Non-coding RNA Research 7 (2022) 217–225

Contents lists available at ScienceDirect

Non-coding RNA Research

journal homepage: www.keaipublishing.com/en/journals/non-coding-rna-research

Original Research Article

Upregulated circulating mir-424 and its' diagnostic value for gram-negative bacteremia after thoracic transplantation

Olga Shevchenko [a,b], Olga Tsirulnikova [b,c], Sofya Sharapchenko [a,*], Olga Gichkun [a,b], Dmitriy Velikiy [a], Nina Gabrielyan [d], Ivan Pashkov [e], Alex Shevchenko [f,b], Sergey Gautier [g,b]

[a] Department of Regulatory Mechanisms in Transplantology, Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation
[b] Department of Transplantology and Artificial Organs, Sechenov University, Moscow, Russian Federation
[c] Department of Therapy, Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation
[d] Department of Endotoxicosis and Purulent-septic Complications, Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation
[e] Department of Surgery, Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation
[f] Department of Cardiology, Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation
[g] Director of Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation

ARTICLE INFO

Keywords:
miR-424
Heart
Lung
Transplantation
Bacteremia
Acute rejection

ABSTRACT

Aims: Early post-transplant complications such as acute graft rejection and infections are associated with high morbidity and mortality of heart and lung transplant recipients who are in vital need of immunosuppressive therapy. MiR-424 is a member of the miR-16 family, which plays an important physiological role in the development of cardiovascular and respiratory pathology, is involved in the regulation of monocyte and macrophage differentiation, and has an immunosuppressive potential. The aim of the study was to determine the diagnostic value of circulating miR-424 as a potential biomarker of post-transplant complications in heart and lung transplant recipients.

Methods: The study enrolled 83 heart transplant recipients, aged 18 to 70 (48 ± 13) years; 26 lung transplant recipients, aged 10 to 74 (36 ± 16) years. The miR-424 plasma expression was detected by real-time PCR (Qiagen, USA). Significance of miR-424 level was assessed through the ΔCt method. Acute graft rejection was verified by the results of endomyocardial or transbronchial biopsy. Post-transplant infectious complications were verified through microbiological identification of bacteremia from blood cultures.

Results: Our study shows miR-424 upregulation in plasma of patients with chronic heart or respiratory failure in comparison with healthy individuals ($p = 0.003$ and $p = 0.04$ resp.). There was a direct correlation of miR-424 expression with red blood cells and hemoglobin levels in patients before heart transplantation ($p = 0.01$ and $p = 0.03$ resp.). After transplantation the expression of plasma miR-424 correlated with the level of C-reactive protein (CRP) both in heart ($r = 0.75$; $p = 0.02$) and lung ($r = 0.50$; $p = 0.04$) transplant recipients. The expression of plasma miR-424 correlated with tacrolimus blood concentration after heart transplantation ($r = 0.38$; $p = 0.04$). The miR-424 level didn't differ in heart or lung transplant recipients with and without acute graft rejection ($p = 0.47$ and $p = 0.78$ resp.), but was significantly higher in heart and lung transplant recipients with gram-negative bacteremia ($p = 0.002$). When the miR-424 level is above a threshold value (−5.72 fold change), the relative risk of bacteremia is RR = 3.84 [95% CI 1.94–7.61]; Se = 60.0%; Sp = 89.2%. CRP concentration above 7 mg/L in duplex test with miR-424 improves the diagnostic characteristics of miR-424 for post-transplant gram-negative bacteremia in heart and lung transplant recipients up to RR = 9.17 [95% CI 1.37–61.46]; Se = 83.3% and Sp = 90.1%.

Conclusion: MiR-424 plasma expression was upregulated in patients with chronic heart and respiratory failure and in heart and lung transplant recipients in the early post-transplant period. The duplex test, including miR-424 and CRP, has a diagnostic value for detecting the high risk of post-transplant gram-negative bacteremia in heart and lung transplant recipients.

* Corresponding author. Department of Regulatory mechanisms in Transplantology, Shumakov National Medical Research Center of Transplantology and Artificial Organs, Shchukinskaya Str. 1, Moscow, 123182, Russian Federation.
E-mail addresses: transplant2009@mail.ru, Nyashka1512@yandex.ru (S. Sharapchenko).

https://doi.org/10.1016/j.ncrna.2022.08.001
Received 17 May 2022; Received in revised form 5 August 2022; Accepted 19 August 2022
Available online 31 August 2022

1. Introduction

Heart and lung transplantations are now an established therapy for end-stage heart and respiratory failure. According to the Registry of International Society for Heart and Lung Transplantation (ISHLT), multiple advances over the past few years improved the survival and quality of life in heart and lung transplant recipients [1,2]. Shumakov National Medical Research Center of Transplantology and Artificial Organs is a leading center of solid organ transplantation in Russian Federation [3].

The post-transplant complications such as acute graft rejection and nosocomial infections are the highest risk factors of primary graft dysfunction and mortality in the first year after heart and lung transplantation. One of the main causes of these complications is immune disorders associated with insufficiency or excess of immunosuppressive therapy [4–6].

Minimally invasive laboratory technologies for early detection of signs of pathological conditions in transplant are being actively developed [7,8]. However, the multifactorial nature of complications doesn't allow to solve the problem of noninvasive diagnosis quickly [9]. With this, the search for new biomarkers of graft damage with proven effectiveness which can reduce the frequency of invasive diagnostic interventions is extremely relevant [10]. Recently, research has been conducted on biological agents that can be used as indicators of the risk of adverse events associated with processes leading to graft injury and dysfunction [11].

Non-coding microRNAs are 18–25 nucleotides long regulatory molecules which are widely known to participate in the regulation of gene expression, proliferation and apoptosis, metabolic disorders, autoimmune diseases and carcinogenesis [12,13]. Circulating microRNAs (miR) are involved in inflammatory processes of the respiratory and cardiovascular systems, besides have a potential value diagnostic and target therapy on post-transplant complications [14,15].

MiR-424 plays an important physiological role in the development of cardiovascular (hypoxia, post ischemic cardiac remodeling) and respiratory (pulmonary arterial hypertension, right ventricular hypertrophy) pathology [16,17]. In addition, miR-424 is involved in the regulation of monocyte and macrophage differentiation and has an immunosuppressive potential [18].

The aim of the study was to determine the diagnostic value of circulating miR-424 as a potential biomarker of post-transplant complications in heart and lung transplant recipients.

2. Materials and methods

2.1. Study setting and population

The study included 109 heart and lung transplant recipients who underwent transplantation and were monitored at Shumakov National Medical Research Center of Transplantology and Artificial Organs: 83 (76%) patients underwent orthotopic heart transplantation (HTx) and 26 patients (24%) – bilateral or single lung transplantation (LTx). All the patients were Russian ethnic descent. The follow-up lasted for 38 ± 14 days after HTx and LTx. Routine examination and treatment of recipients was carried out in accordance with the clinical guidelines of the Russian Transplant Society [16], coincide with the ISHLT consensus guidelines.

The comparison group consisted of 12 healthy individuals: 7 males and 5 females (average age 39 ± 14 years). The study protocol was approved by Shumakov National Medical Research Center of Transplantology and Artificial Organs ethics committee. All of the patients or their guardians gave written informed consent to participate in the study.

Heart transplant recipients have been followed closely and received routine laboratory tests, clinical examination, echocardiography, endomyocardial biopsy, and coronary angiography according to our institution protocols.

Physical examination of lung transplant recipients included instrumental diagnostic methods: computed tomography and chest X-ray, electrocardiography and echocardiogram, body plethysmography, bronchoscopy, transbronchial biopsy, spirometry, and routine laboratory tests.

All the recipients received immunosuppressive therapy including tacrolimus, mycophenolic acid, and methylprednisolone, preventive antimicrobial therapy in the early stages after transplantation and adjuvant therapy, provided for by the standard protocol for the management of heart and lung transplant recipients approved in our center.

The quantification of tacrolimus was performed by chemiluminescence immunoassay by Abbott's Architect i2000 according to the manufacturer's instructions.

2.2. Plasma sampling, miRNA extraction and qRT-PCR

The peripheral blood samples (1–4 samples an average of 1.4 samples from each recipient) were collected in vacuum test tubes containing EDTA, centrifuged for 10 min at 3000 rpm, after which blood plasma was separated from the cell sediment and immediately frozen at −20 °C.

Total RNA was isolated using the miRNeasy Mini Kit (Qiagen, Germany), according to the manufacturer's instructions miRNeasy Serum/Plasma Handbook. The volume of starting material is 100 μl. QIAzol Lysis Reagent (500 μl) is added to plasma samples. Synthetic *C. elegans* miRNA (Syn-cel-miR39, cat no. MSY0000010) was added to samples as a spiked-in control for variations during the preparation of total RNA and subsequent steps. After chloroform was added to the tube and then centrifuged to separate phases, the aqueous phase was transferred to a new tube, and 1.5 times the volume of 100% ethanol was added to it. The solution containing RNA was loaded into the miRNeasy column and further washed according to the manufacturer's instructions. The final volume of elution was 14 μl. Concentration and purity of the obtained RNA were estimated on the NanoDrop 2000 microvolume spectrophotometer (Thermo Fisher Scientific, New York, USA).

Reverse transcription was performed using the MiScript II RT Kit (Qiagen) under the recommended protocol. To obtain cDNA, 300 ng of total RNA extracted from each sample was used, which was added to reaction mixture (4 μl 5x 5x miScript HiFlex Buffer, 2 μl 10X miScript Nucleics Mix, 2 μl of reverse transcriptase mix MiScript and RNAz-free water up to 15 μl) and was incubated for 60 min at 37 °C, followed by an increase in temperature up to 95 °C for 5 min to inactivate transcriptase.

Real-time PCR was performed using the MiScript SYBR Green PCR Kit (Qiagen) and presynthesized miScript Primer Assay (Qiagen) primers in the volume of the reaction mixture of 18 μl (2 μl of cDNA obtained), 10 μl 2x SYBR Green PCR Master Mix, 2 μl 10 × 10x miScript Universal Primer, 2 μl 10x miScript Primer Assay (Hs_miR-424_1 miScript Primer Assay cat no. MS00001486) to the studied microRNA and RNAz-free water up to 18 μl). Negative controls excluding template from the reverse transcription reaction was performed and profiled like the samples. Real-time PCR was performed on the CFX96 Real-Time PCR Detection System (Bio-Rad, USA) according to manufacturer's recommended program (15 min at 95 °C to activate the HotStarTaq DNA Polymerase and 40 three-step cycles (94 °C–15 s, 55 °C–30 s, 70 °C–30 s)). Standard curve was generated by plotting the log copy number miRNeasy Serum/Plasma Spike-In Control (cel-mir 39) used in each PCR against the mean Ct value.

For miRNA-424, a Ct value > 40 was defined as undetectable. Delta Ct method was used to quantify relative miRNA expression. Values were normalized to cel-mir 39 ($\Delta Ct = Ct_{mir424} – Ct_{celmir39}$) and are expressed as $2^{\Delta Ct}\log 10$ [19]. MiR-424 levels were presented as median and interquartile range of relative units.

2.3. Monitoring of HTx and LTx rejection status

Acute graft rejection was verified by the results of endomyocardial or transbronchial biopsy according to the protocol of management of heart and lung transplant recipients (in accordance with the recommendations of the Russian Transplant Society) [20]. The tissue samples had histological and immunohistochemical examination in the Department of Pathology Shumakov Center (The Head of Department – MD, N.P. Mozheiko) (Figs. 1 and 2).

ISHLT-2004 and ISHLT-2013 classifications were used for acute cellular rejection (ACR) and antibody-mediated rejection (AMR) verification [21,22].

2.4. Bacterial isolation and identification

Verification of post-transplant infectious complications was evaluated by routine microbiological assay from blood samples. Positive blood cultures incubated on blood agar to produce enough bacteria biomass (Fig. 3).

The species of the microorganism (gram-negative, gram-positive or fungus) were taken into account. Strain identification, antibiotic resistance sensitivity was automatically determined using the bacteriological analyzer MicroScanWalkAway 96 PlusSystem (USA); the zones of bacterial growth were determined on the Osiris analyzer (Bio-Rad Laboratories, France).

2.5. Statistical analysis

Normally distributed data are reported as mean value and standard deviation. Non-normal data are presented as median and interquartile range for continuous variables and number (%) for categorical variables. The Spearman test was applied as appropriate for the calculation of the correlations between two variables (r; p). For the independent variables, we used the Mann–Whitney *U* test. P-values of less than 0.05 were accepted as significant. The receiver operating characteristic (ROC) curve, the area under the ROC curve (AUC), relative risk (RR) sensitivity (Se), specificity (Sp), diagnostic efficiency (De), positive predictive value (PPV) and negative predictive value (NPV) were utilized to assess the diagnostic value of the identified biomarkers for post-transplant complications. The Youden index was calculated to measure the threshold microRNA expression level [23]. All analyses were carried out using Statistica v. 13.0 (StatSoftInc, USA) or MEDCALC 12.7.5.0 (MedCalc software, Mariakerke, Belgium).

3. Results

3.1. Recipients' characteristics

All the heart transplant recipients included into the study before the transplantation were in New York Heart Association (NYHA) functional class III-IV. Among the heart transplant recipients aged 18 to 70 (48 ± 13) years there were 64 (77%) male and 19 (23%) female recipients (Table 1).

The most of patients with chronic heart failure had dilated cardiomyopathy (60%) or ischemic cardiomyopathy (36%) as an indication for HTx.

Among the group of lung transplant recipients there were 23 adults aged 18 to 74 (39 ± 15) years and 3 pediatric patients aged 10 to 17 (13 ± 3) years; 61.5% were male recipients (Table 2).

The main indications for LTx were cystic fibrosis (39%) and chronic obstructive pulmonary disease (23%).

3.2. MiR-424 expression levels in patients before HTx/LTx

The median of miR-424 expression level in healthy individuals was −8.30 [−9.34; −7.41] relative units. The plasma level of miR-424 varied widely in patients with chronic heart and respiratory failure. A comparative analysis of the miR-424 expression level between healthy individuals and patients before HTx or LTx was carried out (Fig. 4).

MiR-424 expression levels in patients before HTx or LTx were significantly higher in comparison with the healthy individuals ($p = 0.003$, $p = 0.04$ resp.). Median expression of miR-424 didn't differ between patients before HTx −5.46 [-7.54; −1.46] and before LTx −6.71 [-8.37; −5.50], $p = 0.07$.

The miR-424 level in patients before HTx or LTx didn't depend on their gender (Table 3).

Table 4 demonstrates the correlations between miR-424 plasma levels and routine blood parameters in patients before HTx or LTx.

The Spearman test showed that miR-424 expression level did not depend on most general clinical blood parameters in patients before LTx; but revealed the direct correlations of miR-424 with the red blood cells ($r = 0.58$; $p = 0.01$) and hemoglobin ($r = 0.50$; $p = 0.03$) levels in patients with chronic heart failure.

3.3. MiR-424 expression levels in heart and lung transplant recipients

In Table 5 the correlations between miR-424 plasma levels and routine blood parameters after HTx or LTx are shown: complete blood count, blood biochemistry parameters, immunosuppressive drug concentration.

The plasma level of miR-424 didn't correlate with most routine complete blood count and blood biochemistry parameters in patients after HTx and LTx. Only the correlation between the level of miR-424 and C-reactive protein (CRP) was revealed both in heart ($r = 0.75$; $p = 0.02$) and lung ($r = 0.50$; $p = 0.04$) transplant recipients.

The expression of plasma miR-424 correlates with tacrolimus blood concentration in heart transplant recipients ($p = 0.04$), but did not correlate with tacrolimus blood concentration in patients after LTx ($p = 0.23$).

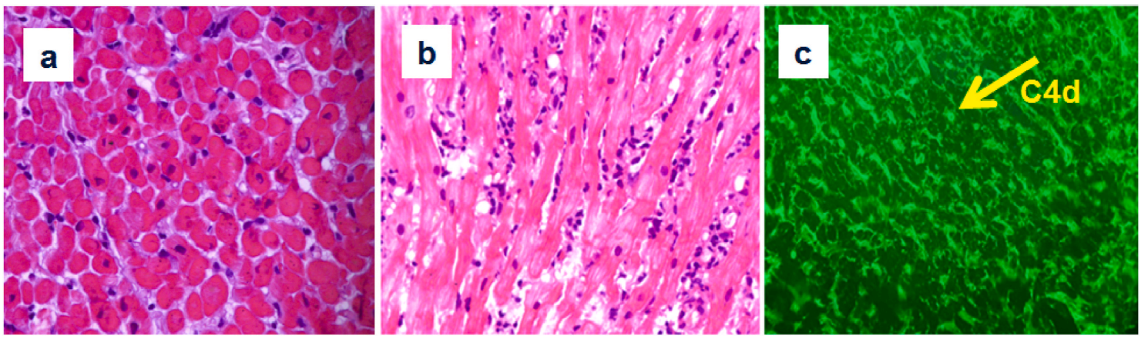

Fig. 1. The samples of endomyocardial biopsies of cardiac grafts: a – no rejection R0G; b –R2G acute cellular rejection; c – antibody-mediated rejection C4d staining.

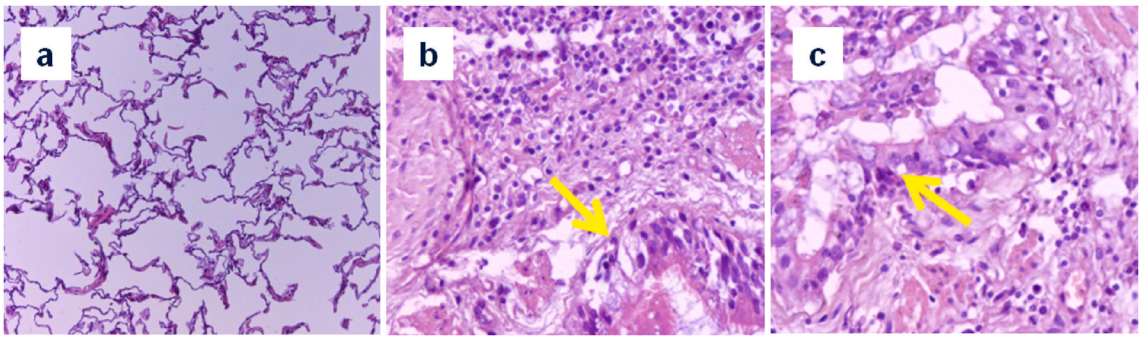

Fig. 2. The samples of transbronchial biopsies of lung grafts: a – no rejection grade A0; b,c –grade B1R acute rejection with an active lesion of the airways (lymphocytic bronchitis), arrows show the lesion of the submucosal layer epithelium with necrosis of individual cells and metaplasia.

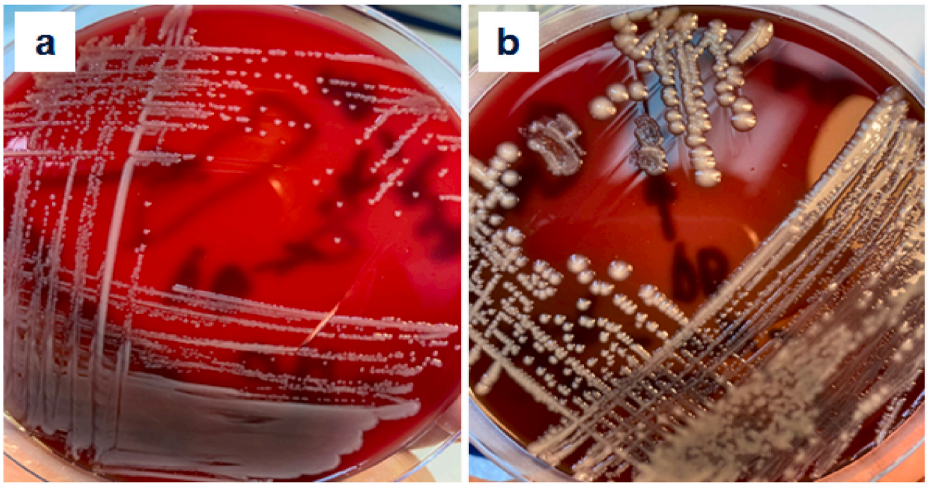

Fig. 3. Blood culture samples on blood agar with microbial growth: a – *Acinetobacter baumannii*; b – *Klebsiella pneumoniae*.

Table 1
Characteristics of heart transplant recipients.

Characteristics	
Heart transplant recipients, n	83
Gender, n (%):	64 (77%)
Male	19 (23%)
Female	
Age, years: range of variation (mean ± std. deviation)	18 to 70 (48 ± 13)
Initial diagnosis, n (%):	50 (60%)
Dilated cardiomyopathy	30 (36%)
Ischemic cardiomyopathy	2 (3%)
Nonrheumatic mitral valve disorders	1 (1%)
Retransplantation	
MiR-424 plasma level before HTx, units median [interquartile range]	−5.46 [-7.54; −1.46]
Tacrolimus blood concentration, ng/ml median [interquartile range]	9.5 [6.1; 11.7]

Table 2
Characteristics of lung transplant recipients.

Characteristics	
Lung transplant recipients, n	26
Gender, n (%):	16 (61%)
Male	10 (39%)
Female	
Age, years: range of variation (mean ± std. deviation)	10 to 74 (36 ± 16)
Initial diagnosis, n (%):	10 (39%)
Cystic fibrosis	6 (23%)
Chronic obstructive pulmonary disease	4 (15%)
Pulmonary arterial hypertension	4 (15%)
Pulmonary fibrosis of various etiologies	2 (8%)
Lymphangioleiomyomatosis	
MiR-424 plasma level before LTx, units median [interquartile range]	- 6.71 [-7.42; −4.43]
Tacrolimus blood concentration, ng/ml median [interquartile range]	8.3 [6.0; 10.8]

3.4. MiR-424 levels and acute graft rejection

According to the ISHLT classifications, 36 recipients with ACR (R1G – R3G degree) and 4 recipients with AMR after HTx were identified. The plasma level of miR-424 didn't differ ($p = 0.47$) in heart transplant recipients with ($n = 40$) and without acute graft rejection ($n = 43$).

Besides, no significant difference of miR-424 levels in heart transplant recipients with ACR ($p = 0.36$) or AMR ($p = 0.83$), compared to recipients without any rejection, was determined (Fig. 5).

In the group of lung transplant recipients only 3 patients with ACR (6 samples) were identified and miR-424 levels in those patients didn't differ between 23 recipients without rejection ($p = 0.78$, Fig. 6).

In 3 lung transplant recipients with ACR the concentrations of CRP were: 305 mg/L, 19 mg/L and 8 mg/L. The median of CRP level in the lung transplant recipients without rejection was 6.50 [5.00; 56.50] mg/L, and did not differ from that in lung recipients with ACR ($p = 0.29$). Thus, the acute rejection after HTx or LTx did not associate with miR-424 or CRP levels.

3.5. MiR-424 levels in recipients with bacteremia

According to the results of bacteriological test of blood cultures there

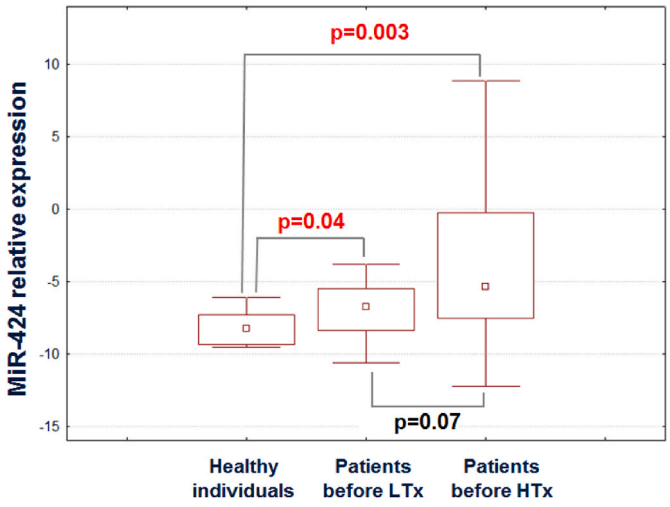

Fig. 4. A comparative analysis of the miR-424 expression level between healthy individuals and patients before LTx or HTx.

Table 3
Median and interquartile range of miR-424 in male and female patients before heart or lung transplantation.

Gender / Group	Male	Female	p-value
HTx	−5.39 [-7.03; 1.36]	−6.26 [-9.40; −4.42]	0.14
LTx	−8.32 [-9.49; 6.31]	−6.61 [-7.59; −3.92]	0.09

The Spearman test showed no significant correlations of miR-424 with the age of patients neither before heart (r = 0.24; p = 0.26) nor before lung (r = 0.05; p = 0.82) transplantation.

were 20 heart and 17 lung transplant recipients with post-transplant bacteremia. The spectrum of microorganisms and their occurrence in blood cultures of heart and lung transplant recipients were shown in Table 6.

The most of heart and lung transplant recipients with bacteremia had gram-negative bacteria in blood cultures: *Acinetobacter baumannii* (55.0% and 52.9% resp.) and *Klebsiella pneumoniae* (40.0% and 52.9% resp.) in the early post-transplant period. All the recipients with post-transplant bacteremia had either gram-negative strains in blood cultures or a combination of gram-negative and -positive bacteria.

MiR-424 expression levels in heart transplant recipients with bacteremia were significantly higher compared to recipients without any bacterial strains in the blood culture (p = 0.02; Fig. 7).

Similar differences were found in the group of lung transplant recipients (Fig. 8).

MiR-424 expression levels in lung transplant recipients with bacteremia were also significantly higher compared to lung recipients without any bacterial strains in the blood culture (p = 0.04).

In Table 7 a comparison of miR-424 plasma level in subgroups was shown: heart transplant recipients with bacteremia were compared with lung transplant recipients with bacteremia; similarly, heart transplant recipients without bacteremia were compared with lung transplant recipients without bacteremia.

The absence of significant differences between the subgroups allows to combine heart and lung transplant recipients for ROC analysis on a larger number of patients, rather than in the heart and lung transplant group separately.

3.6. Diagnostic value of miR-424 and CRP for gram-negative bacteremia

The plasma levels of miR-424 in the group of 37 heart and lung transplant recipients with gram-negative bacteremia was −5.01 [−6.95; −4.16] which significantly exceeded the median level −7.68 [−8.87; −6.61] in the group of 72 heart and lung transplant recipients without any bacteria in blood culture (p = 0.002). The diagnostic value of miR-424 for gram-negative bacteremia in total in heart and lung recipients was assessed. (Fig. 9).

The AUC of miR-424 differed significantly from 0.5 (AUC = 0.75; p = 0.001).The risk of gram-negative bacteremia in heart and lung transplant recipients with miR-424 level above −5.72 fold change was 3.84 times higher than that of the other recipients: RR = 3.84 ± 0.35 [95% CI 1.94–7.61].

Due to the correlation of circulating miR-424 expression with the level of CRP, inflammation indicator of acute phase, in heart and lung transplant recipients, its diagnostic significance for identifying patients with bacteremia have been analyzed.

The AUC of CRP differed significantly from 0.5 (AUC = 0.78; p = 0.001) (Fig. 10).

Similar to miR-424, the risk of gram-negative bacteremia in thoracic recipients with CRP concentration above 7 mg/L was 2 times higher than that of the other recipients: RR = 2.01 ± 0.34 [95% CI 1.03–3.92].

Despite that the sensitivity of miR-424 was almost the same to CRP

Table 4
Spearman test (r; p) of correlation between miR-424 level and complete blood count, blood biochemistry parameters in heart or lung transplant recipients before transplantation

Parameters	Spearman test in groups (r; p)			
	before HTx		before LTx	
Complete blood count				
Red blood cells (10^{12}/L)	**r = 0.58;**	**p = 0.01**	r = 0.27;	p = 0.35
Hemoglobin (g/L)	**r = 0.50;**	**p = 0.03**	r = 0.002;	p = 0.90
White blood cells (10^9/L)	r = −0.30;	p = 0.19	r = 0.20;	p = 0.48
Platelets (10^9/L)	r = −0.11;	p = 0.67	r = 0.10;	p = 0.71
Neutrophils *(%):*				
Segmented	r = −0.18;	p = 0.68	r = 0.25;	p = 0.66
Band	r = −0.14;	p = 0.66	r = 0.20;	p = 0.80
Lymphocytes (%)	r = −0.02;	p = 0.95	r = −0.23;	p = 0.45
Eosinophils (%)	r = 0.14;	p = 0.67	r = 0.18;	p = 0.58
Basophils (%)	r = −0.30;	p = 0.35	r = 0.15;	p = 0.64
Monocytes (%)	r = −0.22;	p = 0.50	r = 0.42;	p = 0.15
Erythrocyte sedimentation rate (mm/h)	r = −0.33;	p = 0.25	r = −0.71;	p = 0.07
Blood biochemistry				
Total protein (g/L)	r = 0.31;	p = 0.19	r = 0.02;	p = 0.93
Total bilirubin (μmol/L)	r = −0.01;	p = 0.97	r = −0.19;	p = 0.47
Blood urea nitrogen (mmol/L)	r = −0.31;	p = 0.18	r = 0.04;	p = 0.87
Creatinine (μmol/L)	r = −0.10;	p = 0.68	r = 0.15;	p = 0.59
Alanine aminotransferase (U/L)	r = −0.21;	p = 0.38	r = −0.40;	p = 0.11
Aspartate aminotransferase (U/L)	r = 0.11;	p = 0.66	r = −0.19;	p = 0.47
Alcaline phosphatase (U/L)	r = 0.33;	p = 0.42	r = 0.28;	p = 0.31
Cholesterol (mmol/L)	r = −0.33;	p = 0.25	r = −0.10;	p = 0.87
Glucose (mmol/L)	r = 0.06;	p = 0.82	r = 0.001;	p = 0.80
C-reactive protein (mg/L)	r = −0.31;	p = 0.45	r = −0.01;	p = 0.98

Table 5
Spearman test (r; p) of correlation between miR-424 level and complete blood count, blood biochemistry parameters and immunosuppressive drug concentration in heart and lung transplant recipients.

Parameters	Spearman test in groups (r; p)			
	after HTx		after LTx	
Complete blood count				
Red blood cells (10^{12}/L)	r = 0.08;	p = 0.80	r = −0.09;	p = 0.69
Hemoglobin (g/L)	r = −0.03;	p = 0.93	r = −0.002;	p = 0.99
White blood cells (10^9/L)	r = −0.23;	p = 0.47	r = 0.40;	p = 0.06
Platelets (10^9/L)	r = 0.30;	p = 0.35	r = −0.18;	p = 0.43
Neutrophils *(%):*				
Segmented	r = 0.29;	p = 0.39	r = −0.05;	p = 0.06
Band	r = −0.40;	p = 0.22	r = −0.11;	p = 0.66
Lymphocytes (%)	r = 0.48;	p = 0.13	r = 0.44;	p = 0.05
Eosinophils (%)	r = 0.25;	p = 0.46	r = 0.26;	p = 0.26
Basophils (%)	r = 0.19;	p = 0.58	r = −0.04;	p = 0.87
Monocytes (%)	r = 0.28;	p = 0.24	r = −0.31;	p = 0.19
Erythrocyte sedimentation rate (mm/h)	r = 0.48;	p = 0.13	r = −0.24;	p = 0.45
Blood biochemistry				
Total protein (g/L)	r = 0.11;	p = 0.73	r = −0.002;	p = 0.99
Total bilirubin (μmol/L)	r = −0.38;	p = 0.22	r = 0.28;	p = 0.20
Blood urea nitrogen (mmol/L)	r = −0.43;	p = 0.16	r = −0.01;	p = 0.96
Creatinine (μmol/L)	r = −0.34;	p = 0.29	r = −0.09;	p = 0.65
Alanine aminotransferase (U/L)	r = −0.13;	p = 0.68	r = −0.37;	p = 0.08
Aspartate aminotransferase (U/L)	r = −0.37;	p = 0.23	r = 0.03;	p = 0.89
Alcaline phosphatase (U/L)	r = −0.32;	p = 0.68	r = 0.31;	p = 0.18
Cholesterol (mmol/L)	r = 0.50;	p = 0.67	r = 0.31;	p = 0.46
Glucose (mmol/L)	r = 0.29;	p = 0.53	r = −0.19;	p = 0.50
C-reactive protein (mg/L)	**r = 0.75;**	**p = 0.02**	**r = 0.50;**	**p = 0.04**
Immunosuppressive drug concentration				
Tacrolimus (ng/ml)	**r = 0.38;**	**p = 0.04**	r = −0.26;	p = 0.23

(60.0% vs 61.9%), miR-424 specificity was 20% higher than that of CRP (89.2% vs 69.2%). In Table 8 the diagnostic characteristics of miR-424, CRP and their combination are shown.

A duplex test, including measurement of miR-424 expression and CRP levels, had the best diagnostic characteristics with respect to identifying heart or lung transplant recipients with development of post-transplant gram-negative bacteremia (RR = 9.17 ± 0.97 [95% CI 1.37–61.46]). The sensitivity and specificity of the duplex test was 83.3% and 90.1%, respectively.

4. Discussion

The outcomes of heart and lung transplantation depend on a wide range of factors. The post-transplant complications significantly reduce the graft function and recipient's survival [24]. Carefully selected and controlled immunosuppression, regular monitoring and timely diagnosis of complications made it possible to achieve long-term survival of recipients maintaining their high life quality.

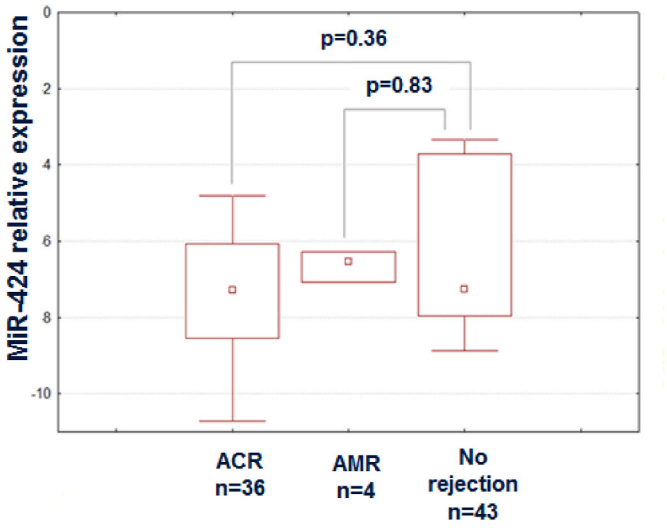

Fig. 5. MiR-424 expression levels in heart transplant recipients with acute cellular rejection (ACR) and antibody-mediated rejection (AMR) compared to recipients without any rejection.
Median of CRP level in heart transplant recipients with ACR and AMR were 6.50 [5.25; 10.00] mg/L and 7.50 [5.50; 18.75] mg/L, resp. The level of CRP in the recipients without rejection was 6.00 [5.00; 13.00] mg/L and did not significantly differ from recipients with ACR and AMR (p = 0.85 and p = 0.92, respectively).

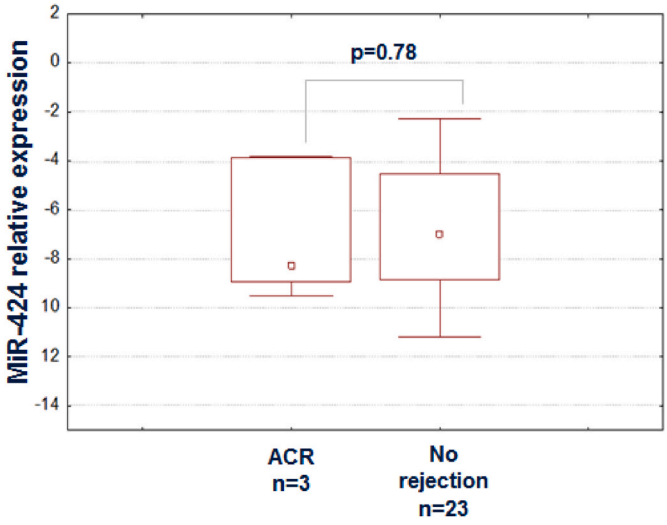

Fig. 6. MiR-424 expression levels in lung transplant recipients with acute cellular rejection (ACR) compared to recipients without any rejection.

A number of studies have shown the regulatory function of signaling molecule miR-424 in many cellular processes, including differentiation of monocytes and macrophages, and its possibility of application as a therapeutic target in the future [25–27]. In this study miR-424 expression levels were evaluated in patients before and after HTx and LTx.

MiR-424 is a member of the miR-16 family. Recent studies have also shown that miR-16 has a particular role in promoting cardiovascular endothelial injury [28]. Marques et al. showed that miR-16–5p might be related to the occurrence of heart failure [29]. R. Malik et al. showed miR-424 properties through its effects at the genetic level and it may be used as a novel next-generation therapeutic option for the prevention and treatment of atherosclerotic coronary artery disease [17]. In our previous studies high expression level of miR-424 along with miR-101, miR-27, miR-339 has been shown in patients with chronic heart failure and chronic obstructive pulmonary disease in comparison with

Table 6
The occurrence of strains in the blood cultures of heart and lung transplant recipients.

Spectrum	*The occurrence of strains in the groups (%)	
	after HTx	after LTx
Gram-negative bacteria		
Acinetobacter baumannii	**11 (55.0%)**	**9 (52.9%)**
Acinetobacter lwoffii	**4 (20.0%)**	**-**
Burkholderia cepacia complex	–	1 (5.9%)
Cedecea davisae	–	1 (5.9%)
Escherichia coli	–	1 (5.9%)
Klebsiella pneumoniae	**8 (40.0%)**	**9 (52.9%)**
Pseudomonas aeruginosa	1 (5.0%)	1 (5.9%)
Ralstonia paucula	–	2 (11.8%)
Ralstonia pickettii	–	1 (5.9%)
Serratia marcescens	–	1 (5.9%)
Serratia rubidaea	–	1 (5.9%)
Gram-positive bacteria		
Enterococcus faecalis	–	1 (5.9%)
Enterococcus faecium	1 (5.0%)	–
Staphylococcus epidermidis	3 (15.0%)	–
Staphylococcus haemolyticus	1 (5.0%)	–
Staphylococcus simulans	–	1 (5.9%)
Streptococcus oralis	1 (5.0%)	1 (5.9%)
Streptococcus mitis	1 (5.0%)	1 (5.9%)
Fungus		
Candida albicans	–	2 (11.8%)

** – % of the total number of heart or lung recipients with gram-negative bacteremia*

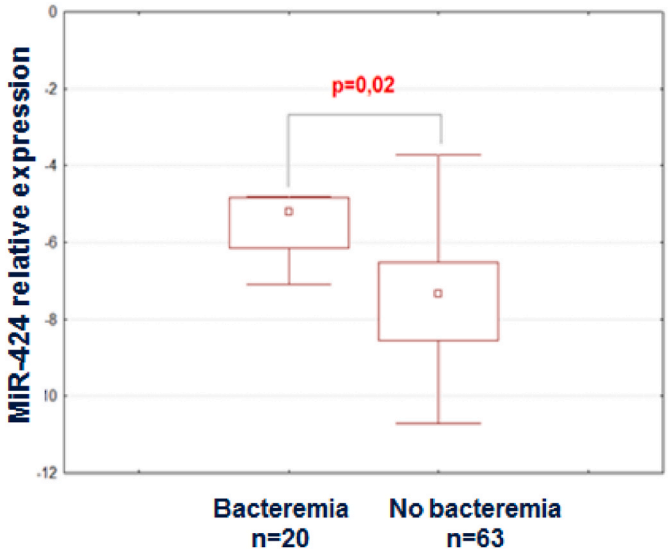

Fig. 7. The expression level of miR-424 higher in heart transplant recipients with bacteremia compared to heart recipients without it.

healthy individuals [30,31]. This study confirms the previously obtained results on miR-424 upregulation in potential heart and lung recipients.

The expression levels of miR-424 did not correlate with the age and gender in patients before and after HTx and LTx. There was no correlation between the level of circulating miR-424 and the most general laboratory and biochemical blood parameters of heart and lung transplant recipients, which may indicate the independence and relatively high specificity of this miR-424.

Moreover, the correlation between the level of miR-424 and the red blood cells and hemoglobin levels we found in patients with chronic heart failure can confirm the assumption of miR-424 participation in hypoxia progression, vascular remodeling and neoangiogenesis [32]. Probably, the expansion of the group of lung transplant recipients, it will be possible to observe any correlation of miR-424 levels with routine blood parameters.

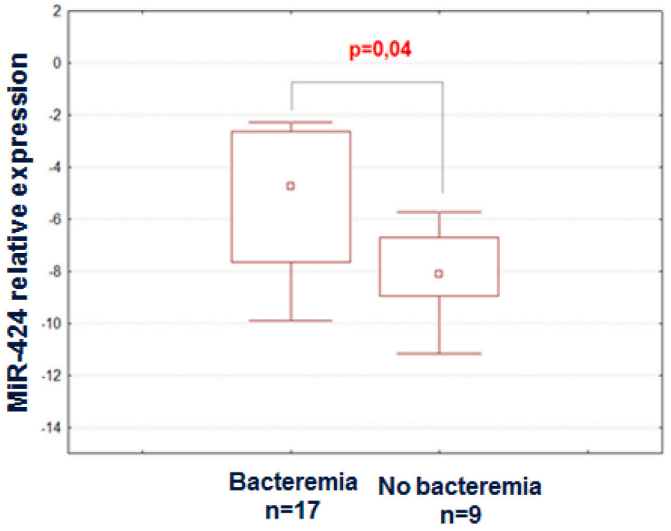

Fig. 8. The expression level of miR-424 higher in lung transplant recipients with bacteremia compared to lung recipients without it.

Table 7
The expression levels of miR-424 in heart and lung recipients with bacteremia compared to recipients without it; data are presented as median and interquartile range.

Groups	MiR-424 level		p-value
	after HTx	after LTx	
No bacteremia	−7.34 [-8.44; −6.57]	−8.10 [-8.92; −6.79]	0.47
Bacteremia	−5.22 [-5.95; −4.89]	−4.74 [-7.48; −2.93]	0.66

MiR-424 plasma levels didn't differ between heart transplant recipients and lung transplant recipients with bacteremia ($p = 0.66$) and between groups of recipients after HTx/LTx without bacteremia ($p = 0.47$).

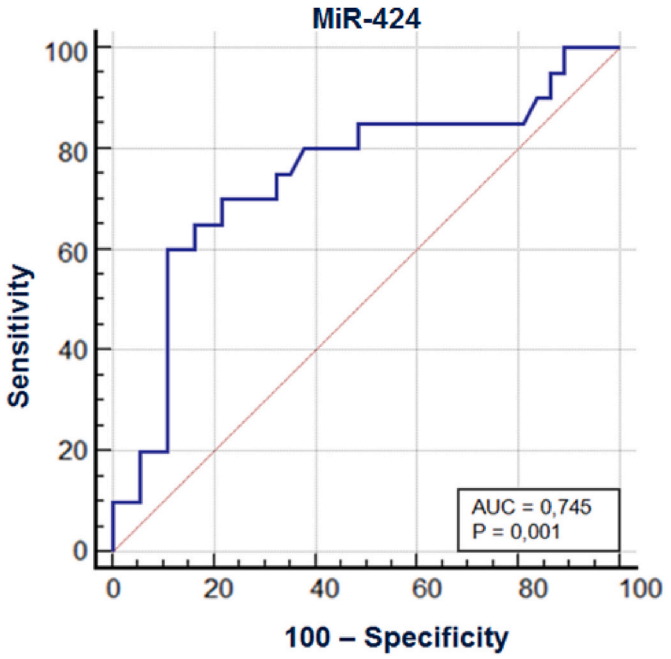

Fig. 9. The ROC curve of miR-424 expression in heart and lung transplant recipients with post-transplant gram-negative bacteremia. Receiver operator characteristic curve of risk score for bacteremia after HTx and LTx. Note: AUC ≥0.5 is considered diagnostically significant.

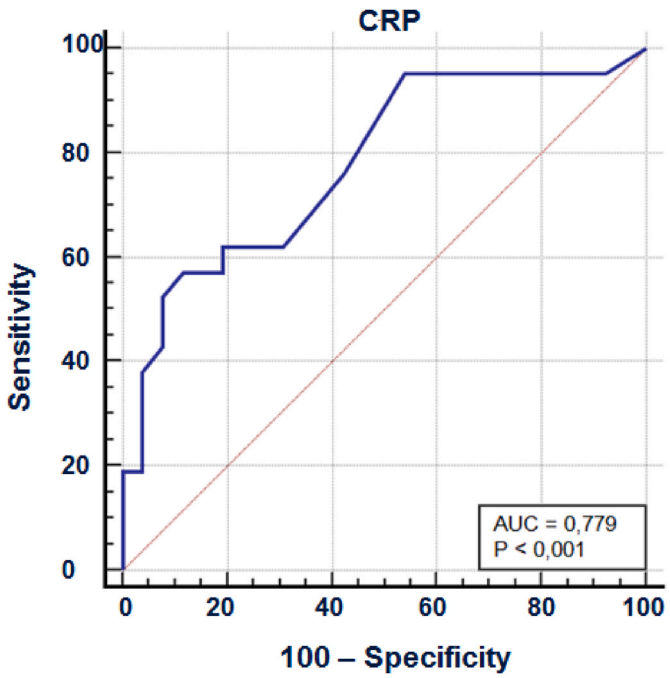

Fig. 10. The ROC curve of CRP concentration in heart and lung transplant recipients with post-transplant gram-negative bacteremia. Receiver operator characteristic curve of risk score for bacteremia after HTx and LTx. Note: AUC ≥0.5 is considered diagnostically significant.

Table 8
Performance of different classifiers.

Classifier	RR	Se	Sp	De	PPV	NPV
miR-424	3.84	60.0%	89.2%	78.9%	75.0%	80.5%
CRP	2.01	61.9%	69.2%	66.6%	61.9%	62.2%
miR-424 + CRP	**9.17**	**83.3%**	**90.1%**	**88.2%**	**83.3%**	**90.9%**

RR – relative risk; Se – sensitivity; Sp – specificity; De – diagnostic efficiency; PPV – positive predictive value; NPV – negative predictive value

According to the S. Schmeier et al., this correlation may be mediated by the gene of transcription factor NFE2L1 (nuclear factor, erythroid 2 like 1) acting as a potential target for hsa-miR-424–5p, which controls the production of globin protein in erythrocytes [33].

Previous studies have shown participation of miRNAs in many physiological and immune functions including the timing of cell death and proliferation, homeostasis, apoptosis and angiogenesis [34,35]. In this work a direct correlation between the miR-424 plasma level and the concentration of CRP in heart and lung recipients was shown. CRP is a classic acute-phase protein, which is widely used in clinical practice as one of the most sensitive laboratory markers of infection, inflammation and tissue damage [30,36]. Both markers indicate the acute phase of inflammation and the correlation between miR-424 and CRP levels either after HTx or LTx may be related to miR-424 involvement in the regulation of CRP production [37].

The correlation between miR-424 plasma level and the calcineurin inhibitor (tacrolimus) blood concentration in heart transplant recipients was revealed. A relationship between miR-424 and enzymes of the cytochrome P450 family, which are responsible for the metabolism of drugs, including immunosuppressants, is shown. S.U. Umu et al. showed due to the suppression of the immune response of lymphocytes mediated through the CDK6-dependent pathway miR-424 has an immunosuppressive potential for the immune relationship of the recipient and the transplant [38].

In this study, the miR-424 level did not differ in recipients with and without acute graft rejection. However I. Sukma Dewi et al. showed that the expression level of miR-424 in heart transplant recipients with acute cellular rejection is higher than in recipients without rejection [32]. The difference in study results may be due to various factors, including study design, immunosuppressive therapy protocols, period after transplantation, ISHLT biopsy grade, gender and age of patients, and others. For more accurate test validation, multicenter studies on more patients are needed.

The expression levels of miR-424 in heart or lung transplant recipients with post-transplant gram-negative bacteremia were higher compared to recipients without it. According to the A. Sahni results, the mechanism of miR-424 action can be associated with the mechanisms of immune response and CX3CL1 expression in infection caused by the gram-negative pathogen *Rickettsia rickettsii* [39], as well as the change of miR-424 expression was specific for recipients with invasive aspergillosis after lung transplantation [40]. D. Cheng et al. described the protective mechanisms of the action of miR-424 on epithelial cells in acute respiratory distress syndrome, which could be the result of acute inflammation processes [41].

The results show the significant high expression of miR-424 level in heart and lung transplant recipients with gram-negative bacteremia. The established differences in miR-424 expression levels in thoracic recipients with and without gram-negative bacteremia made it possible to identify the threshold value and evaluate the diagnostic effectiveness of the studied microRNA. The diagnostic value of miR-424 was evaluated with ROC-analysis.

In study miR-424 showed the highest diagnostic efficacy, unlike CRP, which may be due to the wide specificity of CRP for any acute phase of inflammation. However, the diagnostic characteristics of miR-424 and CRP were significantly increased in duplex test in an unexpected way. This combination can improve the detection rate of bacteremia in heart and lung transplant recipients.

Limitations

However, the number of individuals enrolled in this study is relatively small; therefore, our findings need to be replicated in a larger cohort of patients. This can provide a more complete understanding of the miR-424 role and functions in the pathogenesis of graft damage.

5. Conclusions

MiR-424 plasma expression was upregulated in patients with chronic heart and respiratory failure and in heart and lung transplant recipients in the early post-transplant period.

MiR-424 plasma levels did not differ in recipients with and without acute graft rejection, but was significantly higher in thoracic recipients with gram-negative bacteremia. The duplex test of miR-424 and CRP has a high diagnostic value for detecting the high risk of post-transplant gram-negative bacteremia in heart and lung transplant recipients, that shows the high potential role of studied microRNA in modern transplantology and healthcare technologies.

CRediT authorship contribution statement

Olga Shevchenko: Term, Conceptualization, Methodology, Visualization. **Olga Tsirulnikova:** Resources, Visualization. **Sofya Sharapchenko:** Formal analysis, Writing – original draft, Investigation. **Olga Gichkun:** Writing – review & editing, Investigation. **Dmitriy Velikiy:** Writing – review & editing. **Nina Gabrielyan:** Resources. **Ivan Pashkov:** Resources. **Alex Shevchenko:** Resources. **Sergey Gautier:** Resources, Project administration.

Declaration of competing of interest

The authors have no conflicts of interest to disclose.

References

[1] S. Bos, R. Vos, D.E. Van Raemdonck, et al., Survival in adult lung transplantation: where are we in 2020 Lung transplantation, Curr. Opin. Organ Transplant. 25 (3) (2020) 268.

[2] E.P. Kransdorf, J.A. Kobashigawa, Novel molecular approaches to the detection of heart transplant rejection, Per Med 14 (4) (2017) 293–297.

[3] S.V. Gautier, A.O. Shevchenko, A.Ya Kormer, et al., Three decades of heart transplantation in the Shumakov center: long-term outcomes, Russian J. Transplantol. Artificial Organs 17 (2) (2015) 70–73, https://doi.org/10.15825/1995-1191-2015-2-70-73 (In Russian).

[4] L. Potena, A. Zuckermann, F. Barberini, et al., Complications of cardiac transplantation, Curr. Cardiol. Rep. 20 (9) (2018) 1073.

[5] P. Biderman, Y. Bugaevsky, H. Ben-Zvi, et al., Multidrug-resistant Acinetobacter baumannii infections in lung transplant patients in the cardiothoracic intensive care unit, Clin. Transplant. 29 (9) (2015) 756–762, https://doi.org/10.1111/ctr.12575.

[6] M. Alsaeed, S. Husain, Infections in heart and lung transplant recipients, Crit. Care Clin. 35 (1) (2019) 75–93.

[7] E. Andrikopoulou, P.J. Mather, Current insights: use of Immuknow in heart transplant recipients, Progress in transplantation (Aliso Viejo, Calif.) 24 (1) (2014) 44–50, https://doi.org/10.7182/pit2014664.

[8] P.E. North, E. Ziegler, D.K. Mahnke, et al., Cell-free DNA donor fraction analysis in pediatric and adult heart transplant patients by multiplexed allele-specific quantitative PCR: validation of a rapid and highly sensitive clinical test for stratification of rejection probability, PLoS One 15 (1) (2020), https://doi.org/10.1371/journal.pone.0227385 e0227385.

[9] A. Sen, H. Callisen, S. Libricz, et al., Complications of solid organ transplantation: cardiovascular, neurologic, renal, and gastrointestinal, Crit. Care Clin. 35 (1) (2019) 169–186, https://doi.org/10.1016/j.ccc.2018.08.011.

[10] T. Nováková, T. Macháčková, J. Novák, et al., Identification of a diagnostic set of endomyocardial biopsy microRNAs for acute cellular rejection diagnostics in patients after heart transplantation using next-generation sequencing, Cells 8 (11) (2019) 1400–1406, https://doi.org/10.3390/cells8111400.

[11] M.G. Crespo-Leiro, G. Barge-Caballero, D. Couto-Mallon, Noninvasive monitoring of acute and chronic rejection in heart transplantation, Curr. Opin. Cardiol. 32 (3) (2017) 308–315, https://doi.org/10.1097/HCO.0000000000000400.

[12] S.H. Tsai, P.H. Huang, H.Y. Tsai, et al., Roles of the hypoximir microRNA-424/322 in acute hypoxia and hypoxia-induced pulmonary vascular leakage, Faseb. J. 33 (11) (2019) 12565–12575, https://doi.org/10.1096/fj.201900564RR.

[13] K. Saliminejad, H.R. Khorram Khorshid, S. Soleymani Fard, et al., An overview of microRNAs: biology, functions, therapeutics, and analysis methods, J. Cell. Physiol. 234 (5) (2019) 5451–5465, https://doi.org/10.1002/jcp.27486.

[14] P. Shah, M.R. Bristow, J.D. Port, MicroRNAs in heart failure, cardiac transplantation, and myocardial recovery: biomarkers with therapeutic potential, Curr. Heart Fail. Rep. 14 (6) (2017) 454–464, https://doi.org/10.1007/s11897-017-0362-8.

[15] S. Kalayinia, F. Arjmand, M. Maleki, et al., MicroRNAs: roles in cardiovascular development and disease, Cardiovasc. Pathol. 50 (2021) 107296, https://doi.org/10.1016/j.carpath.2020.107296.

[16] R. Baptista, C. Marques, S. Catarino, F.J. Enguita, M.C. Costa, P. Matafome, M. Zuzarte, G. Castro, A. Reis, P. Monteiro, M. Pêgo, P. Pereira, H. Girão, MicroRNA-424(322) as a new marker of disease progression in pulmonary arterial hypertension and its role in right ventricular hypertrophy by targeting SMURF1, Cardiovasc. Res. 114 (1) (2018 Jan 1) 53–64.

[17] R. Malik, R.S. Mushtaque, U.A. Siddiqui, et al., Association between coronary artery disease and MicroRNA: literature Review and clinical perspective, Cureus 9 (4) (2017), https://doi.org/10.7759/cureus.1188 e1188.

[18] G. Li, Q. Ma, R. Wang, Z. Fan, Z. Tao, P. Liu, H. Zhao, Y. Luo, Diagnostic, Immunosuppressive Potential, Of elevated mir-424 levels in circulating immune cells of ischemic stroke patients, Aging Dis. 9 (2) (2018 Apr 1) 172–181.

[19] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative, PCR and the 2(-Delta Delta C(T)) Method. Methods 25(4) (2001) 402–408, https://doi.org/10.1006/meth.2001.1262.

[20] The Russian Transplant Society, Clinical guidelines: lung transplant and heart-lung transplant. URL. http://transpl.ru/files/rto/transpl_legkih.pdf.

[21] M. Billingham, J.A. Kobashigawa, The revised ISHLT heart biopsy grading scale, J. Heart Lung Transplant. 24 (11) (2005) 1709, https://doi.org/10.1016/j.healun.2005.03.018.

[22] G.J. Berry, M.M. Burke, C. Andersen, et al., The 2013 International Society for Heart and Lung Transplantation working formulation for the standardization of nomenclature in the pathologic diagnosis of antibody-mediated rejection in heart transplantation,, J. Heart Lung Transplant. 32 (12) (2013) 1147–1162, https://doi.org/10.1016/j.healun.2013.08.011.

[23] G. Hughes, Youden's index and the weight of evidence revisited, Methods Inf Med 54(6) (2015) 576–577, https://doi.org/10.3414/ME15-04-0007.

[24] V.P. Nguyen, J.A. Kobashigawa, Antibody-medicated rejection after heart transplantation: diagnosis and clinical implications, Curr. Opin. Organ Transplant. 25 (3) (2020) 248–254, https://doi.org/10.1097/MOT.0000000000000754.

[25] N.J. Sarma, V. Tiriveedhi, S. Ramachandran, et al., Modulation of immune responses following solid organ transplantation by microRNA, Exp. Mol. Pathol. 93 (3) (2012) 378–385, https://doi.org/10.1016/j.yexmp.2012.09.020.

[26] A. Tamara, D.J. Coulson, J.S. Latief, et al., Upregulated anti-angiogenic miR-424-5p in type 1 diabetes (model of subclinical cardiovascular disease) correlates with endothelial progenitor cells, CXCR1/2 and other parameters of vascular health, Stem Cell Res. Ther. 12 (249) (2021), https://doi.org/10.1186/s13287-021-02332-7.

[27] M. Hamdorf, S. Kawakita, M. Everly, The potential of MicroRNAs as novel biomarkers for transplant rejection,, J. Immunol. Res. 2017 (2017) 10–12, https://doi.org/10.1155/2017/4072364.

[28] C.Y. Sun, X.M. She, Y. Qin, et al., MiR-15a and miR-16 affect the angiogenesis of multiple myeloma by targeting VEGF, Carcinogenesis 34 (2) (2013) 426–435, https://doi.org/10.1093/carcin/bgs333.

[29] F.Z. Marques, D. Vizi, O. Khammy, et al., The transcardiac gradient of cardio-microRNAs in the failing heart,, Eur. J. Heart Fail. 18 (8) (2016) 1000–1008, https://doi.org/10.1002/ejhf.517.

[30] O.P. Shevchenko, S.O. Sharapchenko, O.M. Tsirulnikova, et al., MicroRNA expression levels in lung recipients: correlations with clinical and laboratory data, Russian J. Transplantol. Artificial Organs 22 (2) (2020) 86–96, https://doi.org/10.15825/1995-1191-2015-2-70-73 (In Russian).

[31] D.A. Velikiy, O.E. Gichkun, S.O. Sharapchenko, O.P. Shevchenko, A. O. Shevchenko, MicroRNA expression levels in early and long-term period following heart transplantation, Russian J. Transplantol. Artificial Organs 22 (1) (2020) 26–34.

[32] I. Sukma Dewi, Z. Hollander, K.K. Lam, et al., Association of serum MiR-142-3p and MiR-101-3p levels with acute cellular rejection after heart transplantation, PLoS One 12 (1) (2017), https://doi.org/10.1371/journal.pone.0170842 e0170842.

[33] S. Schmeier, C.R. MacPherson, M. Essack, et al., Deciphering the transcriptional circuitry of microRNA genes expressed during human monocytic differentiation, BMC Genom. 10 (2009) 595, https://doi.org/10.1186/1471-2164-10-595.

[34] N. Dastmalchi, R. Safaralizadeh, M.A. Hosseinpourfeizi, et al., MicroRNA-424-5p enhances chemosensitivity of breast cancer cells to Taxol and regulates cell cycle, apoptosis, and proliferation, Mol. Biol. Rep. 48 (2) (2021) 1345–1357, https://doi.org/10.1007/s11033-021-06193-4.

[35] M.S. Ali Sheikh, A. Alduraywish, A. Almaeen, et al., Therapeutic value of miRNAs in coronary artery disease, Oxid. Med. Cell. Longev. (2021) 8853748, https://doi.org/10.1155/2021/8853748.

[36] J. Yu, X. Shi, J. Ma, et al., C-reactive protein is an independent predictor of 30-day bacterial infection post-liver transplantation, Biomolecules 11 (8) (2021) 1195, https://doi.org/10.3390/biom11081195.

[37] C. Leng, J. Sun, K. Xin, et al., High expression of miR-483-5p aggravates sepsis-induced acute lung injury, J. Toxicol. Sci. 45 (2) (2020) 77–86, https://doi.org/10.2131/jts.45.77.

[38] S.U. Umu, H. Langseth, C. Bucher-Johannessen, et al., A comprehensive profile of circulating RNAs in human serum,, RNA Biol. 15 (2) (2018) 242–250, https://doi.org/10.1080/15476286.2017.1403003.

[39] A. Sahni, H.P. Narra, S.K. Sahni, MicroRNA-424 regulates the expression of CX3CL1 (fractalkine) in human microvascular endothelial cells during Rickettsia rickettsii infection, Biochem. Biophys. Rep. 25 (2021) 100897, https://doi.org/10.1016/j.bbrep.2020.100897.

[40] W. Gohir, W. Klement, L.G. Singer, et al., Identifying host microRNAs in bronchoalveolar lavage samples from lung transplant recipients infected with Aspergillus, J. Heart Lung Transplant. (2020), https://doi.org/10.1016/j.healun.2020.07.014.

[41] D. Cheng, C. Zhu, Y. Liang, et al., MiR-424 overexpression protects alveolar epithelial cells from LPS-induced apoptosis and inflammation by targeting FGF2 via the NF-κB pathway, Life Sci. 1 (242) (2020) 117213, https://doi.org/10.1016/j.lfs.2019.117213.