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

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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. 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.). 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).

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 relative risk of bacteremia is RR = 8.58 [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.

# A R T I C L E I N F O

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# A B S T R A C T

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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.
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 monocye 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. MSY0000001) 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 Primer Master Mix, 2 μl 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 miR-Neasy 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 (ΔCt = Ct_miR424–CtCelmir39) and are expressed as 2^ΔCtlog10 [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 = 0.56; 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.
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).

3.5. MiR-424 levels in recipients with bacteremia

According to the results of bacteriological test of blood cultures there

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Table 1
Characteristics of heart transplant recipients.

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

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Table 2
Characteristics of lung transplant recipients.

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

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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.

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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.
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 6.61 was 8.87; 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
the graft function and recipient range of factors. The post-transplant complications significantly reduce
3.83% and 90.1%, respectively.

1.37 – CRP levels, had the best diagnostic characteristics with respect to
CRP and their combination are shown.

(89.2% vs 69.2%). In Table 8 the diagnostic characteristics of miR-424,

miR-424 specificity was 20% higher than that of CRP
(60.0% vs 61.9%), miR-424 – 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

shown that miR-16 has a particular role in promoting cardiovascular
endothelial injury [28]. Marques et al. showed that miR-16

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miR-424 expression levels were evaluated in patients before and after HTx and LTx.

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 expression levels were evaluated in patients before and after HTx and LTx.

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             |          |          |
| Hb (g/L)                            | r = –0.03; p = 0.99            |          |          |
| White blood cells (10^9/L)          | r = –0.23; p = 0.47            |          |          |
| Platelets (10^9/L)                  | r = 0.30; p = 0.35             |          |          |
| Neutrophils (%) Segmented           | r = 0.29; p = 0.39             |          |          |
| Band                                | r = –0.40; p = 0.22            |          |          |
| Lymphocytes (%)                     | r = 0.48; p = 0.13             |          |          |
| Eosinophils (%)                     | r = 0.25; p = 0.46             |          |          |
| Basophils (%)                       | r = 0.19; p = 0.58             |          |          |
| Monocytes (%)                       | r = 0.28; p = 0.24             |          |          |
| Erythrocyte sedimentation rate (mm/h)| r = 0.48; p = 0.13             |          |          |
| **Blood biochemistry**              |                                |          |          |
| Total protein (g/L)                 | r = 0.11; p = 0.73             |          |          |
| Total bilirubin (μmol/L)            | r = –0.38; p = 0.22            |          |          |
| Blood urea nitrogen (mmol/L)        | r = –0.43; p = 0.16            |          |          |
| Creatinine (μmol/L)                 | r = –0.34; p = 0.29            |          |          |
| Alanine aminotransferase (U/L)      | r = –0.13; p = 0.68            |          |          |
| Aspartate aminotransferase (U/L)    | r = –0.37; p = 0.23            |          |          |
| Alkaline phosphatase (U/L)          | r = –0.32; p = 0.68            |          |          |
| Cholesterol (mmol/L)                | r = 0.50; p = 0.67             |          |          |
| Glucose (mmol/L)                    | r = 0.29; p = 0.53             |          |          |
| C-reactive protein (mg/L)           | r = 0.75; p = 0.02             |          |          |
| **Immunosuppressive drug concentration** | r = 0.38; p = 0.04 |          |          |
| Tacrolimus (ng/ml)                  | r = 0.38; p = 0.04             |          |          |

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 diag-
nosis of complications made it possible to achieve long-term survival of
recipients maintaining their high life quality.
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.

### 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%)|
| Cellecta dantae                 |                                             | 1 (5.9%)|
| Esherichia coli                 |                                             | 1 (5.9%)|
| Klebsiella pneumoniae           | 8 (40.0%)                                 | 9 (52.9%)|
| Pseudomonas aeruginosa          | 1 (5.0%)                                  | 1 (5.9%)|
| Balstonia paucula               |                                             | 2 (11.8%)|
| Ralstonia picketti              |                                             | 1 (5.9%)|
| Serratia marcescens             |                                             | 1 (5.9%)|
| Serratia rubidae                |                                             | 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                          |                                           | 2 (11.8%)|

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

### 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 after HTx | MiR-424 level after LTx | p-value |
|-------------------------|-------------------------|-------------------------|---------|
| No bacteremia           | –8.24 [-8.44; -6.57]    | –8.10 [-8.92; -6.79]    | 0.47    |
| Bacteremia              | –7.34 [-8.44; -6.57]    | –8.10 [-8.92; -6.79]    | 0.47    |

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. 7

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

### Fig. 8

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

### 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.
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 immunologic 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 transplantation 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. Dmitriy Velikiy: Writing – review & editing. Nina Gabrielyan: Resources. Ivan Pashkov: Resources. Alex Shevchenko: Resources. Sergey Gautier: Resources, Project administration.

**Declaration of competing interest**

The authors have no conflicts of interest to disclose.
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