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MicroRNAs in urine help to identify acute rejection after kidney transplantation

Introduction

Today, the performance of a graft biopsy remains the golden standard for the diagnosis of transplant-related diseases, including acute kidney rejection. However, the invasiveness of this procedure and the inter-observer variability in the histological evaluation are important limitations. Therefore, there is a need for non-invasive diagnostic tools to detect acute rejection.

MicroRNAs (miRs) are small non-coding RNAs, which represent a relatively novel type of biomarker, due to their stability in body fluids.

Objective

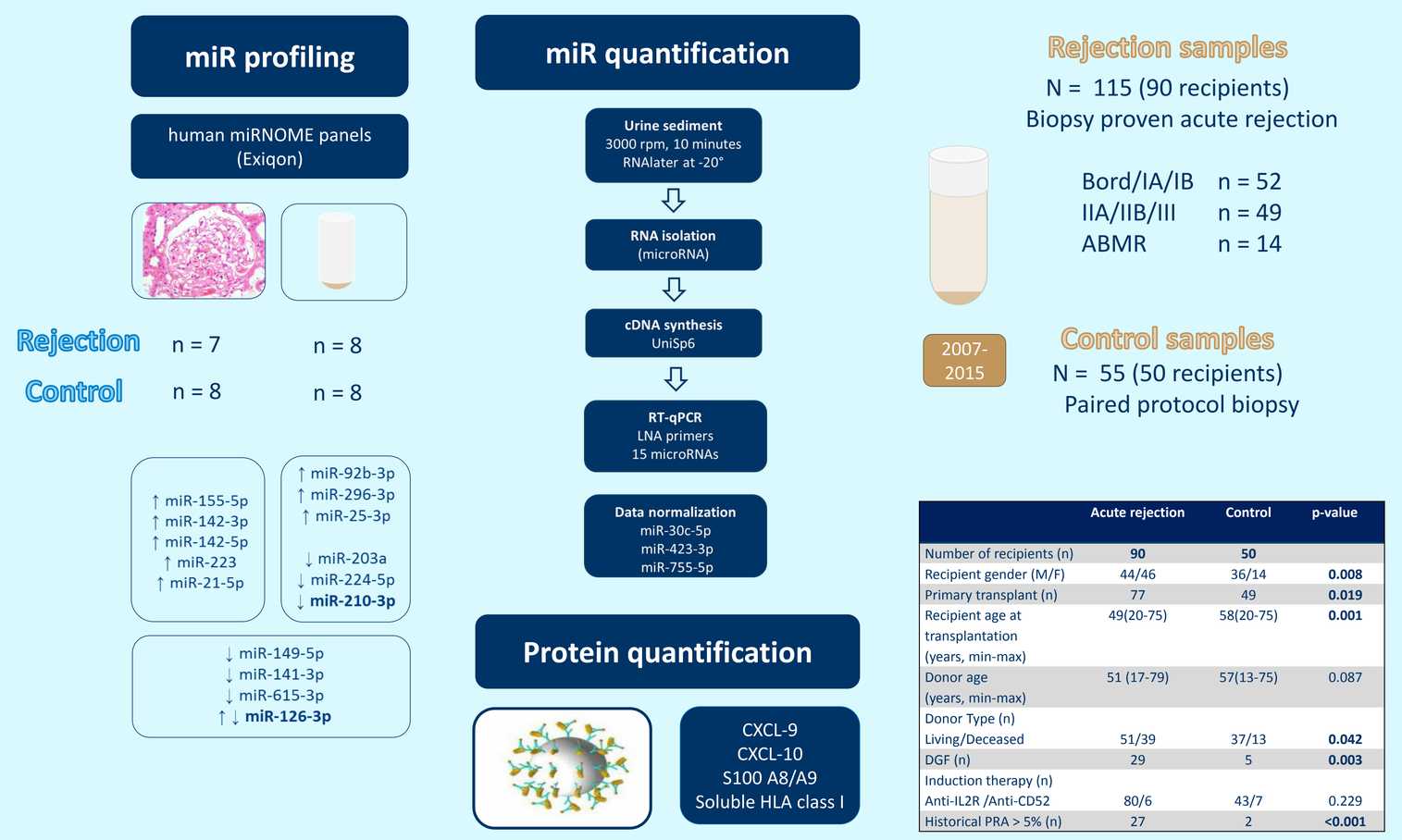
- **To investigate miR and protein expression in urine sediment and supernatant of renal transplant recipients**
- **To determine the predictive value of a combined cellular/molecular biomarker platform in urine for detection of acute rejection**

Methods

- miR expression profiling was performed on RNA isolated from transplant biopsies and urine sediments using commercially available RT-qPCR miR panels.
- The expression of fifteen miRs was quantified with qPCR in an independent set urine sediments.
- Protein levels of CXCL-9, CXCL-10, S100A8/A9 heterodimer, and soluble HLA class I were assessed in paired supernatant.

Results

- A total of 263 ± 26 and 542 ± 53 miRs were significantly expressed (Cq<35 cycles) in biopsy specimens and urine sediments, respectively.
- Five of the fifteen candidate miRs were differentially expressed in urine between the rejection and control group, including miR-155-5p, miR-126-3p, miR-21-5p, miR-25-3p, and miR-615-3p [Fig.1].
- CXCL-9 and CXCL-10 protein levels were significantly elevated (> 8-fold) in urine supernatant from recipients with acute rejection. No significant different expression levels of S100A8/9 heterodimers were measured. The concentration of soluble HLA class I was below the detection limit in 46% of the rejection samples [Fig 2].
- There was no significant difference for any analyte between samples from recipients with T-cell mediated rejection and those with antibody-mediated rejection.
- Each of the analytes was a significant predictor of acute rejection in univariate logistic regression analysis.
- In a multivariate model, three miRs (miR-155p, miR-25-3p, miR-615-3p) along with CXCL-9 levels and recipient age were independent predictors of acute rejection [Table 1, Fig. 3].



	Acute rejection	Control	p-value
Number of recipients (n)	**90**	**50**	
Recipient gender (M/F)	44/46	36/14	**0.008**
Primary transplant (n)	77	49	**0.019**
Recipient age at transplantation (years, min-max)	49(20-75)	58(20-75)	**0.001**
Donor age (years, min-max)	51 (17-79)	57(13-75)	0.087
Donor Type (n)			
Living/Deceased	51/39	37/13	**0.042**
DGF (n)	29	5	**0.003**
Induction therapy (n)			
Anti-IL2R /Anti-CD52	80/6	43/7	0.229
Historical PRA > 5% (n)	27	2	**<0.001**

Fig. 1

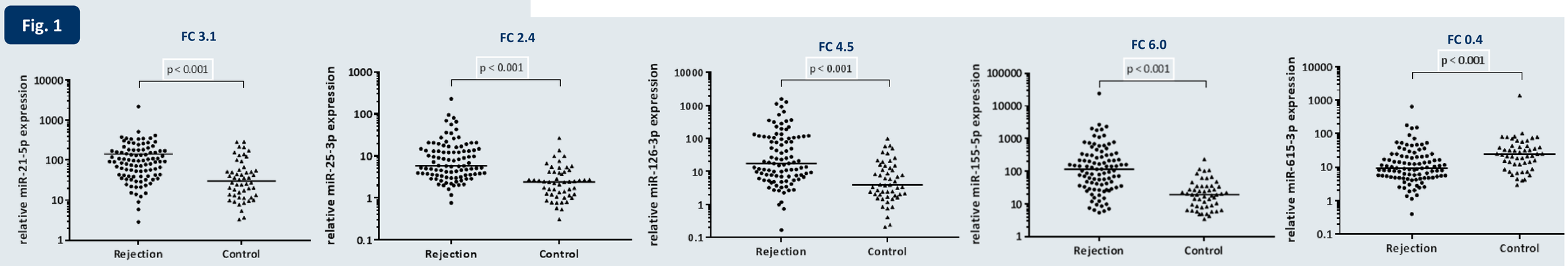


Fig. 2

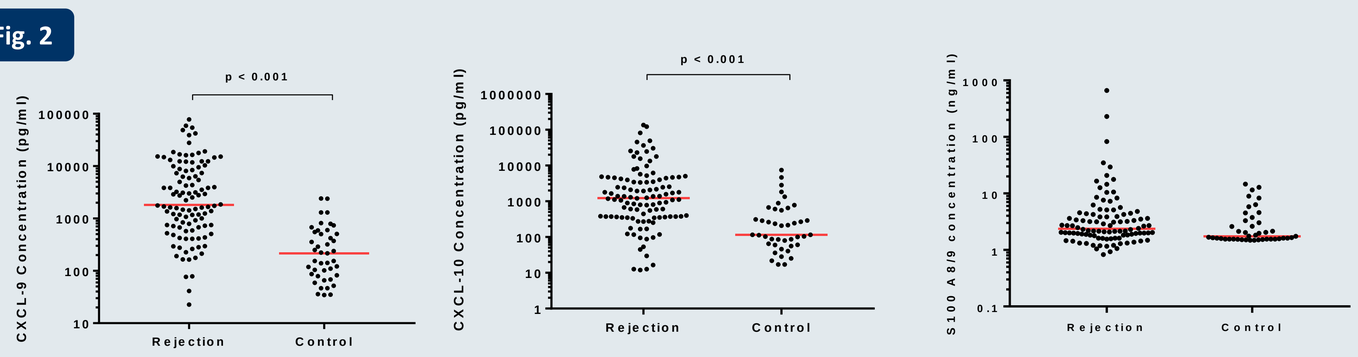
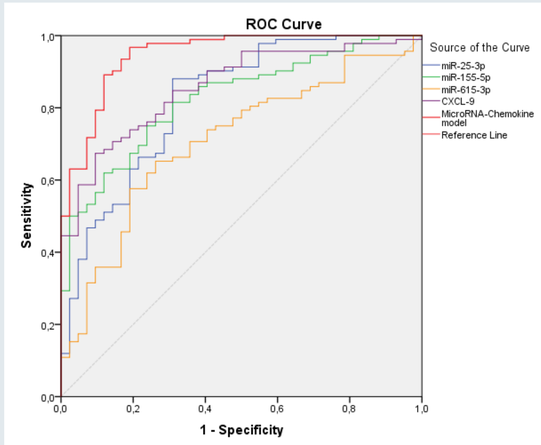


Table 1
Multivariate logistic regression

	Univariate logistic regression		Multivariate logistic regression	
	OR (95 % CI)	p-value	OR (95 % CI)	p-value
miR-21-5p	6.6 (2.9 – 15.1)	**<0.001**		
miR-25-3p	27.2 (8.0 – 93.0)	**<0.001**	5.7 (1.1 – 27.8)	**0.033**
miR-126-3p	4.2 (2.3 – 7.7)	**<0.001**		
miR-155-5p	10.6 (4.6 – 24.6)	**<0.001**	5.0 (1.4 – 18.4)	**0.015**
miR-615-3p	0.3 (0.1 – 0.6)	**<0.001**	0.12 (0.03 – 0.46)	**0.002**
CXCL-9	10.9 (4.8 – 24.9)	**<0.001**	5.9 (2.0 – 17.2)	**0.001**
CXCL-10	4.1 (2.3 – 7.4)	**<0.001**		
Recipient gender	0.3 (0.1-0.6)	**<0.001**		
Primary transplant	0.12 (0.02-0.89)	**0.038**		
Recipient age at transplantation	0.96 (0.93 – 0.98)	**0.001**	0.93 (0.88 – 0.98)	**0.004**
Donor Age	0.97 (0.95 – 1.00)	**0.037**		
Donor Type	2.1 (1.0 – 4.1)	**0.044**		
DGF	4.2 (1.5 – 11.4)	**0.005**		
Induction therapy	0.4 (0.1 – 1.2)	0.112		
hPRA > 5%	11.1 (2.6 – 48.3)	**0.001**		

Fig. 3
ROC analysis



Area Under the Curve

miR-25-3p	0,822
miR-155-5p	0,825
miR-615-3p	0,708
CXCL-9	0,858
MiR-Chemokine model	0,947

Sens 92.4%
Spec 82.9%

Conclusion

A combined measurement miR-25-3p, miR-155-5p, miR-615-3p in the urine sediment and CXCL-9 helps to non-invasively identify acute transplant rejection.

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