cancer and autoimmunity (2-4). Although the majority of miRNAs exist within cells, miRNAs have also been found in extracellular body fluids, including serum and plasma (5-8). The miRNAs in plasma are protected from degradation by binding to carrier proteins or inclusion in microspheres and are stable during storage, facilitating their potential as biomarkers (9). The analysis of circulating miRNAs is being developed for cancer and autoimmune diseases (10), with the potential use as biomarkers for prognosis, diagnosis, disease activity, and response to treatment. Antineutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV) is a group of autoimmune diseases that include granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis. AAV is characterised by inflammation in blood vessels, threatening functioning of vital organs (11). In AAV neutrophil activation, increased cell death, renal impairment and damage of blood vessels potentially alter levels of circulating miRNA, but circulating miRNA has not previously been studied in this disease.

Here, we investigate the pattern of miRNA expression in plasma from patients with systemic vasculitis, in two independent patient cohorts, and compare results with healthy and relevant disease controls, and correlate patterns with clinical parameters.

Patients and methods

Study populations and blood samples

Blood samples were collected from two centres; in Lund 38 AAV patients, 45 healthy controls and 20 renal transplant recipients; in Linköping 67 AAV patients and 27 healthy controls (Table I). The study was approved by the regional ethics review board. Written informed consent was obtained from all subjects. Clinical data were collected from medical records. Cell separation protocols have been published earlier (12), plasma was collected by centrifugation at 1500 g for 15 minutes or on top of Polymorphprep/Lymphoprep at 480 g for 35 minutes (Linköping) or on top of Polymorphprep at 625 g for 45 minutes (Lund). Control experiments comparing the different centrifugation speeds were done for miR-20a, -29a, -34a and -221, showing concordant results (data not shown).

Table I. Characteristics of the patients and controls from the Linköping and Lund cohorts.

		Linkoping cohort	Lund cohort	*p*-value
Healthy controls	Number of individuals (men/women)	27 (15/12)	45 (NA)	
	Median age (IQR), years	41 (26-52)	NA	
Patients	Number of individuals (men/women)	67 (32/35)	38 (18/20)	
	Median age (IQR), years	67 (57-77)	63 (53-72)	ns
	GPA/MPA	44/23	25/13	ns
	PR3-ANCA/MPO-ANCA	40/26*	22/14**	ns
	Median ESR (IQR), mm/hour	12 (8-23)	NA	
	Active disease / remission	6/61	10/28	0.02
	Previous relapses, median number (IQR)	1.0 (0.0-2.0)	1.0 (1.0-2.0)	
	Time since last flare, median number (IQR), months	36 (16-81)	NA	
	p-creatinine, median (IQR), µmol/l	100 (83-155)	99 (79-137)	ns
	eGFR, median (IQR), ml/min/1.73m^2	56 (35-74)	61 (47-77)	ns
	Haemoglobulin, median (IQR), g/l	135 (120-143)	NA	
	Leucocyte count, median (IQR), 10^9/l	8.0 (6.0-9.8)	NA	
	Thrombocyte count, median (IQR), 10^9/l	267 (229-320)	NA	
	Prednisolone dose, median (IQR), mg/day	2.5 (0-5)	1.4 (0-5.6)	ns
	MMF/Aza/Meth % (n)	57 (38)	NA	
Kidney-transplanted patients	Number of individuals (men/women)		20 (13/7)	
	Median age (IQR), years		54 (40-61)	
	eGFR, median (IQR), ml/min/1.73m^2		53 (42-62)	

*Data not available for one patient. **Two patients were ANCA negative.
IQR: Interquartile range; GPA: granulomatosis with polyangiitis; MPA: microscopic polyangiitis; PR3: proteinase 3; MPO: myeloperoxidase; ESR: erythrocyte sedimentation rate; eGFR: estimated glomerular filtration rate; MMF: mycophenolate mofetil; Aza: azathioprine; Meth: methotrexate; NA: data not available; ns: not significant.

RNA isolation and quantitative real-time PCR

Total RNA was extracted at room temperature from plasma using a Total RNA purification kit (Norgen Biotek), according to the manufacturer´s instructions with some minor modifications as described earlier (10), most important being the addition of *Caenorhabditis elegans* synthetic miRNA-39 (miR-39), miR-54 and miR-238 (TAG Copenhagen A/S).

A real-time PCR panel of 35 miRNAs, including 3 miRNAs originating from Caenorhabd (Supplementary Table I) was chosen based on the previous study, excluding those with very low expression (10). RNA was reverse transcribed using a TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems). The RT primer mix consisted of equal volumes of the 35 different miRNA-specific stem-loop primers (Supplementary Table I). The reverse transcription was performed as described earlier on an Applied Biosystems 2720 Thermal Cycler. Specific target amplification was done as described earlier. The preamplification was performed at 95°C for 10 min, followed by 16 cycles at 95°C for 15 sec and at 60°C for 4 min, ending with a hold at 4°C. Next, the preamplified samples and the 35 miRNA TaqMan assays were applied to primed 96.96 Dynamic Array chips using loading and assay reagents according to the manufacturer's instructions (Fluidigm Corp., CA, USA). All samples were run in duplicates. Quantitative real-time PCR (qPCR) was performed with a BioMark RealTime PCR system (Fluidigm) using single probe (FAM-labelled MGB, ROX reference dye) settings and 40 cycles. Data were processed using Fluidigm Real-Time PCR Analysis software (ver. 3.0.2) with the autodetector setting.

RNA was also extracted from peripheral blood mononuclear cells (PBMC) and polymorphonuclear cells (PMN) in samples from the Linköping cohort using mirVana miRNA Isolation Kit (Ambion), according to the manufacturer's protocol. The RNA samples were reverse transcribed using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems) and analysed by quantitative real-time PCR on a 7900HT Fast Real-time PCR System (Applied Biosystems) (12).

Data handling and statistical analysis

For data originating from plasma samples the average raw quantification cycle (Cq) of each duplicate was calculated and Cq>30 were excluded. The data was first normalised with average Cq of cel-miRs (ΔCq=average Cq of cel-miRs for one sample - average Cq of hsa-miR for the same sample). Thereafter, to correct for variations in total input RNA, all ΔCq-values were row-normalised with 23 hsa-miRs which were detected in all samples (hsa-miR-15a-5p, -16-5p, 17-5p, -20a-5p, -24-3p, -29a-3p, -34a-5p, -92a-3p, -106a-5p, -125a-3p, -142-3p, -146a-5p, -146b-5p, -150-5p, -155-5p, -181b-5p, -196a-5p, -203a, -223-3p, -342-3p, -383, -409-3p and -638). For each sample, the average of these 23 miRNAs was subtracted from the above calculated ΔCq-values for all miRNAs.

The qPCR data from PMN and PBMC were analysed in the software package SDS 2.4 (Applied Biosystems) and normalised as described earlier (12). With the statistical package IBM SPSS Sta-

tistics 20 unpaired *t*-test with Welch correction was performed to compare the expression of miRNAs for patients and controls, and the data was plotted in GraphPad Prism 5. Correlation analyses between miRNAs and clinical data, as well as between miRNAs in plasma and intracellular miRNAs, were done with Spearman correlation test. A *p*-value of <0.05 was considered significant. When needed, correction for multiple testing using the false discovery rate (FDR) controlling procedure (13), was used. A programming environment for data analysis and graphics, R (http://www.r-project.org), was used for correlation plots, hierarchical clustering and principal component analysis (PCA) of the data. A number of standard packages as well as others specifically suited for these analyses, such as "pcaMethods", were downloaded at http://CRAN.R-project.org and http://www.bioconductor.org.

Results

Expression of circulating miRNAs in AAV patients

The characteristics of the patients and controls from Linköping and Lund are shown in Table I. The expression of all 32 miRNAs analysed with the Fluidigm system is depicted in the volcano plot in Figure 1. To study patterns of expression a correlation analysis together with hierarchic clustering was performed. This analysis show that patients tended to cluster together with other patients at both centers, even though there was not a complete separation (Fig. 2a-b). Patients with active disease, according to the Birmingham Vasculitis Activity Score (BVAS) (14), did not cluster as a separate group. The correlation clustering analysis divided both Linköping and Lund patients into two groups (marked in Fig. 2a and 2b with group 1 (n=50) and group 2 (n=17) for the Linköping and group 1 (n=31) and group 2 (n=7) for the Lund cohort). Results similar to the clustering analysis were obtained when using principal component analysis (PCA), in which data were compressed to a lower dimension to get an overview of the data. The first two principal components (PC1 and PC2) together explained 54.9% and 41.8% of the variances in the Linköping and Lund data sets, respectively. The PCA score plots showed that the patient group was partially separated from the control group, while the active patients could not be separated from the patients in remission (Fig. 3a-b). The contribution of each miRNA to the first two principal components is shown in the loadings plots in Figure 3c-d, showing a similar pattern in both cohorts.

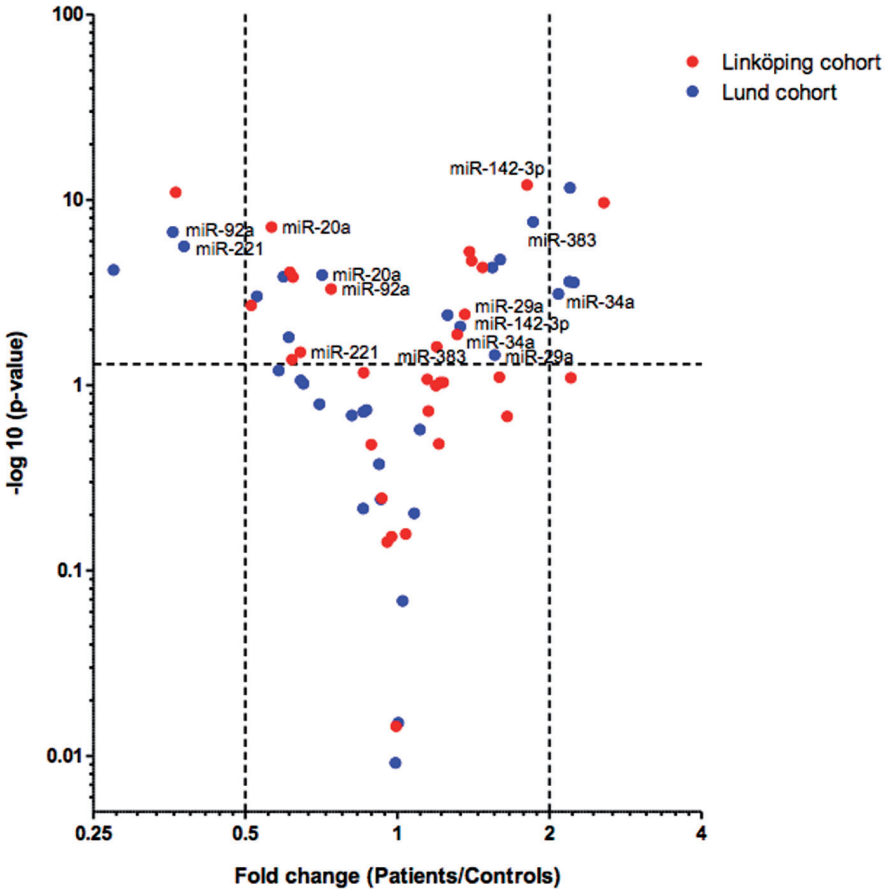

Fig. 1. Volcano plot illustrating the expression of 32 miRNAs in plasma, with fold changes and p-values of each miRNA for both sample cohorts. The broken horizontal line represents a *p*-value of 0.05. The 7 miRNAs that were consistently and significantly different between patients and controls in both cohorts are labelled in the figure.

When analysing individual miRNAs, 16 of them were significantly differently expressed between patients and controls in the Linköping cohort and 17 in the Lund cohort (Supplementary Table I1). Seven of the miRNAs were consistently up- or down-regulated in both cohorts (miR-20a, -29a, -34a, -92a, -142-3p, -221 and -383) (Fig. 4), while 13 were differentially expressed only in one of the cohorts, 3 were dysregulated in both cohorts but in opposite direction and 9 were similarly expressed between patients and controls in both cohorts.

miRNA expression in relation to clinical and laboratory data

The 7 differently expressed miRNAs were compared with clinical data in the Linköping cohort (n=67), as shown in Table II, revealing strong correlations for estimated glomerular filtration rate (eGFR) (where 3 miRNAs correlated positively and 4 negatively with eGFR), and for some of the miRNAs there were also correlations with haemoglobin (Hb), age, C-reactive protein (CRP) and leukocyte count. In the Lund cohort, miR-92a correlated positively (rho=0.502, *p*=0.001) and miR-142-3p correlated negatively (rho= -0.337, *p*=0.039) with eGFR, in line with the results from the Linköping cohort. In addition, miR-20a, -34a and -92a correlated with age (rho= -0.372, *p*=0.021; rho=0.435, *p*=0.006 and rho= -0.346,

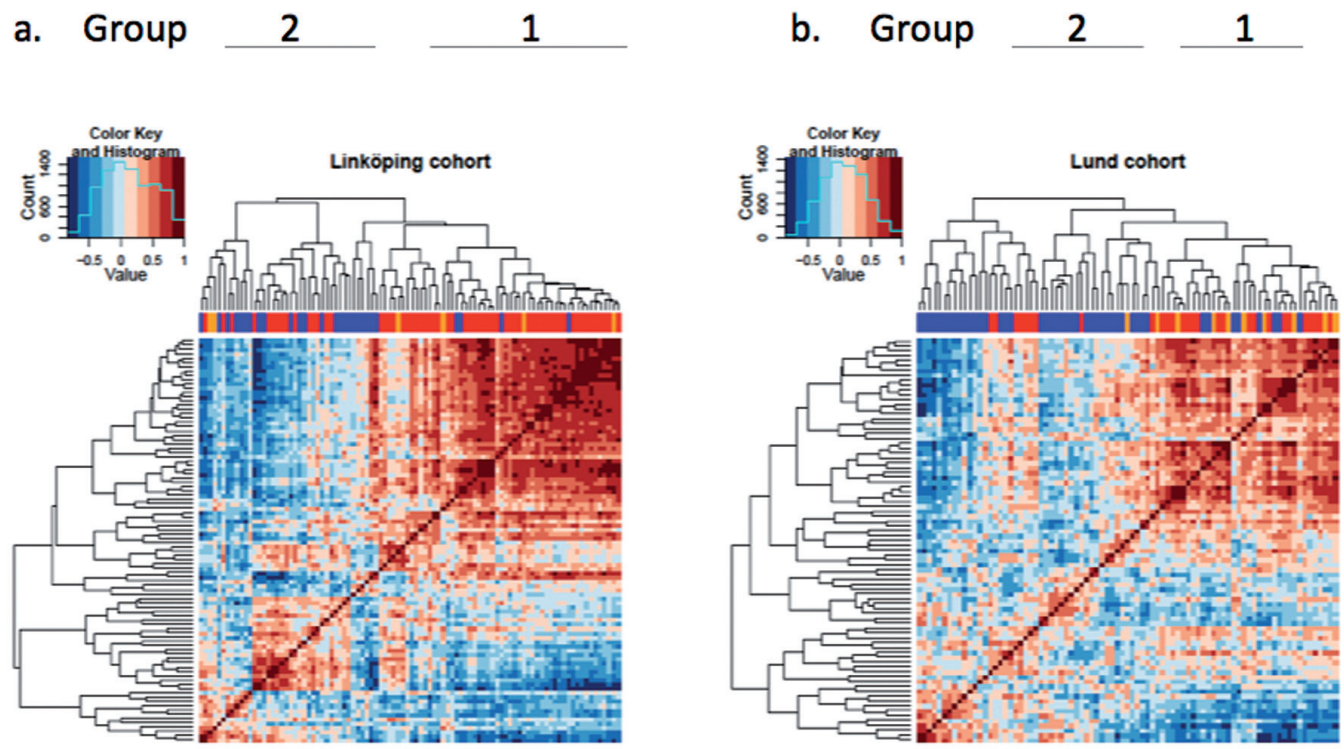

Fig. 2. Correlation plots with hierarchical clustering for the Linköping cohort (a) and Lund cohort (b), showing that patients tended to cluster together with other patients, and controls with other controls, even though there was not a complete separation. In the band below the dendrogram controls are marked in blue, patients in remission in red and active patients in orange. The hierarchical clustering is based on distance of correlation (1-Spearman's rank correlation) and the method "complete linkage". The analysis divided both the Linköping and Lund patients into two groups (group 1 (n=50) and group 2 (n=17) for the Linköping and group 1 (n=31) and group 2 (n=7) for the Lund cohort).

p=0.033, respectively). When comparing the groups indicated by correlation analysis and clustering (group 1 and 2 in Figure 2a and 2b), we found significantly lower levels of eGFR (p<0.001) in group 1 in the Linköping cohort, where almost all individuals with a low eGFR (<60 ml/min/1.73m^2) were assembled in group 1 (Supplementary Fig. 1), while eGFR in group 1 in the Lund cohort did not reach statistical significance (p=0.09).

The strong association between individual miRNAs and eGFR combined with the fact that all patients except two in clustering group 2 in both sample cohorts had eGFR >60 ml/min/1.73m^2 made us investigate the role of eGFR also in disease controls. The same circulating miRNAs were analysed in a previous study in two cohorts of systemic lupus erythematosus (SLE) patients with the same platform (10). eGFR in those disease controls did not correlate in a consistent manner with any of the 7 miRNAs that correlated with eGFR in the Linköping cohort. In both SLE cohorts miR-29a correlated with eGFR, but in opposite directions. Renal transplant recipients (TP) were also used as disease controls. Of the 7 miRNAs that correlated with eGFR in the Linköping cohort, 2 correlated with eGFR in the TP control group (miR-20a rho= -0.570, p=0.009 and miR-92a rho= -0.468, p=0.037), but in the opposite direction. TP patients separated from AAV with about the same degree of separation as for AAV and healthy controls, in both correlation plots with hierarchical clustering and in PCA (Supplementary Fig. 2).

The patients with AAV in the Linköping cohort were divided into subgroups based on diagnosis (GPA and MPA) or ANCA-specificity (PR3-ANCA and MPO-ANCA). Patients with MPA, as well as MPO-ANCA positive patients, had significantly different expression levels of all 7 miRNAs as compared to healthy controls (dysregulated in the same direction as the whole patient cohort) (data not shown). For patients with GPA and PR3-ANCA positive patients, the expression levels of 3 of the 7 miRNAs were significantly different (miR-142-3p, -20a and -92a for GPA and miR-142-3p, -20a and -29a for PR3-ANCA, data not shown). Thus, subgroups of patients behave in a similar manner as the whole patient group, which can also be seen in PCA plots of patients with PR3-ANCA or MPO-ANCA, where the patients cluster together and the controls cluster together (Supplementary Fig. 3).

Correlations between intracellular and circulating miRNAs

For some of the 7 differentially expressed miRNAs the levels in plasma were reflected by the intracellular levels in PBMCs and PMNs. miR-20a in plasma was positively correlated with miR-20a in PMNs (p<0.001, rho=0.374). When studying the intracellular levels of miRNAs in patients compared to controls, miR-34a was upregulated in both PBMCs and PMNs (p<0.001 and p<0.001, respectively), and miR-20a and -92a were down-regulated in PMNs (p=0.001 and p=0.008, respectively). However, miR-142-3p in plasma was negatively correlated with

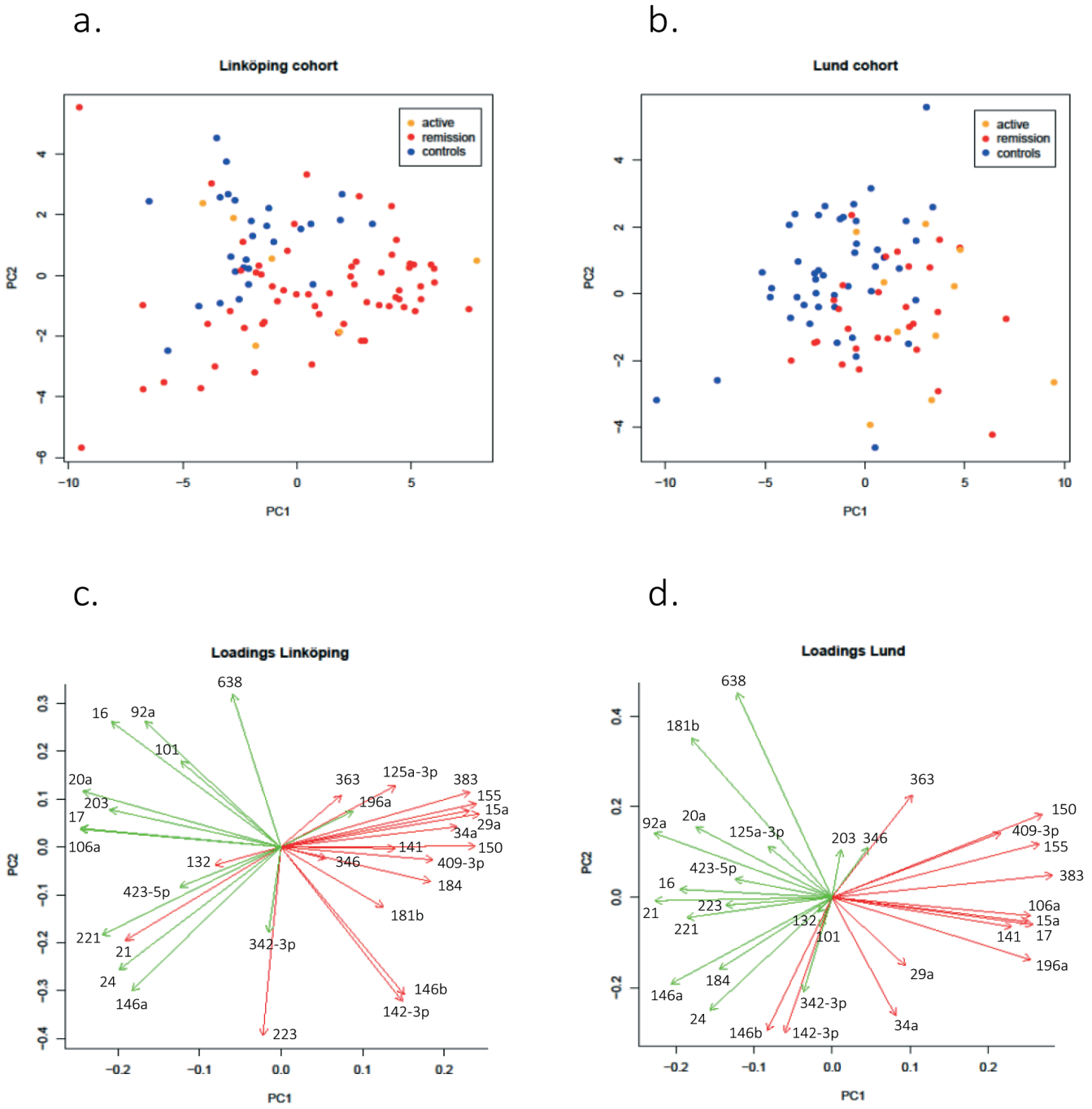

Fig. 3. Principal component analysis, which compresses the data to a lower dimension to get an overview, for the two sample cohorts. The score plots (a-b), summarising the relationship among the samples, show partial separation between patients and controls, and the loadings plots (c-d) show the contribution of individual miRNAs to principal component (PC) 1 and PC2. In the loadings plots, the miRNAs with a higher expression in patients than in controls are marked in red and miRNAs with a lower expression are marked in green.

miR-142-3p in PBMCs (p=0.003, rho= -0.308) and was down-regulated in PMNs from patients compared to controls, while miR-221 was up-regulated in patients in both PMNs and PBMCs, contradictory to the levels in patient plasma.

Discussion

The main finding from this study was that a set of miRNAs could distinguish AAV patients from healthy controls, both with correlation-cluster analysis and PCA. The results could be reproduced in an independent cohort. Further, loadings plots showed that largely the same individual miRNAs contribute to the separation of controls and cases in the two cohorts. This is, to our knowledge, the first study to examine the expression of circulating miRNAs in plasma from AAV patients.

The separation between patients and controls was present also when studying GPA and MPA patients separately, as well as PR3-ANCA and MPO-ANCA positive patients. Seven miRNAs were significantly and consistently up- or down-regulated in patients compared to controls in both of the sample cohorts.

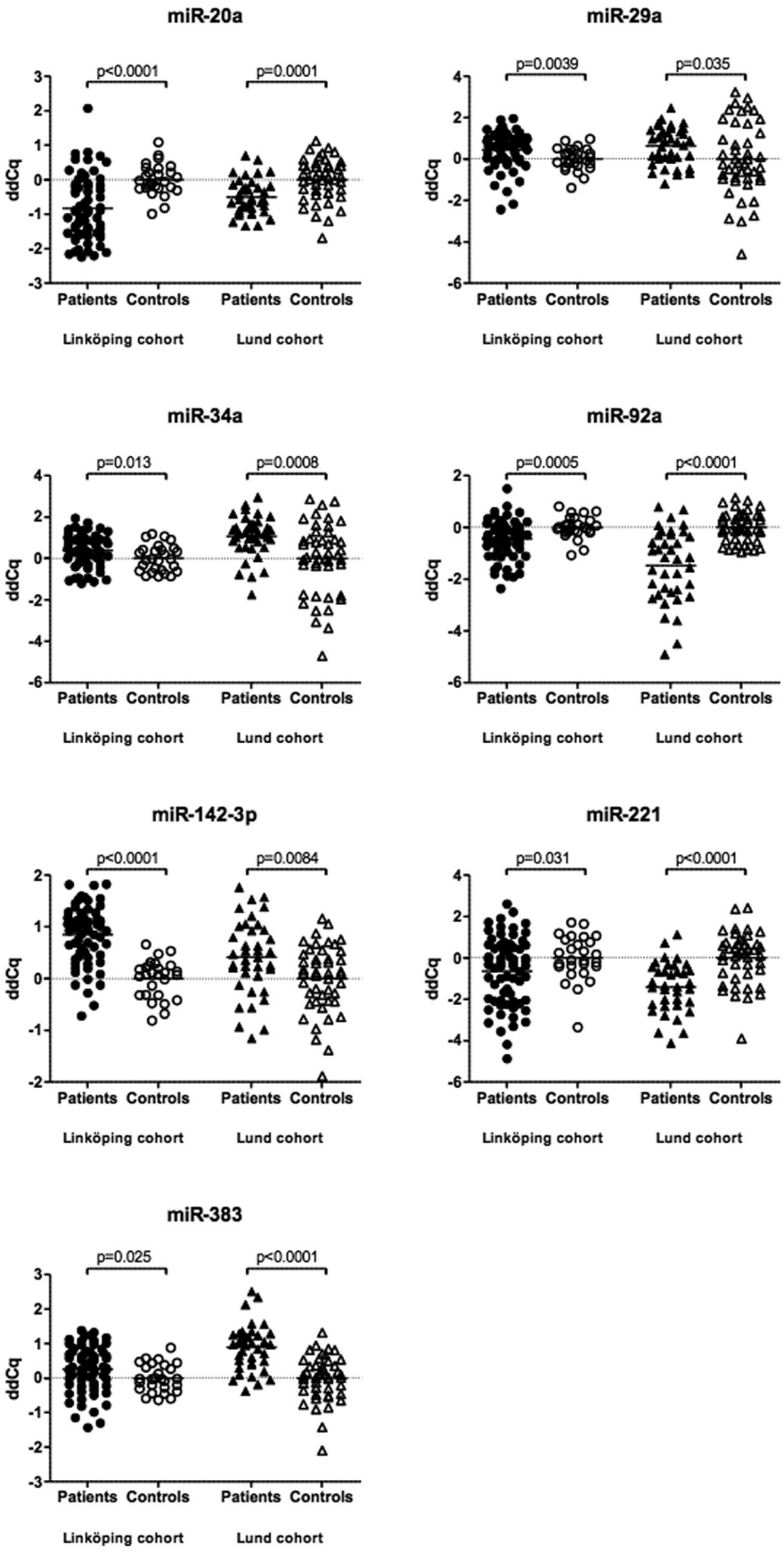

Fig. 4. Dot plots showing ddCq values of the 7 miRNAs that were consistently up- or down-regulated in patients compared to controls in the two sample cohorts.

When miRNAs enter the circulation they are packed in shedding vesicles, such as microvesicles and exosomes (5), or bound to RNA-binding proteins, including HDL (16) and Ago2 (16), which prevents degradation. The altered expression pattern of circulating miRNAs found in AAV patients could be caused by different factors including vascular dysfunction, inflammation, cell death, renal dysfunction, degrading enzymes in the circulation, lipoprotein disturbances, pharmacological therapy and handling of samples.

AAV is characterised by inflammation in small blood vessels, leading to endothelial cell damage and vascular dysfunction. When damage occurs, the endothelial cells are detached from the endothelial monolayer and in addition microparticles are released to the blood stream (17). Others have shown that patients with active AAV have higher levels of microparticles compared to patients in remission and healthy controls (18-21). In one of these studies not only active patients but also those in remission had higher levels of neutrophil microparticles compared to healthy controls (21). Vascular dysfunction is a common feature in many autoimmune diseases (17, 22). In line with this, a similar change in the miRNA pattern as was found in our study has been observed in other diseases, such as rheumatoid arthritis (RA) and SLE. For example, three differently expressed miRNAs in the AAV patients were concordantly regulated in plasma samples from SLE patients (10), *i.e.* up-regulation of miR-142-3p and down-regulation of miR-20a and -92a. Similarly, another study showed up-regulation of miR-142-3p and down-regulation of miR-92a in plasma samples from both SLE patients and RA patients (23). In addition, in serum from patients with systemic sclerosis the expression of miR-142-3p was up-regulated compared to healthy controls (24).

Activation of inflammation cascades as well as the coagulation system may alter the expression of miRNA. It is well known that serum samples contain more miRNA as compared to simultaneously drawn plasma samples where no coagulation has occurred *in vitro* (25). In the present study, however, we

Table II. Correlations between 7 miRNAs and clinical parameters for all AAV patients in the Linköping cohort.

	eGFR	Hb	CRP	Leuko	Pred	Thrombo	Age	Sex
miR-20a	$p<0.001$ r=0.646	$p=0.005$ r=0.383	n.s.	($p=0.041$) r=-0.285	n.s.	n.s.	$p=0.004$ r=-0.345	n.s.
miR-92a	$p<0.001$ r=0.504	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
miR-221	$p=0.001$ r=0.437	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
miR-29a	$p<0.001$ r=-0.531	($p=0.036$) r=-0.291	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
miR-34a	$p<0.001$ r=-0.484	($p=0.049$) r=-0.275	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
miR-142-3p	$p=0.001$ r=-0.393	$p=0.001$ r=-0.436	($p=0.025$) r=0.302	$p=0.003$ r=0.408	n.s.	n.s.	($p=0.027$) r=0.270	n.s.
miR-383	$p<0.001$ r=-0.551	$p=0.006$ r=-0375	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

eGFR: estimated glomerular filtration rate; Hb: haemoglobin; CRP: C reactive protein; Leuko: leukocyte count; Pred: prednisolone; Thrombo: thrombocyte count; dark grey: positive correlation; light grey: negative correlation. *p*-values within brackets are not significant after correction for multiple testing using the false discovery rate (FDR) controlling procedure, where $p<0.011$ were considered significant.

found no strong correlation between markers of inflammation (*i.e.* CRP and erythrocyte sedimentation rate) and any individual miRNA in plasma. Only miR-142-3p correlated positively with CRP, but the correlation was stronger with leukocytosis.

Altered cell death might lead to changes in miRNA levels, for example through release of apoptotic bodies containing miRNAs into the circulation (26). In SLE, removal of apoptotic cells is compromised (27), and it has been shown that patients with SLE have increased numbers of microparticles tagged for removal (28). In patients with AAV decreased apoptosis has been observed (29), maybe paralleled with an increase in other forms of cell death, such as necrosis and NETosis.

The miRNAs not contained in microparticles may leave the blood stream through glomerular filtration. Some cardiac specific miRNAs released to the circulation after myocardial infarction can be retrieved in the urine (30). The importance of such urinary clearance in patients not experiencing massive necrosis is unknown. In the present study we found that eGFR and several individual miRNAs were correlated, but correlations went in both directions (4 up-regulated and 3 down-regulated). This suggests other effects than mere urinary retention owing to reduced GFR. No consistent eGFR correlations for miRNAs could be seen among disease controls such as renal transplant recipients and when re-analysing two SLE cohorts from a previous publication. Wang *et al*. (31) reported that miR-146a and miR-155 in serum from SLE patients correlated with eGFR, both being lower in patients with reduced eGFR. From this we conclude that renal disease in AAV does contribute to the pattern of circulating miRNAs, but alterations in the GFR itself is probably not the main mechanism. This interpretation is further underlined by the fact that cluster analysis has the same ability to differentiate between AAV and transplant recipients as between AAV and healthy controls.

Studies have shown that in patients with impaired kidney function the levels of circulating RNases are increased (32). In patients with severe kidney disease (chronic kidney disease stage IV and V) the rate of miRNA degradation in plasma was increased (33), which might be explained by the increased levels of circulating RNases. In our study, only 17 of the patients (6 in Lund and 11 in Linköping) had eGFR below 30 ml/min/1.73m^2, and subsequently it is unlikely that the altered miRNA expression stems from changes in miRNA degradation.

Lipoprotein abnormalities are common in renal disease but have not been studied in detail in AAV patients (34). Vickers *et al*. (15) have studied miRNAs associated with HDL, and the top 10 list of the most abundant miRNAs associated with HDL in healthy individuals included miR-106a and -17. Both of these showed less expression in patients from the Linköping cohort (but not from the Lund cohort). Thus, changes in level of HDL may contribute to our findings.

We have recently shown that pharmacological therapy with prednisolone affects the intracellular levels of miRNAs (12). Although we found some correlations between circulating and intracellular miRNAs in the present study, the circulating miRNA levels were not associated with prednisolone treatment.

Technical factors might also influence the results, but as both cohorts were accompanied by controls collected and studied in parallel this is not a likely explanation for the observed differences between cases and controls. The influence of the different centrifugation speeds that were used when collecting plasma in the two cohorts was directly addressed in separate experiments.

This study has some limitations, including the fact that we included only 32 of the >180 miRNAs that have been found in plasma. Although this selection was based on miRNAs deemed important in other autoimmune diseases, it is possible that other miRNAs might be more informative. Another shortcoming is that there were a few technical variations between the runs of the two study sets, such as small differences in plasma preparation and new batches of miRNA assays. Also, there are a relatively small number of patients with active disease in our study, and samples from patients with active disease were collected both before and after start of therapy.

Our results, showing that a set of miRNAs can separate patients with AAV from healthy controls, have the potential to be clinically useful in the future. However, a number of issues remain to

be studied, such as the best choice of miRNAs and optimisation of the technique. Future research should include parallel measures of HDL as well as isolation and quantitation of different microvesicles to relate these levels to the miRNAs. It would be interesting to include all 180 miRNAs that have been detected in plasma, so as to find possible candidates missed in the present study. Another approach would be to sequence the whole population of small RNAs in the patients to potentially identify unique miRNAs not present in healthy controls. Also, prospective studies in larger cohorts of untreated vasculitis patients are warranted. Finally, the role of the kidney and circulating miRNA-turnover should also be addressed in future work.

In conclusion, we report that a set of circulating miRNAs could distinguish patients with AAV from healthy controls in two independent cohorts. The miRNA expressions were related to some clinical features in AAV, especially eGFR, and the levels of circulating miRNAs could partly be traced to intracellular levels. Future research will be needed to establish the putative clinical utility of these findings.

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