Upregulation of microRNA-21 is a poor prognostic marker in patients with childhood B cell acute lymphoblastic leukemia

Hany Abedelmalik Labib\textsuperscript{a}, Neveen G. Elantouny\textsuperscript{b}, Nevin F. Ibrahim\textsuperscript{b} and Ahmed A. Alnagar\textsuperscript{c}

\textsuperscript{a}Clinical Pathology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt; \textsuperscript{b}Internal Medicine Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt; \textsuperscript{c}Medical Oncology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt

ABSTRACT

Objectives: Many studies have demonstrated that microRNA-21 (miR-21) is an oncogene and is upregulated in tumor tissue. However, its association with B-cell acute lymphoblastic leukemia (B-ALL) remains poorly understood.

Methods: The expression of miR-21 was detected by real-time quantitative PCR in 75 children with de novo B-ALL as well as in 50 healthy controls. This study was conducted to evaluate the miR-21 as a biomarker for risk assessment, diagnosis and prognosis.

Results and discussion: Compared with normal controls, miR-21 expression was significantly upregulated in childhood B-ALL patients. Using the receiver operating characteristic curve, 3.23 was selected as the cut-off value of miR-21 expression in distinguishing patients from controls. Patients group with High miR-21 expression was significantly associated with those aged <2 and >10 years, lower platelets count, more incidence of CNS infiltration and poorer treatment outcome also, they showed a significantly poorer disease-free survival (DFS) and overall survival (OS) compared to those with low miR-21 expression group. Its expression was an independent prognostic marker according to multivariate analysis.

Conclusion: This is the first report demonstrating the upregulation of miR-21 in childhood B-ALL, and its association with poor response to induction therapy, shorter DFS and OS. These results suggest that miR-21 upregulation represent an unfavorable prognostic marker in Childhood B-ALL.

KEYWORDS
Childhood; B-ALL; miR-21

Introduction

MicroRNAs (miRNAs), which are small and non-coding RNAs, regulate the translation of specific protein-coding genes. Since their discovery in 1993 \cite{1}, altered expressions of miRNAs have been associated with several diseases and tumor they are involved in tumorigenesis and the development of various types of cancer. MiRNAs have been implicated in multiple cellular processes including differentiation, proliferation, migration, and apoptosis \cite{2}. Detection of extracellular miRNAs in blood and other body fluids and the feasibility of their quantification provide evidence that these tiny molecules may serve as promising molecular biomarkers in diagnosis, differential diagnosis, prognosis and therapy of hematologic cancers \cite{3,4}.

Oorschot and his colleagues reported that 14 miRNAs are upregulated, whereas five are downregulated in lymphoblast compared to normal CD34 + cells \cite{5}. Studies concerning the role of miRNA in childhood acute lymphoblastic leukemia (ALL) are few and agreed that assessment of miRNA expression may aid in understanding the development of different phenotypes and the biological functions of miRNA in childhood ALL \cite{6}. Because the outcomes of patients who relapse are dismal, the logical next step is to find more useful leukemic markers to identify those patients at high risk of relapses early as possible \cite{7}.

MicroRNA-21 (miR-21) is one of the most prominent miRNAs implicated in the genesis and progression of human cancer. It is an oncogenic miRNA ‘oncomir’ since it promotes tumor growth, invasion, angiogenesis, and metastasis by targeting and suppressing several apoptotic and tumor suppressor gene \cite{8}.

ALL is a malignant clonal disorder of lymphoid progenitor cells that proliferate and replace the normal hematopoietic cells of the bone marrow and has a cure rate of around 80% with current treatments \cite{9}. Several studies have focused on understanding its pathogenic events however, the progress in developing early diagnosis and predicting clinical behavior of ALL represents an important clinical need that could provide information for devising new therapeutic approaches.

Few studies have been conducted until now concerning the role of miRNA in childhood ALL. To the best of our knowledge, this is the first work investigated the clinical significance of miR-21 expression in childhood B cell ALL so, the aim of this study was to
test the potential value of serum miR-21 expression level as a diagnostic and prognostic biomarker in those patients.

**Subjects and methods**

Bone marrow and peripheral blood samples were obtained at diagnosis from 75 consecutive patients with childhood B-ALL classified, treated and followed up from February 2011 to May 2016. In addition, control PB samples were obtained from 50 apparently healthy subjects with matched age and sex were analyzed.

The present study was conducted in the Department of Clinical Pathology Faculty of Medicine, Zagazig University, Egypt. Informed consent was obtained from the persons responsible for the children according to the Ethical Committee for Human Research in the Zagazig University Hospitals. The diagnosis was made by standard morphological analysis and flowcytometry in BM/PB samples at diagnosis, with all patients presenting more than 50% of blasts cells.

Patient received one cycle of induction chemotherapy in the form of intravenous vincristine 1.5 mg/m² on days 0, 7, 14 and 21, intramuscular l-Asparaginase 6000 IU/m²/thrice/week and daily steroids then assessment of the response was performed on day 14 and day 28, from the start of induction therapy. Minimal residual disease (MRD) was detected by immunophenotyping on days 14 and 28.

**RNA extraction**

Mononucleated cells were separated by a Ficoll-Hypaque centrifugation gradient and the total RNA, including small RNA, was isolated using the Qiagen miRNeasy Serum/Plasma Kit (Qiagen, Catalog no. 217184) following the protocol supplied by the manufacturer. RNA concentration was determined using the Nano Drop ND-1000 spectrophotometer (NanoDrop Tech., Inc. Wilmington, DE, U.S.A.) [10]. The RNA was stored in DEPC-treated water at −80°C till use.

**Reverse transcription**

The TaqManTM MicroRNA Reverse Transcription kit (Applied Biosystems, P/N 4366596, Egypt) and miRNA-21 specific stem-loop primers (Applied Biosystem, assay ID000397, Egypt) were used for miRNA reverse transcription (RT) reaction. RT primer for small nuclear RNA U6 (RNU6B) (Applied Biosystems, assay ID 001093, Egypt) was used as an endogenous control. For each sample, 10 ng of total RNA was used in a 15 µl reaction mixture containing RNA extract, 100 mM of each deoxynucleotide triphosphate, MultiScribe reverse transcriptase (50 U/IL), RT buffer, RNase inhibitor (20 U/ml), gene-specific TaqMan primer and nuclease-free water. RT was carried out in a Mastercycler Gradient Thermocycler (Eppendorf, Hamburg, Germany) 42°C for 30 min, and 85°C for 5 min, then held at 4°C. A negative control was included in each experiment, using all the reagents except reverse transcriptase.

**qRT-PCR analysis**

Quantitative real-time PCR (qPCR) was performed using miScript SYBR Green PCR Kit (cat. no.218073, QIAGEN, Germany) according to the manufacturer’s protocol. The miRNA levels for the investigated miRNAs were measured using StepOne (Applied Biosystems, U.S.A.). The run program was adjusted according to real-time PCR cycling conditions following the manufacture instruction. All reactions were run in duplicate and included a no-template control (with water instead of cDNA), and a no-reverse transcriptase control.

The expression of the target gene was normalized by setting appropriate thresholds to obtain accurate Ct values that were provided from the real-time PCR instrumentation. The CT value is related to the amount of PCR product.

**Statistical analysis**

All statistical calculations were performed using SPSS 17.0 for Windows (SPSS Inc, IL, U.S.A.). MiR-21 expression levels in samples were shown by mean and standard deviation (mean ± SD). The two-tailed Chi squared test was employed to associations between variables. Survival rates were calculated according to the Kaplan–Meier method and survival curves were plotted; statistical differences were analyzed using the log-rank test. Multivariate analysis of the prognostic factors was performed with Cox regression mode with respect to disease-free survival (DFS) and overall survival (OS). The level of statistical significance was set at 5% ($p$-value < 0.05). The receiver operating characteristic (ROC) curve was plotted and the area under the curve was calculated analyzed by the Hanley and McNeil method. The sensitivity and specificity were calculated using a web calculator (VassarStats; Vassar College, Poughkeepsie, NY, U.S.A.) to assess the best sensitivity and specificity for prediction of the optimum cut-off values for serum miR-21 expression level.

**Results**

We compared miR-21 expression between patients ($n = 75$) and healthy controls ($n = 50$). Serum miR-21 is
significantly up regulated in patients with childhood B-ALL compared to healthy controls (9.62 ± 3.23 and 2.56 ± 0.83, respectively) \((p < 0.001)\). Patients were divided into two groups on the basis of their miR-21 expression levels high and low. The cut-off level was set at 9.83, a value corresponding to the upper quartile level detected in patients (Figure 1).

The optimal cut-off value of miR-21 expression identified by ROC curve analysis in distinguishing ALL patients from normal controls was 3.23 and had acceptable sensitivity and specificity (88.7 and 71.8, respectively) (Figure 2).

Patients with high miR-21 expression were significantly associated with those aged <2 or >10 years \((p = 0.002)\), lower platelets count \((p = 0.003)\), more incidence of CNS infiltration \((p = 0.001)\) and risk of drug resistance \((\text{MRD}^+)\) at 14 and 28 days \((p = 0.012\) and 0.025, respectively) compared with low expression group (Tables 1 and 2).

A Kaplan–Meier curve was performed to further assess the prognostic value of miR-21 expression for childhood B-ALL patients. It showed a clear demarcation between miR-21 high and miR-21 low patients groups; the patients with high miR-21 expression level had a significant shorter DFS, Figure 3, and OS time, Figure 4, compared to the patients with low miR-21 expression \((p = 0.031\) and \(p = 0.022\), respectively).

The multivariate Cox proportional hazards model indicated that serum miR-21 expression was an

### Table 1. Comparison between low and high miR-21 expression groups in patients.

| Parameter          | Low (48) | High (27) | \(p\) |
|--------------------|----------|-----------|------|
| Sex                | Male     | 27        | 25   | 0.88 |
|                    | Female   | 21        | 44   |      |
| Age (years)        | <2, ≥10  | 12        | 25   | 0.002\* |
|                    | 2–9      | 36        | 75   | 0.27 |
| Lymphadenopathy    | –ve      | 20        | 42   | 0.63 |
|                    | +ve      | 28        | 58   |      |
| Hepatomegaly       | –ve      | 22        | 46   | 0.61 |
|                    | +ve      | 26        | 54   | 1.73 |
| Spleenomegaly      | –ve      | 19        | 40   | 0.88 |
|                    | +ve      | 29        | 60   | 0.59 |
| CNS Infiltration by blast | –ve | 41 | 85 | 0.027\* |
|                    | +ve      | 7         | 15   | 0.27 |
| TLC (×10^9/l)      | <50      | 30        | 63   | 0.72 |
|                    | ≥50      | 18        | 37   | 0.44 |
| Hb (g/dl)          | <10      | 41        | 85   | 0.75 |
|                    | ≥10      | 7         | 15   | 0.15 |
| Platelets (×10^9/l) | <50  | 19        | 42   | 0.003\* |
|                    | ≥50      | 29        | 58   | 0.22 |
| IPT                | pro-B ALL | 7        | 15   | 0.84 |
|                    | C-B ALL  | 17        | 37   | 0.41 |
|                    | Pre-B ALL | 14   | 31   | 0.33 |
|                    | Mature-B ALL | 7    | 17   | 0.15 |
| Karyotyping        | Normal   | 20        | 42   | 0.77 |
|                    | Hyperdiploid | 8    | 17   | 0.19 |
|                    | Hypodiploid | 5    | 10   | 0.27 |
|                    | t(9;22)  | 3         | 6    | 0.03 |
|                    | Other    | 3         | 8    |      |
|                    | Insufficient | 3   | 8    |      |

Note: Hb: hemoglobin, TLC: total leucocytic count, IPT: immunophenotyping.

\*p < 0.05.

### Table 2. Association between the miR-21 expression levels and response to therapy.

| miR-21 expression | Response to therapy (at 14 d) | MRD\* | \(p\)-value | Odds ratio |
|-------------------|------------------------------|-------|-------------|------------|
| Low (48)          | 18                           | 30    | 0.012\*     | 6.21       |
| High (27)         | 19                           | 8     |             |            |

Note: MRD, minimal residual disease.

\*p < 0.05.
independent poor prognostic factor for both DFS (HR = 4.01; 95% CI = 1.74–9.34; p = 0.005) and OS (HR = 4.63; 95% CI = 1.96–10.98; p = 0.001) (Table 3).

Discussion

ALL, the most common malignancy in children, has achieved remarkable treatment improvements by virtue of recent molecular biomarkers [11]. Experimental works have shed light on the diagnostic and prognostic role of miRNAs in hematologic disorders [12,13]. It has been demonstrated to play a key role in tumorigenesis. miRNAs may offer a new regulatory model of gene expression, and its expression levels correlate closely with specific clinical features of cancer, so that they can be used to classify normal and cancerous tissues, as well as for prognosis [14,15].

However, few studies have been conducted until now concerning the role of miRNA in childhood ALL. These studies have shown that differential miRNA expression analysis may help to understand the development of different phenotypes and the biological functions of these miRNAs in childhood ALL. The primary aim of the present study was to assess the expression level of miR-21 in childhood B-ALL. In addition, we tried to identify the relations between miR-21 and clinicopathological features and other prognostic markers to explore the usefulness of its expression level in predicting prognosis in childhood B-ALL.

We found that, serum miR-21 was significantly overexpressed in childhood B-ALL patients compared to healthy controls. The ROC curve analysis, in this study, revealed that serum miR-21 expression levels could differentiate B-ALL patients from healthy controls at a cut-off 3.23 with 88.7% sensitivity and 71.78% specificity. Some investigators reported its role in other types of cancer, including gastric, esophageal, colorectal, pancreatic, lung, prostate, and ovarian cancer [16–18].

It has been reported that miR-21 plays a role in the development of tumor via regulating the expression of the tumor suppressor, such as PDCD4, PTEN and TPM1 [19,20]. The effects of miR-21 on a specific target are context and sequence specific. A perfect match of ≥6 nucleotides is required to bind miR-21 to the 3′-UTR of the target gene and form the RNA induced silencing complex [21]. STATs family serves an important role in malignant transformation and oncogenesis by regulating cell proliferation, differentiation and invasion. Members of the miR-17 cluster family regulate STAT3, which in turn regulates certain cancer-associated miRNAs, including miR-155 and miR-21, by binding to the promoters of these genes [22,23].

Extracellular miRNAs existing in the circulation of cancer patients may be present as a result of the fast turnover of tumor cells resulting in cell death and lysis [24]. Several studies demonstrated that miRNAs

![Figure 3. Kaplan–Meier curves of DFS of childhood patients with newly diagnosed B-cell acute lymphatic leukemia (B-ALL) stratified by the level of microRNA-21 (miR-21) expression (high versus low).](image)

![Figure 4. Kaplan–Meier curves of OS of childhood patients with newly diagnosed B-ALL stratified by the level of microRNA-21 (miR-21) expression (high versus low).](image)

Table 3. Multivariate analyses of prognostic variables of DFS and OS in ALL patients.

| Variables       | Recurrence-free survival |        |         | OS           |        |
|-----------------|--------------------------|--------|---------|--------------|--------|
|                 | HR       | 95% CI | p value | HR            | 95% CI | p value |
| Age             | 1.045    | 0.82–3.78 | 0.314 | 0.87          | 0.89–3.96 | 0.67    |
| CNS Infiltration| 2.86     | 0.95–5.62 | 0.083 | 2.06          | 1.04–5.76 | 0.032*  |
| Platelets (x107/l) | 1.14    | 0.64–2.46 | 0.792 | 1.20          | 0.67–2.74 | 0.78    |
| miR-21 expression | 4.01    | 1.74–9.34 | 0.005* | 4.63          | 1.96–10.98 | 0.001*  |

*Significant: 2-sided Pearson’s chi-square test, p < 0.05.
are increased in plasma/serum as tumor-derived miRNAs are resistant to endogenous ribonuclease activity because these may be protein-bound, such as to the argonaut-2 protein and high density lipoproteins [25, 26] or packaged by secretory particles, including apoptotic bodies and exosomes, in plasma/serum [27].

For better understanding of the clinical relevance of miR-21 expression in childhood B-ALL patients group, we divided it into a high expression group \((n = 27)\) and a low expression group \((n = 48)\), according to the expression level of miR-21. High miR-21 expression group was significantly associated with those had <2 or >10 years, lower platelets count, more incidence of CNS infiltration and more risk of drug resistance (MRD\(^+\)). Many studies demonstrated that, miR-21 deregulation represented a global cancer phenomenon; associated with poor prognosis, poor response to therapy and survival in other cancer types including lung cancer [30], colorectal carcinoma [28, 29], gastric cancer [30], pancreatic cancer [16], diffuse large B-cell lymphoma [31].

To determine whether elevated serum miR-21 was a potential predictor of prognosis in our patients, we proved that miR-21 expression was significantly associated with DFS and OS by Kaplan–Meier analysis and log-rank test. Patients group with high level of miR-21 expression had worse DFS and OS compared with those with low level.

According to multivariate Cox regression analysis, serum miR-21 expression could be used as an efficient independent prognostic marker for DFS and OS. Lawrie et al. found that, expression of miR-21 was increased in DLBCL cells and was used as an independent prognostic indicator for DLBCL patients [32] also, Mao et al. revealed that, serum miR-21 was used as an independent and powerful predictor of OS for primary central nervous system lymphoma [33].

These findings highlight the potential utility of miR-21 as a prognostic biomarker. Its use could facilitate decision-making to select for prospective patients needing meticulous follow-up for early detection of relapse and additional or alternative treatments.

Although there is an evidence indicating that elevated miR-21 expression is a prognostic factor in ALL patients, several issues need to be addressed when interpreting our results. The sample size in our study was not large enough to fully explore the synergistic relationship between a miR-21 expression profile and different combinations of confounding factors that may affect its behavior. Response rate to therapy seems to be lower than expected, because of economic reasons, we cannot included some effective recent expensive drugs in the protocol of therapy. Adding to that, ALL is a heterogeneous disease at the molecular levels, even within the same patient.

In conclusion, the results found in this study, taken in the context of the role of miR-21 suggesting that, miR-21 serum profiling can potentially serve as a promising non-invasive biomarker for risk assessment and prognosis in childhood B-ALL.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Notes on contributors**

**Dr Hany Abedermalik Labib** was born in 1971 and is a professor of Clinical and chemical pathology, faculty of Medicine since 2011 Zagazig University, Egypt. He is a consultant of the specialized hematology oncology unit. He has coauthored over 15 publications including Hematology oncology disorders diagnosis, genetic studying of different diseases using different cytogenetic and molecular techniques.

**Dr Neven G. Elantouny** is an MD, PhD with 10 years of research experience in the oncology area. She has a special interest in elucidating the molecular mechanisms of oncogenesis and many other immunological diseases and now she is interested in gene and drug delivery system. She has coauthored over 10 publications. She was co-principal investigator in renal cancer research project.

**Dr Nevin F. Ibrahim** obtained the doctor’s degree from Zagazig University, Egypt. She has coauthored in many publications including how to overcome the side effects of chemotherapy and personalized it. She is a postdoctoral fellow in Prof. Hany A. Labib’s group.

**Dr Ahmed A. Alnagar** earned his PhD degree in medical oncology from national cancer institute, Egypt, where he worked on development and personalized a new protocols of therapy in Leukemic patients. He is co-principal investigator in hepatic cancer research project. He has many posters and international publications.

**References**

[1] Lee RC, Feinbaum RL, Ambros V, et al. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993;75:843–854.

[2] Yin Y, Jun L, Shujie C, et al. MicroRNAs as diagnostic biomarkers in gastric cancer. Int J Mol Sci. 2012;13:12544–12555.

[3] Li Q, Liu L, Li W. Identification of circulating microRNAs as biomarkers in diagnosis of hematologic cancers: a meta-analysis. Tumor Biol. 2014;35:10467–10478.

[4] Godfrey A, Zongli X, Clarice R, et al. Serum microRNA expression as an early marker for breast cancer risk in prospectively collected samples from the Sister Study cohort. Breast Cancer Res. 2013;15:350–351.

[5] Oorschot D, Kuipers E, Arentsen-Peters S, et al. Differentially expressed miRNAs in cytogenetic and molecular subtypes of pediatric acute myeloid leukemia. Pediatr Blood Cancer. 2012;58:715–721.

[6] de Oliveira C, Scrideli A, Brassesco S, et al. Differential miRNA expression in childhood acute lymphoblastic leukemia and association with clinical and biological features. Leuk Res. 2012;36:293–298.

[7] Suhaq V, Solomon S, Malkovksva V. Acute myelogenous leukemia. In: Rodgers P, Young N, editors. Bethesda handbook of clinical hematology. New York: Lippincott Williams & Wilkins; 2005. p. 131–147.
[8] Si H, Xiaoming S, Yingjian C, et al. Circulating microRNA-92a and microRNA-21 as novel minimally invasive biomarkers for primary breast cancer. J Cancer Res Clin Oncol. 2013;139:223–229.

[9] Bassan R, Gatta G, Tondini C, et al. Adult acute lymphoblastic leukaemia. Crit Rev Oncol Hematol. 2004;50:223–261.

[10] Heneghan H, Miller N, Lowery A, et al. Circulatory microRNAs as novel minimally invasive biomarkers for breast cancer. Ann Surg. 2010;251(3):499–505.

[11] Friedmann AM, Weinstein HJ. The role of prognostic features in the treatment of childhood acute lymphoblastic leukemia. Oncologist. 2000;5(4):321–328.

[12] Han W, Feng D, Li G, et al. A set of miRNAs that involve in the pathways of drug resistance and leukemic stem-cell differentiation is associated with the risk of relapse and glucocorticoid response in childhood ALL. Hum Mol Genet. 2010;20(3):499–505.

[13] Li P, Wang X. Role of signaling pathways and miRNAs in chronic lymphocytic leukemia. Chin Med J (Engl). 2013;126(21):4175–4182.

[14] Huang Y, Shen XJ, Zou Q, et al. Biological functions of microRNAs: a review. J Physiol Biochem. 2011;67:129–139.

[15] Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer. 2006;6:857–866.

[16] Ali S, Khaldoun A, Wei C, et al. Differentially expressed miRNAs in the plasma may provide a molecular signature for aggressive pancreatic cancer. Am J Transl Res. 2011;3(1):28–47.

[17] Zhao H, Jie S, Leonard M, et al. A pilot study of circulating miRNAs as potential biomarkers of early stage breast cancer. PLoS One. 2010;5(10):e13735. DOI:10.1371/journal.pone.0013735

[18] Zeng Z, Wang J, Zhao L, et al. Potential role of microRNA-21 in the diagnosis of gastric cancer: a meta-analysis. PLoS One. 2013;8(9):e73278. DOI:10.1371/journal.pone.0073278

[19] Zhu S, Si ML, Wu H, et al. MicroRNA-21 targets the tumor suppressor gene tropomyosin1 (TPM1). J BiolChem. 2007;282:14328–14336.

[20] Lou Y, Yang X, Wang F, et al. MicroRNA-21 promotes the cell proliferation, invasion and migration abilities in ovarian epithelial carcinomas through inhibiting the expression of PTEN protein. Int J Mol Med. 2010;26:819–827.

[21] Sontheimer EJ. Assembly and function of RNA silencing complexes. Nat Rev Mol Cell Biol. 2005;6:127–138.

[22] Kohanbash G, Okada H. MicroRNAs and STAT interplay. Semin Cancer Biol. 2012;22:70–75.

[23] Carraro G, El Hashash A, Guidolin D, et al. MiR 17 family of microRNAs controls FGF10 mediated embryonic lung epithelial branching morphogenesis through MAPK14 and STAT3 regulation of E Cadherin distribution. Dev Biol. 2009;333:238–250.

[24] Turchinovich A, Ludmilas W, Anne L, et al. Characterization of extracellular circulating microRNA. Nucleic Acids Res. 2011;41:1–11.

[25] Arroyo JD, Chevillet JR, Kroh EM, et al. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc Natl Acad Sci USA. 2011;108:5003–5008.

[26] Vickers KC, Palmisano BT, Shoucri BM, et al. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nat Cell Biol. 2011;13:423–433.

[27] Hasselmann DO, Rappl G, Tilgen W, et al. Extracellular tyrosinase mRNA within apoptotic bodies is protected from degradation in human serum. Clin Chem. 2001;47:1488–1489.

[28] Kishore A, Jana B, Jana P, et al. Review article novel insights into miRNA in lung and heart inflammatory diseases. Mediat Inflamm. 2014;2014:27.

[29] Tokarz P, Blasiak J. The role of microRNA in metastatic colorectal cancer and its significance in cancer prognosis and treatment. Acta Biochim Pol. 2012;59(4):467–474.

[30] Komatsu S, Ichikawa D, Tsujiura M, et al. Prognostic impact of circulating miR-21 in the plasma of patients with gastric carcinoma. Anticancer Res. 2013;33(1):271–276.

[31] Lawrie C, Gal S, Dunlop H, et al. Detection of elevated levels of tumor-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br J Haematol. 2008;141(5):672–675.

[32] Lawrie CH, Soneji S, Marafioti T, et al. MicroRNA expression distinguishes between germinal center B cell-like and activated B cell-like subtypes of diffuse large B cell lymphoma. Int J Cancer. 2007;121:1156–1161.

[33] Mao X, Sun Y, Tang J. Serum miR-21 is a diagnostic and prognostic marker of primary central nervous system lymphoma. Neurol Sci. 2014;35:233–238.