BRITISH JOURNAL OF BIOMEDICAL SCIENCE, 2017
VOL. 74, NO. 4, 187–192
https://doi.org/10.1080/09674845.2017.1333265

Role of miR-29 as marker of risk of acute rejection after heart transplant

S Guo[a], X Guo[b], S Wang[b], Q Nie[c], G Ni[d] and C Wang[e]

[a]Department of Clinical Medicine, Harbin Medical University, Harbin, China; [b]Departmentment of Anesthesia, Guangdong Cardiovascular Institute, Guangdong Provincial Key Laboratory of Coronary Heart Disease Prevention, Guangdong General Hospital, Guangdong Academy of Medical Science, Guangzhou, China; [c]State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China; [d]Department of Heart Transplantation, The Affiliated Hospital of Xiamen University, Xiamen, China; [e]Department of Heart Transplantation, Wuhan Union Hospital, Wuhan, China

ABSTRACT

Background: Circulating miRNAs are potential biomarkers of the pathogenesis of certain diseases and in monitoring therapeutic responses. We hypothesized that serum miR-29 can determine risk of acute cardiac allograft rejection.

Methods: Peripheral vein blood was collected from 50 healthy volunteers and 506 patients during post-transplant surveillance. Serum cardiac troponin I (cTnI) and miR-29 was detected by ELISA and qRT-PCR assay respectively. Rejection risk was defined as International Society of Heart and Lung Transplant score from leukocyte infiltration of an endomyocardial biopsy. No evidence of rejection was defined as grade R0, mild as R1, moderate as 2R and severe as 3R. Specificity and sensitivity of miR-29 to discriminate rejection was determined by the area under the curve (AUC) of receiver operating characteristic curve analysis. Correlations between miR29 and rejection grade were compared.

Results: Serum miR-29 was 100.8 ± 42.4 copies/μl in R0 groups (P = 0.164 versus controls), 537.5 ± 84.3 copies/μl in R1 groups (P = 0.024) and 1478.4 ± 198.7 copies/μl in the joint R2/R3 groups (P = 0.001). MiR-29 was 1963.5 ± 214.7 six months after transplantation, 1242.5 ± 103.8 after a year, 825.6 ± 58.2 after 2 years, 413.8 ± 61.9 after 3 years and 270.6 ± 34.6 ng/mL after 4 years (P < 0.001). The level of miR-29 correlated positively with cTnI, NT-proBNP, white blood cell counts, and negatively with lymphocyte counts (all P < 0.001). The AUC values (95% CI) for discriminating R0 and R1 was 0.81 (0.75–0.89), and was 0.79 (0.72–0.86) for R0 and R2/R3 (both P < 0.01).

Conclusion: miR-29 is a promising predictor of the risk of heart transplant rejection.

ARTICLE HISTORY
Received 11 April 2017
Accepted 27 April 2017

KEYWORDS
Allograft rejection; biomarker; miR-29

Introduction

Despite best medical care, up to 30% of heart transplant recipients experience a rejection episode during the first year [1]. Currently, international guidelines recommend that periodic endomyocardial biopsy is the standard method for the surveillance of the risk of acute allograft rejection during the first 6 to 12 months after transplantation, with the potential for annual assessments [2]. This calls for up to 16 repeated endomyocardial biopsies, a process which is clinically dangerous and psychologically troubling. Although the endomyocardial biopsy remains the gold standard for determining the risk of acute rejection surveillance, this invasive technique also suffers from inter-observer variability, high cost, potential clinical complications, and significant patient discomfort [3,4].

Accordingly, there has been considerable effort in improving the ability to predict acute allograft rejection noninvasively using various biomarkers. Repeated measurement of N-terminal pro-B-type natriuretic peptide (NT-proBNP) or C-reactive protein (CRP) has been reported to be ineffective in predicting acute cellular rejection [5], although high-sensitivity cardiac troponin I (cTnI) showed promising results with a good correlation with acute cellular rejection [6,7]. However, significant overlap of cTnI between groups with or without acute allograft rejection, with poor sensitivity and specificity, in addition to conflicting data of cardiac troponin T (TnT), prevents clinicians from adopting these biomarkers as a full substitute for endomyocardial biopsy [7].

MicroRNAs (miRNAs) are non-coding RNAs of 19–25 nucleotides that recognize complementary MRNAs and inhibit their translation into functional protein via negatively regulating gene expression [8]. It has recently demonstrated that the circulating miRNAs are stable to RNase digestion, repeated freeze-thawing and other

CONTACT X Guo gxgggzyy@126.com

harsh conditions [9]. Under physiological or pathological condition, miRNAs are actively secreted or passively released into blood [10]. As potential biomarkers, miRNAs may be used in the evaluation of several cardiovascular diseases [11–14].

miR-29 is a novel marker associated with metabolic and cardiovascular diseases [15], and is associated with the severity of cardiomyocytes injury [16]. We hypothesized that miR-29 is a non-invasive marker for predicting the risk of acute cardiac allograft rejection.

Subjects and methods

All patients who underwent a heart transplant from October 2008 to August 2015 were included. From 506 patients (108 cases from Beijing fuwai hospital, 42 cases from the affiliated hospital of Xiamen University,183 cases from the Wuhan Union hospital and 173 cases from Guangdong General Hospital in the same period), 3544 endomyocardial biopsies were obtained either for clinically suspected rejection, or as a diagnostic tool for cases with allograft dysfunction or as routine surveillance protocol biopsies. 50 healthy subjects were included as a control group. All the patients with an informed consent form agreed to supply their blood samples collected at the time of endomyocardial biopsy for the research. A complete blood screening was performed before all procedures. A total of 1824 stored blood samples of 506 transplant cases were analysed. The study protocol was approved by the Institutional Review Board of our institute. The patients were treated with antiviral prophylaxis and immunosuppression in a standardized post-transplant therapy. Maintenance immunosuppression was tacrolimus-based for all the patients. Information on drug treatments and dosing is described elsewhere [17]. Endomyocardial biopsies were graded according to the International Society for Heart and Lung Transplantation (ISHLT) 2004 revised grading scale (0, 1R, 2R, 3R) [18]. No evidence of cellular rejection (leukocyte infiltration, standard H&E staining) was defined as grade R0, mild as R1, moderate as 2R and severe as 3R. Significant rejection was defined as a rejection grade of ≥2R (2R/3R). In routine clinical practice, rejection scores ≥2R usually lead to a treatment intervention. All transplant recipients were monitored for acute rejection by surveillance endomyocardial biopsy performed at scheduled intervals after transplant, these being at 2,4, 6, 12, 24, 36 and 48 months. Biopsies were performed via a right internal jugular vein approach, and 5 to 7 specimens were acquired from the right ventricle each time.

Laboratory procedures were as follows. Six ml venous blood was obtained from the patients investigated at the time of biopsy. Total RNA was isolated from the blood using the mirVana microRNA Isolation Kit (Life Technology, Grand Island, NY USA). RNA concentrations were read using a Nanodrop 2000 (Thermo Scientific, Shanghai, China). Total RNA was converted into cDNA with the TaqMan MicroRNA Reverse Transcription Kit according to manufacturer's instructions (Life Technology, Shanghai, China). Subsequently, cDNAs were stored at −80 °C.

For the miR-29 studies, a total of 6 ng of total RNA was used to reverse transcribe miR-29 and U6 snRNA control into cDNA following the TaqMan miRNA protocol (Applied Biosystems, Foster City, CA, USA), using hairpin primers directed to miR-29 and U6 snRNA as a control in a thermocycler (GeneAmp PCR System 9700, Applied Biosystems) for 30 min at 16 °C, 30 min at 42 °C, 5 min at 85 °C. Real-time quantitative polymerase chain reaction was then performed using miRNAs specific TaqMan probe assays in a Chromo4 thermal cycler (Bio-Rad Laboratories LTD, Hemel Hempstead, UK). miR-29 specific primer was purchased from Qiagen(Shanghai,China) (forward, AGTGAATGAGGCCTTCGAGA; reverse, GCATCTGAGTCGCCACTGTA). miR-29 expression levels were normalized to U6 snRNA and calculated using the $2^{-\Delta\Delta Ct}$ method [19] using commercially available normal colon RNA as a calibrator.

Heparinised plasma samples were stored at −80 °C after centrifugation until analysed. White blood cell (WBC) counts, lymphocyte counts, serum creatinine, CRP and cTnI were measured by standard routine techniques. Serum NT-proBNP levels were determined using the commercially available Elecsys proBNP sandwich, electrochemiluminescence immunoassay on an Elecsys 2010 Analyzer (Roche Diagnostics, Mannheim, Germany). The results are expressed as pg/ml. The lower detection limit was 5 pg/ml, and intra-assay variation was 2.6%.

Statistical methods were as follows: All data are presented as mean with standard deviation (SD) or median and interquartile range. Statistical comparisons between two groups used student's *t*-test or Mann–Whitney. Correlations were tested by Spearman's method. Comparison of receiver operator characteristic (ROC) curves was done by the method of Ruopp et al. [18]. The area under the ROC curve (AUC) identifies optimal sensitivity and specificity levels for predicting risk of rejection. Sensitivity, specificity, accuracy, and positive and negative predictive values (PPV and NPV) of miR-29 in distinguishing acute rejection from non-acute rejection samples were also calculated along with 95% confidence intervals (95% CI). Analysis was performed using SAS v 9.3 (SAS Institute Inc., Cary, USA). $p < 0.05$ was considered statistically significant.

Results

Subjects' characteristics were as follows: From the Guangdong Cardiovascular Institute, 520 patients had

1570 visits with paired EMB histopathology and rejection grades. Male patients accounted for 76.8% (389/506) and were 51.6 (47.7 ± 12.6) years old; female patients accounted for 23.2% (117/506) and were with 50.8 (46.3 ± 13.2) years old. The controls were 31 men aged 51.9 (13.1 year and 19 females aged 51.6 (12.9) years] age $P = 0.584$, sex $P = 0.763$). Of 3544 biopsies, 41% showed no evidence of cellular rejection (scored grade R0). The mild (grade 1R), moderate (grade 2R) and severe (grade 3R) rejection was seen in 1665 (47%), 218 (6.1%) and 99 (2.8%), respectively. The remainder of 109 (3.1%) contained no myocardial tissue in the harvested biopsy fragments or anatomically or technically not possible. Thus 109 (3.1%) biopsy samples and 14 cases corresponding the samples were excluded from the study. The 3535 biopsy samples and 506 patients were used for further study. No evidence of cellular rejection (scored grade R0) was found in 231/506 (45.6%) of patients. The mild (grade 1R) cellular rejection and moderate to severe acute cellular rejection (grade 2R or 3R) represented 224/506 (44.2%) and 51/506 (10%) of patients, respectively (see Table 1).

One thousand, seven hundred and twenty biopsy samples from 452 patients were harvested at ≥2–6 month, whilst 1815 biopsy samples from 406 patients were harvested at >6 months post-transplantation. Of the 506 patients, no relationship was found between cellular rejection grade and indication for cardiac transplantation, cytomegalovirus serology (IgG) status and the visits occurred prior to time post-transplantation ($P > 0.05$, respectively). In the first 6 months post-transplantation, 452 of 506 patients (89.3%) visited our hospital. Of these, 1240 of 1720 (72%) of biopsies [representing 173/452 (38.2%) of patients] suggested mild (grade 1R) cellular rejection; 260/1720 (15.1%) of biopsies [representing 37/452 (8.18%) of patients] indicated moderate to severe acute cellular rejection. Six months post-transplantation, the mild (grade 1R) cellular rejection was 425/1815 (23.4%) [representing 51/406 (12.5%) of patients]; moderate to severe acute cellular rejection (grade 2R/3R) was 57/1815(3.14%) [representing 14/406 (3.4%) of patients].

Clinical variables associated with miR-29 after heart transplant were as follows. Serum miR-29 was 47.6 ± 28.4 copies/µl in 50 healthy controls. Compared to control levels, miR-29 was 100.8 ± 42.4 copies/µl ($P = 0.164$) in the R0 group, 537.5 ± 84.3 copies/µl ($P = 0.024$) in the R1 group and 1478.4 ± 198.7 copies/µl ($P = 0.001$) in the combined R2/R3 group (Figure 1(A)). miR-29 correlated positively with the level of cTnI ($r = 0.46$, $P < 0.001$) (Figure 1(B)) pub NT-proBNP ($r = 0.64$, $P < 0.001$) as well as the WBC counts ($r = 0.51$, $P < 0.001$) and negatively correlated with lymphocyte counts ($r = -0.46$, $P < 0.001$). Serum Creatinine (Cr) level showed weak association with miR-29 ($r = -0.082$, $P = 0.045$). miR-29 showed no statistical correlation with patients' age and CRP level ($r = 0.046$, $P = 0.627$; $r = 0.163$, $P = 0.194$, respectively).

miR-29 was measured at specific time points after transplantation in accordance with the predefined biopsy protocol. miR-29 levels at each time points were 1963.5 ± 214.73 after 6 months, 1242.5 ± 103.8 after a year, 825.6 ± 58.2 after 2 years, 413.8 ± 61.9 after three years and 270.6 ± 34.6 copies/µl after four years ($P < 0.001$). miR-29 was negatively correlated with time after transplantation ($r = -0.65$, $P < 0.001$) (Figure 1(C)). In multiple linear regression analysis, lymphocyte counts, WBC counts, cTnI and NT-proBNP remained as independent variables associated with miR-29 (all $P < 0.001$) with R^2 value of 0.51 of the model ($P < 0.001$).

The miR-29 levels was an significant predictor of acute cellular rejection (2R/3R) in multivariate models ($r = 0.08$, $P = 0.004$). Overall diagnostic accuracy of serum miR-29 is represented by the AUC of the ROC curve. The mean (95%) AUC values for discriminating between healthy control and R0 was 0.54 (0.43–0.67) with sensitivities of 32.1% and specificities of 83.4% (Figure 2(A)). The AUC values for discriminating between R0 and R1 was 0.81 (0.75–0.89) with sensitivities of

Table 1. Baseline characteristics of study patients.

Characteristics	*n* = 506	R0 (*n* = 231)	R1 (*n* = 224)	R2/R3 (n = 51)	*P*-value
Age, years, mean (standard deviation)	51.6 (12.9)	50.8 (13.6)	48.9 (13.1)	47.3 (13.5)	0.13
Male sex (*n*)	389	223	218	48	0.628
Indication for cardiac transplantation					
Coronary artery disease (*n*)	190	87	91	17	0.873
Non-ischaemic cardiomyopathy (*n*)	278	128	113	29	1.00
Other indications (*n*)	38	16	20	5	0.794
Study visit occurred prior to time post-transplantation					
<6 months (*n*)	452	214	201	37	0.571
6–12 months (*n*)	30	9	11	7	0.638
13–24 months (*n*)	21	7	10	3	0.540
25–36 months (*n*)	2	1	2	2	0.384
37–48 months (*n*)	1	0	1	2	0.397
Cytomegalovirus serology (IgG) status					
Donor and recipient positive (*n*)	152	70	72	15	0.740
Donor and recipient negative (*n*)	80	38	30	8	0.657
Donor positive and recipient negative (*n*)	83	40	34	8	0.519
Donor negative and recipient positive (*n*)	154	73	70	16	0.668
Unknown (*n*)	37	20	18	4	0.573

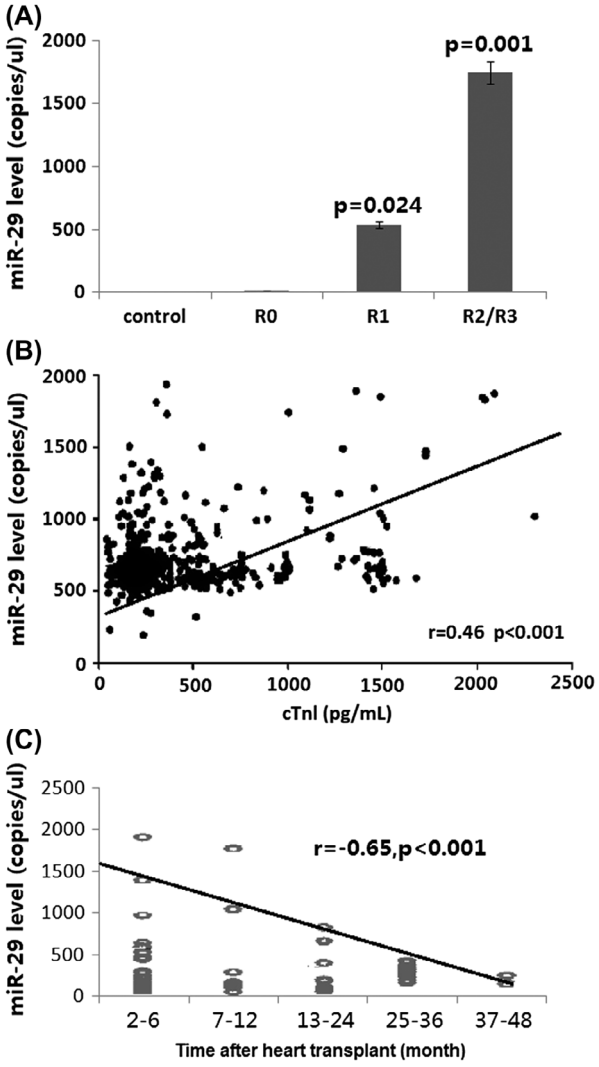

Figure 1. ROC curves calculated on the serum miR-29. Receiver operating characteristic (ROC) curves are a widely-accepted indicator of diagnostic utility. Measure of accuracy is the corresponding area under the ROC curve, denoted as AUC. It ranges in value from 0.5 (chance) to 1.0 (perfect discrimination).

74.4% and specificities of 42.3% (Figure 2(B)). The AUC values for discriminating between R0 and R2/R3 was 0.79(0.72–0.86) with sensitivities of 79.6% and specificities of 53.8% (Figure 2(C)).

In order to determine whether the serum miR-29 had clinical value risk of rejection, the PPV (positive predictive value), NPV (negative predictive value), and diagnosis efficiency were calculated. The PPV of serum miR-29 to diagnose R0, R1 and R2/R3 was 84.6, 71.8 and 87.4%; The NPV of serum miR-29 to diagnose R0, R1 and R2/R3 was 62.7, 67.5 and 73.4%: the diagnosis efficiency of serum miR-29 was 82.6, 71.8 and 79.4%, respectively.

Discussion

miRNAs are becoming increasingly recognized as potential tools in the biomedical science of cardiovasculat [11–14] and other disease [20–25]. Our study shows that the serum miR-29 level after heart transplant correlated with rejection score, cTnI, NT-proBNP, WBC counts, and negatively correlated with lymphocyte counts and post-transplant months. Detecting allograft rejection after transplantation via noninvasive methods such as biomarkers is highly sought after. Biomarkers such as CRP, NTproBNP, TnT or their combinations could not successfully predict acute allograft rejection [26–28]. High-sensitivity cardiac hs-TnI has been shown to have good relationship with acute allograft rejection, although re-categorized by ISHLT score, significant overlap was shown [29]. In their study, the median value of cTnI was higher in the patients with acute rejection than that of patients without acute rejection, although a statistical difference was not observed. Thus noninvasive testing with cardiac troponin I or T as the current guidelines recommend cannot substitute for endomyocardial biopsy in adult heart transplant recipients. Studies to identify noninvasive rejection biomarkers have increased significantly during the last years, However, new accurate markers than the routine used are still lacking.

miRNAs may impact on lymphocyte development or function and play important roles in transplant immunology. Recent studies revealed that miRNAs might participate in the regulation of the HLA-G gene expression through a putative miRNA binding site at its 3′ UTR region [30]. Specific miRNAs could govern expression of genes relevant to allograft rejection, tolerance induction and post-transplant infection [31]. Besides, they were also monitored as biomarkers in organ quality, ischaemia-reperfusion injury, acute rejection, tolerance and chronic allograft dysfunction [32–35]. Ye et al. showed that in rat hearts subjected to ischaemia reperfusion injury, mir-29 antagomiRs significantly reduced myocardial infarct size and apoptosis. These observations favour the notion that suppression of miR-29 is protective for cardiac tissue in stress conditions such as ischaemia-reperfusion injury [36]. Arnold et al. reported that immunosuppressant rapamycin treatment increased the expression of cardiac miR-29 family miRNAs in ZDF rats [37]. In our study, whether immunosuppressants regulated miR-29 expression and influenced our results need further investigation. Emerging studies suggest that virus-derived miRNAs function to regulate viral and host gene expression specifically to enhance survival of the virus. However, whether CMV affects miR-29 during HT need further investigation.

Our data add to knowledge of miRNAs. Hulsmans et al. reported that the miRNAs in inflammatory microvesicles in association with metabolic and cardiovascular diseases were found to be the let-7 family, miR-17/92 family, miR-21, miR-29, miR-126, miR-133, miR-146 and miR-155 [38]. Wang et al. found that serum microRNA-124 levels were positively related with liver necroinflammation. Furthermore, antiviral therapy decreased serum microRNA-124 levels followed by histological improvement [39]. Huang et al. found serum miR-29 levels to be negatively correlated with liver fibrotic stages and

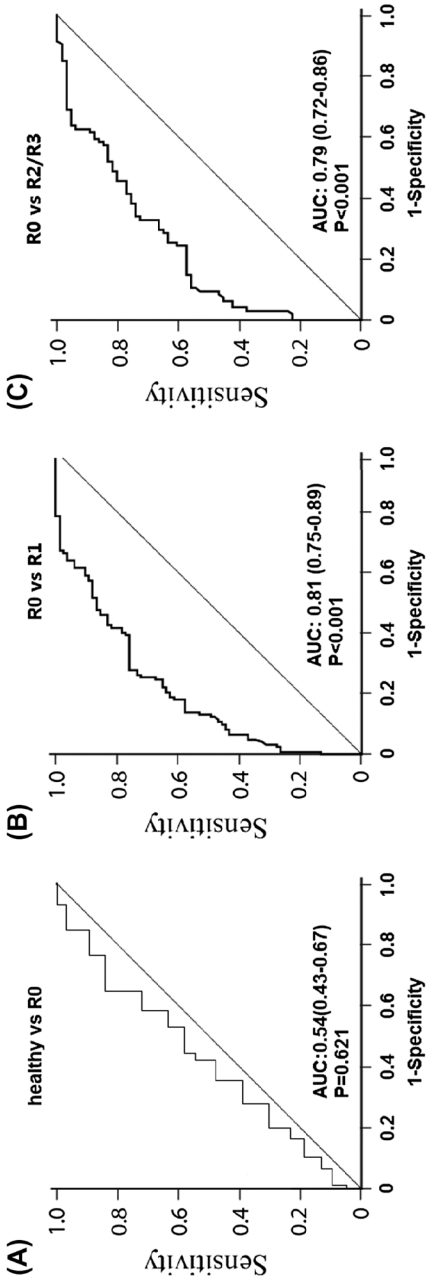

Figure 2. ROC curves calculated on the serum miR-29**.** Receiver operating characteristic (ROC) curves are a widely- accepted indicator of diagnostic utility. Measure of accuracy is the corresponding area under the ROC curve, denoted as AUC. It ranges in value from 0.5 (chance) to 1.0 (perfect discrimination). A, Healthy vs R0; B, R0 vs R1; C, R0 vs R2/R3.

necroinflammation in patients with chronic HBV infection [40]. The correlation of miR-29 and WBC/lymphocyte counts supports the possible link between inflammation and serum miR-29 levels.

Our observations were based on the retrospective analysis in a single-centre, and not all patients had all consecutive samples taken at each time point, although we present data only where both miR-29 and biopsies were assessed. Lack of pretransplant haemodynamic data was another limitation because of the presence of acute rejection detected by biopsy surveillance. Furthermore, the follow-up duration of the included patients had a limited time duration of 1–2 years after transplantation. Hence, development of overall long term cardiovascular mortality or chronic vasculopathy after transplant cannot be discussed.

In conclusion, we show that serum miR-29 levels are significantly increased after cardiac transplantation, correlate negatively with time after transplant, and positively with the grades of risk of acute rejection. Our study is an advance in biomedical science as it shows that serum miR-29 could be used as a non-invasive marker to predict acute cardiac allograft rejection.

Summary table

What is known about this subject
- Endomyocardial biopsy is an expensive and invasive procedure for cardiac allograft monitoring.
- miRNAs are freely circulating in human plasma and link with varying pathologies.
- Circulating miR-29 is increased in several cardiovascular diseases.

What this study adds
- Increased levels of circulating miR-29 are present after heart transplantation and reflect increasing risk of rejection.
- miR-29 levels fall in a four-year period after transplantation
- miR-29 is a sensitive predictor of the risk of acute allograft rejection.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] Stehlik J, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung Transplantation: 29th official adult heart transplant report – 2012. J Heart Lung Transplant. 2012;31:1052–1064.

[2] Costanzo MR, Dipchand A, Starling R, et al. The International Society of Heart and Lung Transplantation Guidelines for the care of heart transplant recipients. J Heart Lung Transplant. 2010;29:914–956.

[3] Saraiva F, Matos V, Gonçalves L, et al. Complications of endomyocardial biopsy in heart transplant patients: a retrospective study of 2117 consecutive procedures. Transplant Proc. 2011;43:1908–1912.

[4] Terzi A, Sezgin A, Tunca Z, et al. A single-center retrospective clinicopathologic study of endomyocardial biopsies after heart transplant at Baskent University Hospital in Ankara, 1993–2014. Exp Clin Transplant. 2015;13:346–351.

[5] Battes LC, Caliskan K, Rizopoulos D, et al. Repeated measurements of NT-pro-B-type natriuretic peptide, troponin T or C-reactive protein do not predict future

allograft rejection in heart transplant recipients. Transplantation. 2015;99:580–585.
[6] Patel PC, Hill DA, Ayers CR, et al. High-sensitivity cardiac troponin I assay to screen for acute rejection in patients with heart transplant. Circ Heart Fail. 2014;7:463–469.
[7] Ahn KT, Choi JO, Lee GY, et al. Usefulness of high-sensitivity troponin I for the monitoring of subclinical acute cellular rejection after cardiac transplantation. Transplant Proc. 2015;47:504–510.
[8] Olivieri F, Capri M, Bonafè M, et al. Circulating miRNAs and miRNA shuttles as biomarkers: perspective trajectories of healthy and unhealthy aging. Mech Ageing Dev. 2016;16:30185–30193.
[9] Barwari T, Joshi A, Mayr M. MicroRNAs in cardiovascular disease. J Am Coll Cardiol. 2016;68:2577–2584.
[10] Ghai V, Wang K. Recent progress toward the use of circulating microRNAs as clinical biomarkers. Arch Toxicol. 2016;90:2959–2978.
[11] Ahlin F, Arfvidsson J, Vargas KG, et al. MicroRNAs as circulating biomarkers in acute coronary syndromes: a review. Vascul Pharmacol. 2016;81:15–21.
[12] Cao W, Guo Q, Zhang T, et al. Prognostic value of microRNAs in acute myocardial infarction: a systematic review and meta-analysis. Int J Cardiol. 2015;189:79–84.
[13] Vegter EL, Schmitter D, Hagemeijer Y, et al. Use of biomarkers to establish potential role and function of circulating microRNAs in acute heart failure. Int J Cardiol. 2016;224:231–239.
[14] Heymans S, Eriksson U, Lehtonen J, et al. The quest for new approaches in myocarditis and inflammatory cardiomyopathy. J Am Coll Cardiol. 2016;68:2348–2364.
[15] Kalyani A, Sonawane PJ, Khan AA, et al. Post-transcriptional regulation of renalase gene by miR-29 and miR-146 MicroRNAs: implications for cardiometabolic disorders. J Mol Biol. 2015;427:2629–2646.
[16] Zhu H, Fan GC. Role of microRNAs in the reperfused myocardium towards post-infarct remodelling. Cardiovasc Res. 2012;94:284–292.
[17] De Vlaminck I, Khush KK, Strehl C, et al. Temporal response of the human virome to immunosuppression and antiviral therapy. Cell. 2013;155:1178–1187.
[18] Taylor DO, Stehlik J, Edwards LB, et al. Registry of the International Society for Heart and Lung Transplantation: twenty-sixth official adult heart transplant report-2009. J Heart Lung Transplant. 2009;28:1007–1022.
[19] Pfaffl MW.A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 2001;29:45e
[20] Sun BL, Liu X, Gao Y, et al. Downregulation of miR-124 predicts poor prognosis in pancreatic ductal adenocarcinoma patients. Br J Biomed Sci. 2016;73:152–157.
[21] Ren X, Shen Y, Zheng S, et al. miR-21 predicts poor prognosis in patients with osteosarcoma. Br J Biomed Sci. 2016;73:158–162.
[22] Yadegari ZS, Akrami H, Hosseini SV, et al. miR - 146a gene polymorphism and susceptibility to gastric cancer. Br J Biomed Sci. 2016;73:201–203.
[23] Nation BR. MicroRNAs: the future of genomic science? Br J Biomed Sci. 2016;73:151.
[24] Fooladinezhad H, Khanahmad H, Ganjalikhani-Hakemi M, et al. Negative regulation of TIM-3 expression in AML cell line (HL-60) using miR-330-5p. Br J Biomed Sci. 2016;73:129–133.
[25] Rao JR, Nelson D, Moore JE, et al. Non-coding small (micro) RNAs of Pseudomonas aeruginosa isolated from clinical isolates from adult patients with cystic fibrosis. Br J Biomed Sci. 2010;67:126–132.
[26] Ruopp MD, Perkins NJ, Whitcomb BW, et al. Youden index and optimal cut-point estimated from observations affected by a lower limit of detection. Biom J. 2008;50:419–430.
[27] Hulsmans M, Holvoet P. Downregulation of miR-29 by antisense inhibitors and a PPAR-gamma agonist protects againstmyocardial ischaemia-reperfusion injury. Cardiovasc Res. 2010;87:535–544.
[28] Battes LC, Caliskan K, Rizopoulos D, et al. Repeated measurements of NT-pro-B-type natriuretic peptide, troponin T or C-reactive protein do not predict future allograft rejection in heart transplant recipients. Transplantation. 2015;99:580–585.
[29] Arora S, Gullestad L, Wergeland R. Probrain natriuretic peptide and C-reactive protein as markers of acute rejection, allograft vasculopathy, and mortality in heart transplantation. Transplantation. 2007;83:1308–1315.
[30] Battes LC, Caliskan K, Rizopoulos D. Repeated measurements of NT-pro-B-type natriuretic peptide, troponin T or C-reactive protein do not predict future allograft rejection in heart transplant recipients. Transplantation. 2015;99:580–585.
[31] Patel PC, Hill DA, Ayers CR. High-sensitivity cardiac troponin I assay to screen for acute rejection in patients with heart transplant. Circ Heart Fail. 2014;7:463–469.
[32] Veit TD, Chies JA. Tolerance versus immune response – MicroRNAs as important elements in the regulation of the HLA-G gene expression. Transpl Immunol. 2009;20:229–231.
[33] Harris A, Krams SM, Martinez OM. MicroRNAs as immune regulators: implications for transplantation. Am J Transplant. 2010;10:713–719.
[34] Mas VR, Dumur CI, Scian MJ, et al. MicroRNAs as biomarkers in solid organ transplantation. Am J Transplant. 2013;13:11–19.
[35] Hu J, Wang Z, Tan CJ, et al. Plasma MicroRNA, a potential biomarker for acute rejection after liver transplantation. Transplantation. 2013;95:991–999.
[36] Ye Y, Hu Z, Lin Y, et al. Downregulation of microRNA-29 by antisense inhibitors and a PPAR-γ agonist protects against myocardial ischaemia–reperfusion injury. Cardiovasc Res. 2010;87:535–544.
[37] Arnold N, Koppula PR, Gul R, et al. Regulation of cardiac expression of the diabetic marker MicroRNA miR-29. PLoS One. 2014;9:e103284.
[38] Hulsmans M, Holvoet P. MicroRNA-containing microvesicles regulating inflammation in association with atherosclerotic disease. Cardiovasc Res. 2013;100:7–18.
[39] Wang JY, Mao RC, Zhang YM, et al. Serum microRNA-124 is a novel biomarker for liver necroinflammation in patients with chronic hepatitis B virus infection. J Viral Hepat. 2015;22:128–136.
[40] Huang C, Zheng JM, Cheng Q, et al. Serum microRNA-29 levels correlate with disease progression in patients with chronic hepatitis B virus infection. J Dig Dis. 2014;15:614–621.