Investigation of Circulating MicroRNA Levels in Antibody-Mediated Rejection After Kidney Transplantation

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ABSTRACT

Background. One of the most important possible complications determining long-term graft survival after kidney transplant is antibody-mediated rejection (ABMR). The criterion standard approach to recognize ABMR is currently the kidney biopsy with histopathologic analysis. However, this test has limitations because of difficulties in timing of sampling, the evaluability of histology because of the questionable representativeness of specimens, and the limited number of this intervention. Hence, new reliable, noninvasive biomarkers are required to detect the development of ABMR in time.

Methods. In this study, we analyzed the clinical data of 45 kidney transplant patients (mean age of 44.51 years, 20 male and 25 female subjects). These participants were recruited into 5 subcohorts based on their clinical status, histologic findings, and level of donor-specific anti-HLA antibodies. Circulating microRNAs (miR-21, miR-181b, miR-146a, miR-223, miR-155, miR-150) in plasma samples were quantified by quantitative polymerase chain reaction and their levels were correlated with the clinical characteristics in different subgroups.

Results. The relative expression of plasma miR-155 (P = .0003), miR-223 (P = .0316), and miR-21 (P = .0147) were significantly higher in patients who had subsequent histology-approved ABMR with donor-specific anti-HLA antibody positivity (n = 10) than in the "triple negative" group (n = 21), and miR-155 showed the highest sensitivity (90%) and specificity (81%) to indicate ABMR development based on receiver operating characteristic analysis.

Conclusions. According to our preliminary data, plasma miR-155, miR-21, and miR-223 can indicate the development of ABMR after kidney transplant in correlation with classic clinical parameters. However, future studies with larger number of participants are necessary to further evaluate the diagnostic properties of blood miRNAs in prediction of this life-threatening condition.

KIDNEY transplantation has become a routine surgical procedure for the treatment of end-stage kidney disease. Although long-term clinical outcomes are improving as a result of current administration of immunosuppressive therapy and advanced patient care, antibody-mediated graft rejection (ABMR) still occurs in many cases. The T-cell-mediated or cellular-type alloimmunity is largely controlled by currently used immunosuppressants, but the role of B cells and donor-specific anti-HLA antibodies (DSAs) is less understood as yet. Measurement of DSAs has become a practice in the daily routine, but their specificity is still questionable. Although kidney

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biopsy specimens obtained in the presence of high-titer of DSAs can show histologic abnormalities, which are typical for ABMR, early-stage or early-onset fibrosis may already develop at an almost irreversible stage. ABMR is generated in approximately 10% of kidney transplant recipients, which may result in a graft loss in up to 30% of recognized cases [1]. According to recent publications, the incidence of pathologic histology findings in kidney biopsy specimens increases sharply in about 6 months after transplant and in about 50% to 60% of follow-up samples after 4 to 5 years [2]. The diagnosis and the determination of types of rejection are based on the Banff criteria, which is a classification for the structural and immunohistochemical abnormalities [3,4]. The disadvantages of biopsy are that it can potentially cause unwanted adverse effects (e.g., intraparenchymal arteriovenous shunt) and is invasive. It is even more difficult to make the patients accept this intervention, especially if repeated biopsy is needed to monitor disease progression. Timing of the intervention is also important because sampling in an inappropriate time point does not provide any advantages. That explains the clinical need for blood biomarkers that can aid the timing of histologic sampling and predict or at least confirm the development of ABMR. MicroRNAs (miRNAs) are small noncoding RNAs consisting of about 18 to 24 nucleotides that post-transcriptionally regulate gene expression through promoting messenger RNA degradation, leading to the interference and attenuation of target protein expression [5]. These RNA molecules are involved in the regulation of key biochemical events and disease progression, such as lipid metabolism [6], cell differentiation [7], cardiovascular diseases [8], and cancer [9]. Furthermore, miRNAs have been intensively investigated as novel pathophysiological mediators and laboratory parameters in a number of diseases, such as sepsis [10] and intraventricular hemorrhage [11]; thus, they may be considered to be potential biomarkers for predicting allograft rejection as well. According to the www.mirbase.org database, 2693 mature hsa-miRNAs are currently known in humans [12]. Several types of miRNAs are detectable in peripheral blood and other body fluids and thus may be suitable for noninvasive diagnostics [13]. In addition, these RNA molecules can also represent a promising research field into miRNA-based therapy for certain diseases [14,15]. Currently, only limited data are available on altered miRNAs as laboratory biomarkers in the context of kidney injury and transplant [16]; thus, we sought to investigate the expression of some selected circulating plasma miRNAs in the prediction of the development in ABMR after kidney transplantation.

MATERIAL AND METHODS

Patient Groups

The clinical data of 45 patients followed up by the Department of Organ Transplantation of the University of Debrecen were analyzed in this study. Twenty male and 25 female participants had a mean (SD) age of 44.51 (13.77) years. Of them, 30 had a primary kidney transplant with a median time since kidney transplant of 3.6 years. We defined 5 study subgroups (groups A-E) based on the presence of DSAs, clinical and laboratory signs suggestive of ABMR, such as decreased estimated glomerular filtration rate (eGFR) with proteinuria, and histologic findings (Table 1, Table 2). The causes of end-stage kidney disease, which were the indications for kidney transplant, can be seen in Table 3. Group A was the main ABMR ("triple positive") study group that was defined by having relevant clinical symptoms, the presence of DSA with increased mean fluorescence intensity (MFI) values, and histologically confirmed disease. This group consisted of 10 patients (6 male, 4 female) with a mean age of 40.97 years, and 4 of them underwent a primary transplant

Table 1. Patient Subgroups Based on DSA Level, Clinical Symptoms, and Histologic Diagnosis

Variable		Group A (n = 10)	Group B (n = 6)	Group C (n = 6)	Group D (n = 2)	Group E (n = 21)
De novo DSA*		+	+	+	–	–
Clinical signs		+	+	–	+	–
	Decreased eGFR	+	+	–	+	–
	Proteinuria	+	+	–	+	–
Histological result overall: ABMR		+	–	–	+	–
Histologic signs according to Banff classification (2017) (average score of items within group)	Glomerulitis, g	1.44			1	
	Transplant glomerulopathy, cg	1.33			0	
	Tubulitis, t	0.22			0.5	
	Tubular atrophy, ct	0.89			0.5	
	Interstitial inflammation, i	0.56			1.5	
	Peritubular capillaritis, ptc	1.75			1.5	
	Hyaline arteriolar thickening, ah	1			0	
	Intimal arteritis, v	0.11			0	
	Arterial fibrous intimal thickening, cv	0.89			0.5	
	Mesangial matrix increase, mm	0.67			0	
	Interstitial fibrosis, ci	1			0.5	
	Tubulointerstitial inflammation, ti	0.67			0	
	Peritubular capillary C4d staining	0.89			0	
Electron microscopy	7/12†	7/10			0/2	

ABMR, antibody-mediated rejection; DSA, donor-specific antibody; eGFR, estimated glomerular filtration rate.

* DSA was considered to be present if at least 2 tests revealed it with a mean fluorescence intensity value > 1000.

† No. of cases within group where a double layer basal membrane was identified.

Table 2. Main Clinical Features of Recruited Patients

			PRA		Donor	
	Sex, female ratio (%)	Age, mean (SD), y	Negative, %	Average, % (If Positive)	Age, y	LRKT/ DDKT
Group A	4/10 (40)	40.97 (17.12)	4/10 (40)	52.99	47.30 (16.73)	1/9
Group B	1/6 (17)	42.8 (9.75)	3/6 (50)	76.14	40.50 (12.49)	0/6
Group C	5/6 (83)	50.2 (10.63)	5/6 (83)	89.83	51.50 (17.42)	0/6
Group D	0/2 (0)	52.20 (12.59)	1/2 (50)	52.95	49.50 (12.02)	0/2
Group E	10/21 (48)	44.47 (14.42)	18/21 (86)	55.71	47.10 (12.48)	2/21

DDKT, deceased donor kidney transplant; LRKT, living-related kidney transplant; PRA, panel-reactive antibody.

Table 3. Causes of Chronic Kidney Failure in the Study Subpopulations

Etiology	Group A	Group B	Group C	Group D	Group E
Unknown etiology	4	2	1		7
Polycystic kidney disease	2		1	1	3
Alport syndrome	1			1	
Diabetic nephropathy	1	1	2		2
Congenital kidney hypoplasia	2				
Acquired obstructive uropathy		1			1
IgA nephropathy		1			1
Membranoproliferative glomerulonephritis		1			
Diffuse membranous glomerulonephritis			1		
Pyelonephritis/interstitial nephritis			1		1
Kidney tubulointerstitial disease, unspecified					1
Wegener granulomatosis					1
Systemic lupus erythematosus					1
Focal segmental glomerulosclerosis					1
Amyloidosis					1
Vesicoureteral reflux					1

with a mean time since kidney transplant of 4.6 years. Group B with 6 patients (5 male, 1 female) having an average age of 42.8 years, was defined by relevant clinical symptoms and presence of DSA (at least with 1000 MFI level) but with negative histologic results. In 3 cases in this cohort primary transplant occurred with an average 4.17 years elapsed since operation. Group C was defined by the lack of clinical symptoms (i.e., stable, good kidney function) and negative histology but the presence of high MFI values of DSA. This group included 6 patients (1 male, 5 female) with a mean age of 50.2 years. Five of them were after primary transplant, and the mean time since surgery was 3.5 years. The definition of group D was the presence of clinical symptoms and no DSA detected in the serum but positive histologic finding. This group consisted of only 2 male patients with a mean age of 52.2 years, with 1 primary transplant and a mean time since surgery of 2.5 years. Finally, Group E was the "triple negative" control group with the lack of clinical, DSA, and pathologic findings. It consisted of 21 patients (11 male, 10 female) with a mean age of 44.33 years. Of them, there were 17 primary transplants showing an average interval of 3.1 years since kidney transplant. Formation of subgroups was defined to follow all the possible alternative combinations of indicators in connection with plasma level of miRNAs to predict ABMR.

Light Microscopy, Electron Microscopy, and Immunofluorescence Staining

In most cases, native and transplanted kidney biopsies were divided into 3 parts to perform conventional light microscopy, electron microscopy, and immunofluorescence analyses. The fixative for light microscopy of kidney biopsy specimens was neutral buffered formaldehyde. Paraffin wax processing of kidney biopsy specimens was carried out with the routine diagnostic workload. High magnification light microscopy was crucial to evaluating glomerular disease, so thin slices were produced. Given the small size of a needle biopsy specimen, paraffin wax slices were cut at 2- to 3-μm thickness. Thicker slices were necessary for some stains and investigations, such as Congo red or immunoperoxidase studies. Slices were routinely stained by a technique that highlighted cells, such as hematoxylin and eosin, and one that highlighted the basement membranes and connective tissue matrix, such as Jones silver, trichrome, or periodic acid Schiff (PAS) stain. A Congo red stain was used for detection of amyloid, while elastin stain was applied for evaluating vascular disease. In kidney transplant pathology, the development of the Banff classification has laid great emphasis on the use of the PAS stain to delineate tubular basement membranes and to identify tubulitis, the principal marker of acute rejection. For this purpose, PAS staining is certainly equal to Jones silver and was technically easier.

Electron microscopy has become less used in recent years in many aspects of diagnostic pathology but has retained its importance in kidney pathology. It might detect unsuspected method failures, as when electron microscopy shows electron dense deposits in a case with apparently negative immunohistochemical results. There is evidence that ultrastructural examination of the basement membranes of peritubular capillaries for splitting gives a relatively specific marker of chronic rejection. Fixation of electron microscopy specimens conventionally uses glutaraldehyde or a glutaraldehyde/formaldehyde mixture, such as

Table 4. Sequences of Primers for the Analysis of Mature Plasma miRNAs

Mature miRNA	Stem Loop Primers for Reverse Transcription	Forward Primers for RT-qPCR	Universal Reverse Primer for RT-qPCR
miR-21-5p	5′ – GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAAC TCAACA – 3′	5′ – GTTTGGTAGCTTATCAGACTGA – 3′	5′ – GTGCAGGGTCCGAGGT – 3′
miR-181b-5p	5′ – GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAAC ACCCAC – 3′	5′ – GTTTGAACATTCATTGCTGTCG – 3′	5′ – GTGCAGGGTCCGAGGT – 3′
miR-146a-5p	5′ – GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAAC AACCCA – 3′	5′ – GTTTGGTGAGAACTGAATTCCA – 3′	5′ – GTGCAGGGTCCGAGGT – 3′
miR223-3p	5′ – GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAAC TGGGGT – 3′	5′ – GTTGGGTGTCAGTTTGTCAAAT – 3′	5′ – GTGCAGGGTCCGAGGT – 3′
miR155-5p	5′ – GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAAC ACCCCT – 3′	5′ – GTGGGTTAATGCTAATCGTGAT – 3′	5′ – GTGCAGGGTCCGAGGT – 3′
miR-150-3p	5′ – GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACCTGT – 3′	5′ – TTGCTGGTACAGGCCTGG – 3′	5′ – GTGCAGGGTCCGAGGT – 3′
cel-miR-39-3p	5′ – GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAAC AATCAG – 3′	5′ – GTGAACTTATTGACGGGCG - 3′	5′ – GTGCAGGGTCCGAGGT – 3′

miRNA, microRNA; RT-qPCR, real-time quantitative polymerase chain reaction.

Karnovsky's fixative, with postfixation using osmium tetroxide. Processing is conventionally into an epoxy resin. Semithin slices for block selection were stained using toluidine blue. Ultrathin slices were stained with uranyl acetate and lead citrate before examination under the electron microscope. In our institute, electron microscopy is performed on all biopsy specimens of native and transplanted kidney.

Immunohistochemical investigations are a routine part of the investigation of native and transplanted kidney biopsy specimens. A basic panel of antibodies is used for the detection of tissue deposits of IgG, IgA, IgM, kappa, lambda, albumin, and complement (usually C3, C1q, C4d). This represents a minimum. Additional antibodies can be used at the pathologist's discretion.

The list of antibodies that might be used in special cases is extensive. A selection includes those directed against the following: (1) specific amyloid types (amyloid-a, transthyretin); (2) specific α (2, 3, 4, 5) chains of type 4 collagen (Alport syndrome); (3) fibronectin (fibronectin glomerulopathy); (4) type 3 collagen (collagenous glomerulopathy); and (5) viral antibodies (SV40, adenovirus, cytomegalovirus, Epstein-Barr virus).

Immunofluorescence

This approach has the advantage of simplicity and reliability because plasma proteins can be removed from frozen slices simply by washing. The essential panel of antibodies against immunoglobulins and complement is used with a direct immunofluorescence method, which is extremely simple and quick. Immunofluorescence has several disadvantages. A separate frozen specimen is taken at the time of biopsy. A cryostat and epifluorescence microscope is required. Relevant images are stored digitally.

Immunohistochemistry Using Paraffin Wax Slices

After formaldehyde fixation, proteolytic digestion is essential to remove plasma proteins and to "unmask" epitopes in glomerular immunoglobulin deposits. Hence, slices were incubated with a proteolytic enzyme (trypsin) for variable periods. They all were stained subsequently with a single antibody. For these kidney transplant biopsy specimens, SV40, HLA-DR, C4d, and LCA immunohistochemical staining were used.

Analysis of Plasma miRNAs

Peripheral blood samples were drawn into a Vacutainer tube containing 3.2% Na-citrate (Becton Dickinson, San Jose, Calif, United States), and were subsequently centrifuged at 1500 *g* for 15 minutes at room temperature to obtain platelet-poor plasma. These samples then stored at −80° C. Relative expression of cell-free miRNAs was analyzed from platelet-poor plasma samples. Briefly, thawed plasma samples were first centrifuged at 10,000 *g* for 1 minute at room temperature, and 400 μL of cell-free supernatants were spiked-in with 5 pmol mirVana cel-miR-39 mimic (Ambion, Austin, Tex, Untied States, ID:MC10956). Circulating miRNAs were then isolated with miRNeasy Kit (Qiagen, Hilden, Germany). The isolated total RNA was then reverse transcribed into complementary DNA using miRNA-specific stem-loop reverse transcriptase primer (500 nM, Integrated DNA Technologies, Leuven, Belgium) and TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Vilnius, Lithuania) according to the manufacturer's instructions. Quantification of miRNAs was performed using real-time quantitative polymerase chain reaction (RT-qPCR), for which universal reverse primer (100 μM, Integrated DNA Technologies), miRNA-specific forward primer (100 μM, Integrated DNA Technologies), Universal Probe

Library probe #21(10 μM, Roche Diagnostics, Mannheim, Germany), Taq DNA polymerase (5 U/μL, Thermo Scientific, Vilnius, Lithuania), and dNTP Mix (2.5 mM, Thermo Scientific, Vilnius, Lithuania) were used. Each measurement was performed in triplicates using QuantStudio 12K Flex qPCR instrument (Applied Biosystems by Thermo Fisher Scientific, Waltham, Mass, United States). To normalize the results of different miRNAs, cel-miR-39 reference gene was measured in all the samples with the same RT-qPCR method. Oligonucleotides and RT-qPCR assays were designed by the software developed by Czimmerer et al, and sequences of primers that were used in this study are listed in Table 4 [17].

Ethical Statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Scientific and Research Ethics Committee of the University of Debrecen (protocol no.: DE RKEB/IKEB 5228-2019) in accordance with the Declaration of Helsinki.

Statistical Analysis

Kolmogorov-Smirnov test was used for evaluation of the normality of data. To compare the data of 2 groups, we applied Mann-Whitney *U* test. In case of those miRNAs which showed a significant difference between 2 subgroups, receiver operating characteristic (ROC) curve analysis was performed. Youden index was calculated to determine the cutoff values of each baseline miRNA for sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) to predict the development of ABMR. Statistical significance was defined when P value was $< .05$. Statistical analyses were performed using GraphPad Prism software (version 6.01, La Jolla, Calif, United States).

RESULTS

The typical histopathologic characteristics of ABMR are shown on Figs 1 and 2. The electron microscopic alteration in ABMR showed thickening of the peritubular capillary membrane (Fig 1). Peritubular capillaritis was characterized by the presence of both mononuclear and polymorphonuclear inflammatory cells, which is typical in ABMR. Furthermore, glomerulitis represents endothelial enlargement and inflammatory cell infiltration, often resulting in capillary luminal narrowing and destruction. The double or multilayering of glomerular basal membrane are more typical to chronic ABMR. Because complement C4d is a product of complement activation, immunofluorescent staining showed a complement activation and degradation product of C4d in the peritubular capillary after antibody binding (Fig 2). Acute tubular injury may also be a sign of active ABMR if it is presented in the absence of other known causes. These observations, together with the clinical signs proved the occurrence of ABMR. Among these participants, those with histology positive cases were enrolled into group A.

Of 6 different plasma miRNAs, miR-155 ($P = .0003$), miR-21 ($P = .0147$), and miR-223 ($P = .0316$) demonstrated a significant difference between the triple positive and triple negative groups because the expression of all these miRNAs were higher in those who had clinical symptoms, DSA, and histopathologic findings than in participants with negative results for any of these alterations (Fig 3 A-C). Furthermore, miR-155 already showed elevated levels ($P = .0185$) in patients with DSA and clinical signs but still without histology (group B) (Fig 3A). In case of miR-21, there was a gradually increasing expression in plasma from "triple negative" individuals toward "triple positive" participants but did not reach a statistical significance, probably because of the low number of cases (Fig 3B). Similar tendency was observed in miR-223, and a substantial change ($P = .0316$) was seen only between groups A and E (Fig 3C). No significant difference with any tendency was observed in the relative levels of miR-146a, miR-181, and miR-150 among these study subgroups (Fig 3D-F). Our data suggest that circulating miR-155, miR-21, and miR-223 indicated the

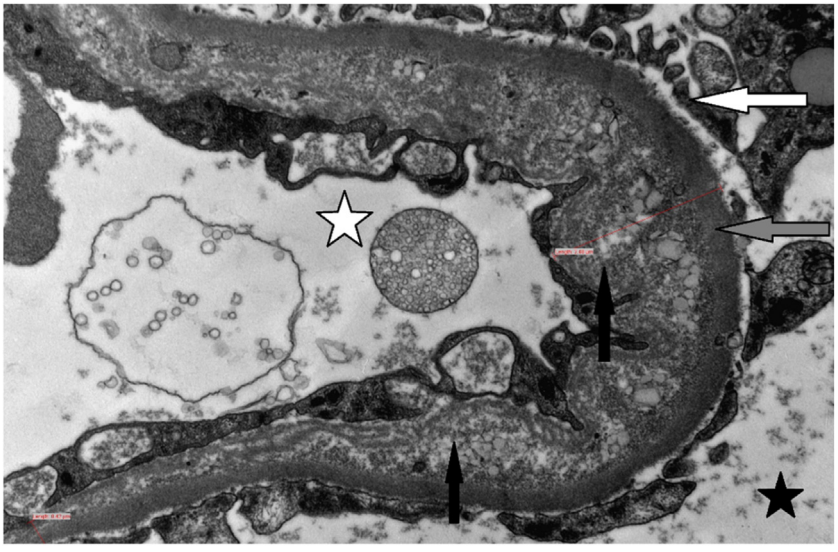

Fig 1. Electron microscopic investigation of peritubular capillary membrane. The black arrows point the subendothelial multilayering of the glomerular basement membrane because of repetitive sublethal endothelial cell injury. The white star represents the capillary lumen, the black one shows the urinary space. The white arrow points to podocyte foot processes, the gray one to the original glomerular basement membrane (JEOL 1010; 15,000×).

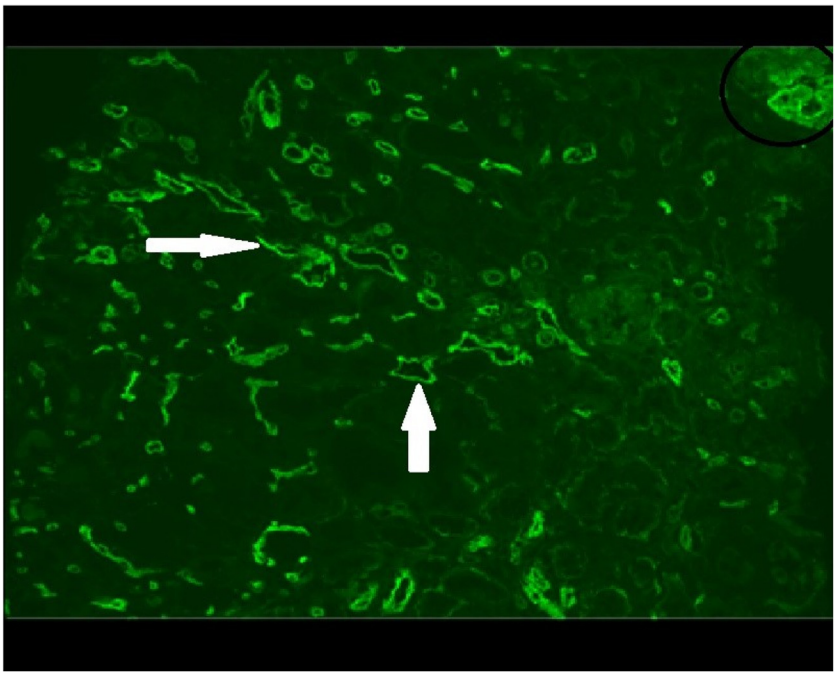

Fig 2. Immunofluorescence staining of kidney parenchyma showing prominent, bright, linear peritubular capillary basement (white arrows) (Olympus BX51 U-RFL-T fluorescence microscope; 200×).

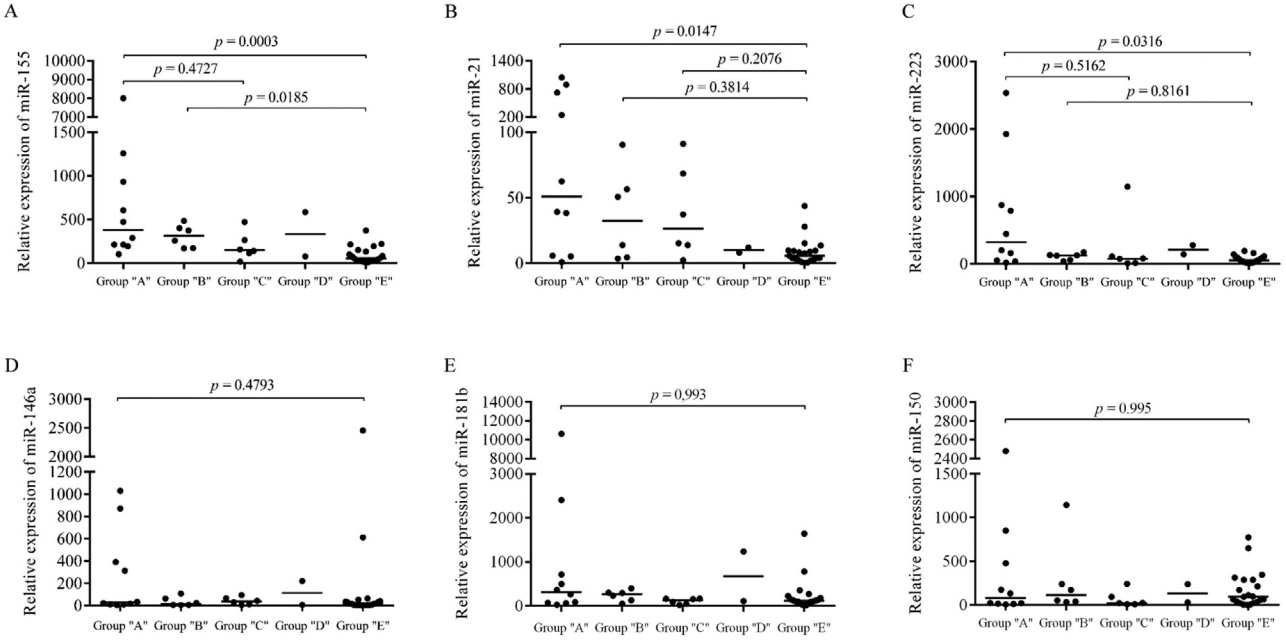

Fig 3. The relative expression of miR-155 **(A)**, miR-21 **(B)**, miR-223 **(C)**, miR-146a **(D)**, miR-181b **(E)**, and miR-150 **(F)** measured by RT-qPCR in pre-ABMR plasma samples of 45 patients after kidney transplant. Dots represent single expression values. Median values are depicted with horizontal lines. To compare the data of 2 groups, we applied Mann-Whitney *U* test.

ABMR, antibody-mediated rejection; miRNA, microRNA; qPCR, quantitative polymerase chain reaction.

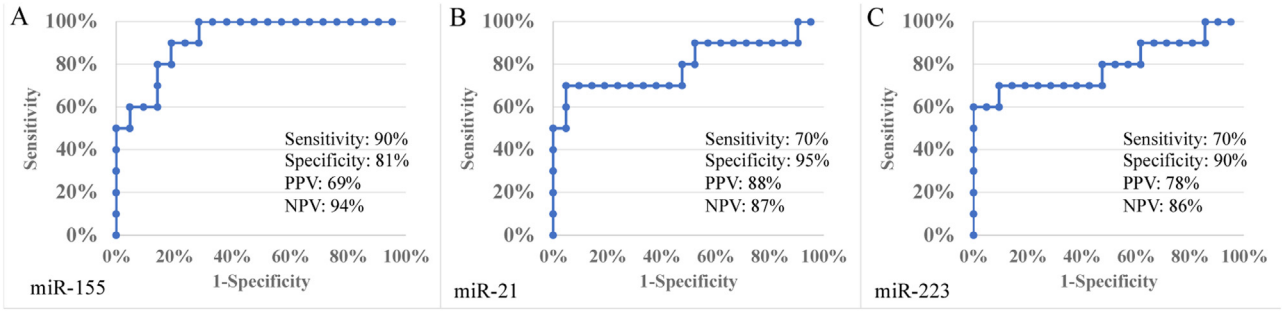

Fig 4. Analysis of ROC curves for plasma miR-155 **(A)**, miR-21 **(B),** and miR-223 **(C)** to analyze their predictive value in the development of ABMR. Among these circulating miRNAs, miR-155 showed the highest sensitivity and specificity to indicate that ABMR may develop in patients after kidney transplant.

ABMR, antibody-mediated rejection; miRNA, microRNA; ROC, receiver operating characteristic.

development of ABMR with higher plasma levels in pre-ABMR samples.

Furthermore, in case of miR-155, miR-21, and miR-223, ROC curve analysis was performed. The ideal cut-off value for miR-155 was found at a relative expression value of 152.46, with sensitivity of 90%, specificity of 81%, PPV of 69%, and NPV of 94%. The optimal cut-off value for miR-21 was determined at an expression level of 27.88, with sensitivity of 70%, specificity of 95%, PPV of 88%, and NPV of 87%. Examining the statistical results of miR-223, the most appropriate cut-off value was at the expression level of 144.32, where sensitivity was 70%, specificity was 90%, PPV was 78%, and NPV was 86%. Overall, miR-155 showed the highest sensitivity and specificity to indicate the development of ABMR (Fig 4A-C).

DISCUSSION

We know that extracellular vesicles (EVs) play a role in the kidney (ischemic) injury. This is observed in that miR-21 is a profibrotic mediator that can also be shuttled by tubular EVs. These EVs might represent a biomarker for graft rejection [18]. Angiotensin-converting enzyme inhibitor treatment was proven to decrease the tissue expression of miR-21 and transforming growth factor β, thus inhibiting tissue fibrosis in kidney transplant recipients. It might also explain the protective effect of angiotensin-converting enzyme inhibitors on allograft function by reducing mediators of fibrosis [19]. Also, miR-21 takes part in the correction of ischemia-reperfusion injury via contributing to a protective angiogenesis pathway induced by hypoxia-inducing factor 1 α [20]. Thus, the increase in miR-21 expression may be also a sign of an ongoing repair mechanism, by novel angiogenesis in an ischemia-reperfusion−injured kidney graft. The exact mechanism of ABMR is still under investigation. However, it is clear that both ischemic and compensatory angiogenetic factors might play a role in this complication. During the hypothermic machine perfusion of extended criteria donor graft kidney, an increased miR-21 expression in the kidney tissue was demonstrated after acute kidney injury, and miR-21 quantification after certain time of hypothermic machine perfusion correlated with eGFR at 6 and 12 months post transplant, which suggested that miR-21 might be a predictor of graft function [20]. In parallel, the role of miR-155 in a rat kidney transplant model was confirmed. Authors found that the expression of miR-155 in plasma was consistent with the dynamic change of acute rejection degree [21]. Urinary expression of miR-155 was studied to be a prognostic factor for acute rejection in logistic regression model, and the authors proved it even in the presence of immunosuppressive drug exposure. Thus, urinary miR-155 detection could be very useful to identify patients with a potential high risk of rejection at the early stages of the post-transplant period [22]. Uromodulin and certain miRNAs (such as miR-29c, miR-126, miR-146a, miR-150, miR-155, and miR-223) might be potential biomarkers for kidney graft−associated pathology and outcome. However, these miRNAs did not correlate with eGFR in this study, in contrast to uromodulin, which did [23]. There are interesting approaches of miR-155 in context with systemic lupus erythematosus (SLE); miR-155-deficient mice had lower serum autoantibody levels with less prominent T-cell response development. Thus, antagonizing miR-155 might be a future approach in treating SLE [24]. Others confirmed the possible role of miR-223 in SLE, too. In a mice model, the deletion of miR-223 exacerbated the lupus phenotypes associated with increased population of S1PR1+CD4+T in spleen, suggesting compensatory role of miR223 in the pathogenesis of lupus nephritis [24]. This is in accordance with our findings that a high miR-155 and miR-223 expression were parallel with high activity of DSA. Also, miR-223 is also known as one the cardiovascular-associated microRNAs. It was observed in a Japanese population that expression levels of some miRNAs (miR-126, miR-197, miR-223) in participants with chronic kidney disease were significantly lower than of in those with normal kidney function. In addition, a significant linear association between the cumulative score of these miRNAs and chronic kidney disease was found [25].

We also observed that plasma miR-21, miR-155, and miR-223 showed a significant difference between the 2 “triple” groups. These circulating miRNAs had high relative expression in patients with verified ABMR (group A compared with completely stable participants with negative results (group E). Furthermore, based on the ROC curve analysis, miR-155 had

the highest sensitivity (90%) and specificity (81%) to indicate ABMR development; however, comparable values were determined in case of miR-21 and miR-223 (Fig 4). The expression of miR-21, miR-142, and miR-221 is associated with high fibrosis score (Banff classification), and these miRNAs were more expressed in plasma exosome, indicating high-grade interstitial fibrosis and tubular atrophy after kidney transplant [19].

CONCLUSIONS

In summary, post-transplant miRNA profiling may help us to make an early and prognostic diagnosis of ABMR. The usefulness of the plasma levels of these markers for timing a histology sampling is inevitably proven. However, classic diagnostic tools cannot be currently replaced by these new biomarkers. A weakness of the study is the low number of cases. Despite this fact, it seems that the analysis of heterogeneous circulating miRNA levels is important and may play a role in the follow-up of the disease course. A deeper understanding of regulation of miRNAs may also lead us to miRNA-based diagnostics, possibly targeted therapies, and thus the development of personalized immunosuppression in time, to prevent the generation of ABMR.

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