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# MicroRNA as a diagnostic biomarker in childhood acute lymphoblastic leukemia; *systematic* review, meta-analysis and recommendations

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ABSTRACT

Several studies detected abnormal mi-RNAs expression levels in childhood Acute Lymphoblastic Leukemia (ALL) with potential diagnostic value. We conducted a systematic search on certain microRNAs in childhood ALL. We included 17 studies with a total of 928 ALL children and 307 controls. Ten studies provided miRNAs expression levels and seven provided frequency data. Sensitivity and specificity of a single miRNA ranged from 46.55% to 100% and from 71.8% to 100%, respectively. The highest diagnostic odds ratio (DOR) was for the diagnostic panel (miR-128a and miR-223) reaching 546 [95% CI: 73.768–4041.282]. Also, miR-128a, miR-128b, miR-223, let-7b, miR-155 and miR-24 can be used as diagnostic discriminatory biomarkers between ALL and AML. Further large cohort studies are needed to confirm our results.

## 1. Introduction

microRNA (miRNA) is endogenous noncoding RNA that contains 19–25 nucleotides. It has a regulatory effect on gene expression on both transcription and post-transcription levels (Bartel, 2004). Fifty percent of annotated human miRNAs are located at fragile sites or cancer-related genomic regions (amplification, breakpoint and heterozygosity) (Calin et al., 2004).

Worldwide, acute lymphoblastic leukemia (ALL) is the most common childhood malignancy; It accounts for one fourth of all childhood cancers and approximately 75% of all childhood leukemias (Pui, 1995). ALL is a complex disease characterized by clonal proliferation of B-cell precursors (BCP) and T-cell precursors and results in accumulation of leukemic lymphoblast in bone marrow (BM) and various extramedullary sites. The gold standard diagnostic method in childhood ALL is invasive (Haferlach et al., 2005). Therefore, researchers keep seeking for molecular changes that can diagnose patients with early cancer or precursor lesions by minimally invasive samples such as blood, stool, and urine for extraction of DNA or RNA (Iacobucci and Mullighan, 2017; Roberts and Mullighan, 2015).

Identification and molecular profiling of novel miRNAs in childhood ALL have been accessible in the era of great advances in different technologies used for gene expression analysis. Correlating the great miRNA databases with the clinical application in childhood ALL is the optimum benefit that is urgently needed by clinicians. Many studies have been published on either an individual miRNA or a diagnostic panel of miRNAs as a valuable diagnostic biomarker in childhood ALL. Only one meta-analysis study about circulating miRNAs as a diagnostic biomarker in any type of hematological cancers has been published (Li et al., 2014). But no systematic review or meta-analysis has been published on miRNA as a diagnostic biomarker in pediatric ALL.

The aim of this systematic review and meta-analysis is the identification of miRNAs as diagnostic biomarkers in childhood ALL. Also, we recommend specific modifications in setting up miRNAs research studies that will help in clinical translation that is eagerly needed.

## 2. Methods

### 2.1. Research strategy

We have searched PubMed (2007–2018), The Cumulative Index to Nursing & Allied Health Literature (CINHAL) in addition to both International Society of Paediatric Oncology (SIOP) and American Society of Hematology (ASH) abstract meetings (2007–2017). Also, a parallel search of Google Scholar was conducted. Other databases of miRNA such as, miRbase.org were screened for relevant miRNAs. The

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following combinations were used in PubMed: microRNA OR miRNA OR miR AND Acute Lymphoblastic Leukemia. Based on these results a more focused search was built: miR-17-3p OR miR-17-5p OR miR-18a OR miR-19 OR miR-21 OR miR-24 OR miR-30e-5p OR miR34b OR miR-99a OR miR-100 OR miR-101 OR miR-125b OR miR126 OR miR-128a OR miR-128b OR miR-130b OR miR-142 OR miR-146a OR miRNA-149 OR miR-191 OR microRNA-193b-3p OR microRNA-196a OR microRNA-196b OR let-7b; OR miR-203 OR miR-204 OR miR-210 OR miR-222 OR miR-223 OR miR-181a OR miR-331 OR miR-339, and miR-142-3p OR miR-708 AND Acute Lymphoblastic Leukemia.

### 2.2. Inclusion and exclusion criteria

The search was limited to English literature only. We also scanned the references in the extracted studies and included what was considered relevant. The eligibility criteria were: (i) using a case –control design; (ii) cases are childhood ALL patients (iii) contained frequency data. The major exclusion criteria were (i) no control population; (ii) complete data is not available; (iii) duplicate publication; (iv) the data in cell lines or any non-human experimental approach was excluded.

Two investigators independently (WMR, AS) followed a standardized research protocol and extracted the data. The following information was extracted: the surname of the first author, year of publication, ethnicity, age group, 2 × 2 contingency table for specificity and sensitivity. For studies including subjects of different ethnic groups, data were extracted separately for each ethnic group whenever possible. Ethnicity was categorized as country where the study was conducted.

### 2.3. Statistical analysis

Diagnostic odds ratios (DOR) were pooled using random-effects methods for pooling odds ratios. We considered positive test where it is detectable. We estimated summary ROC curves using the Moses-Littenburg regression-based method on STATA package 14.2. Comparative functions of effect size for binary data are the log-odds ratio or relative risk. Heterogeneity quantity Q was calculated across the studies based on DerSimonian and Laird (DerSimonian and Laird, 1986).

## 3. Results

### 3.1. Baseline characteristics of studies

Our search yielded a total of 602 records, twenty-four studies were found potentially relevant. Causes of exclusion were outlined in the graphical abstract. Two studies conducted on adult patients were excluded (Ninomiya et al., 2012; Zhu et al., 2012). The final eligible studies were 17 case-control studies. Ten studies provided the expression levels of the over/under-expressed miRNAs and seven studies provided frequency data namely, sensitivity and specificity (Tables 1 and 2). We also synthesized the diagnostic odds ratio for some studies (Table 2). For simplicity we will discuss both groups separately.

#### 3.1.1. Studies providing the expression levels

A total of ten case-control studies included 556 patients of childhood ALL and 209 normal controls. In most studies, samples were taken from BM, and RNA was extracted using TRIZOL assay. Three studies (Duyu et al., 2014; Ju et al., 2009; Li et al., 2013b) used initially microarray analysis that was further validated using qRT-PCR, other studies used qRT-PCR for quantification of specific microRNA panel. The number of differentially expressed microRNAs ranges from 9 to 144. One study (Li et al., 2013c) investigated the expression of two microRNAs, namely miR-100 and miR-99a, that were found to be down-regulated in ALL patients while they were reported to be upregulated in another study (Schotte et al., 2011). Two studies investigated the expression of only one miRNA (Cao et al., 2016; Organista-Nava et al., 2015). In one study, the median expression level of miR-24 was 0.84 compared to 1.25 in the normal control ($p < 0.001$) (Organista-Nava et al., 2015). In the other, the mean expression of miR-34b was 1.65 ± 0.69 with a significant difference compared to controls ($p = 0.012$) (Cao et al., 2016). Four studies reported all the altered miRNAs (Duyu et al., 2014; Ju et al., 2009; Li et al., 2013b; Zhang et al., 2009). The range of up-regulated miRNAs was 6 to 77, and the range of down-regulated miRNAs was 3 to 67. It is worthy to mention that all cases included in these studies are the two ALL subtypes (B- and T-lineages) except three studies that included only one ALL subtype (B-lineage) (Ju et al., 2009; Li et al., 2013c; Ramani et al., 2017). In addition, four studies compared miRNAs expression ALL with AML and control and consequently proved some miRNAs as diagnostic discriminatory biomarkers between ALL and AML (Cao et al., 2016; Mi et al., 2007; Organista-Nava et al., 2015; Schotte et al., 2011).

#### 3.1.2. Studies providing data about sensitivity and specificity

Seven studies reported sensitivity and specificity of 19 microRNAs. They recruited 372 children with ALL and 161 normal controls. The frequency data are outlined in Table 2. The sensitivity and specificity of a single miRNA ranged from 46.55% to 100 and from 71.8% to 100%, respectively. The highest diagnostic odds ratio was for combination of miR-128a and miR-223 reaching 546 [95%CI: 73.77–4041.28]. The frequency per case of six altered microRNAs were provided in two studies (Mi et al., 2007; Swellam et al., 2018), the pooled DOR of those six miRNAs was 83.048 [95%CI: 28.95–238.22] see Fig. 1. The pooled estimated sensitivity and specificity were 0.81 (95% CI: 0.74–0.87) and 0.91 (95% CI: 0.87-0.94), respectively (Fig. 2). The area under the curve of the receiver operator curve (ROC) of the six microRNAs was more than 0.8 (Fig. 3) and the positive likelihood ratio was 6.502 [95%CI: 2.12–19.97] (Fig. 4). Moreover, Fig. 5 shows the summary operating point, i.e., summary values for sensitivity and specificity.

## 4. Discussion

We found that many dysregulated microRNAs have been reported to be used as diagnostic discriminatory biomarker between ALL and AML. Both miR-128 and miR-155 were proven to be significantly highly expressed in childhood ALL (Duyu et al., 2014; Ramani et al., 2017). In addition, miR-128a, miR-128b, miR-155 were reported in the literature (Mi et al., 2007; Wang et al., 2010; Zhu et al., 2012) that they are significantly highly expressed in ALL compared with AML. On the other hand, miR-24, let-7b and miR-223 are significantly expressed at a lower level in ALL compared to AML(Mi et al., 2007; Organista-Nava et al., 2015). Consequently, miR-128a, miR-128b, miR-155, miR-24, let-7b, and miR-223, can be used as diagnostic discriminatory biomarkers between ALL and AML.

In addition, when we ranked the diagnostic odds ratio (DOR) calculated (Table 2): the diagnostic panel **(miR-128a + miR-223)** for childhood ALL was the highest (DOR = 546, 95% CI = [73.38, 4041.28]); followed by **miR-203** (DOR = 280, 95% CI = [27.38, 2863.38]); then the diagnostic panel **(miR-128a + 128b)** (DOR = 202, 95% CI = [39.36, 1100.79]); then **miR-181a** (DOR = 89.23, 95% CI = [22.59, 352.43]); then the diagnostic panel **(miR-128b + let7b)** (DOR = 62.07, 95% CI = [17.60, 218.85]); then the diagnostic panel **(miR128a + let-7b)** (DOR = 44.1, 95% CI = [13.32, 146.03]); then **miR-21** (DOR = 18.86, 95% CI = [7.44, 47.83]); then **miR-326** (DOR = 11.45, 95% CI = [3.47, 37.8]); and, finally, **miR-200c** which was the lowest (DOR = 4.84, 95% CI = [1.39, 16.77]).

Historically, the identification of miRNA profile signatures in diagnosis or prognosis of human cancers started in 2004 in CLL patients, then research investigated other common cancers such as breast and lung. In 2007, Mi et al performed the first genome-wide miRNA expression analysis on ALL and AML samples (Mi et al., 2007). They have found four types of miRNAs that discriminate ALL from AML (Table 2).

**Table 1**
Characteristics of studies reporting the expression level of dysregulated microRNAs.

| Study | Sample size (population) | RNA extraction (sample source: case/control) | Quantification method | Cut-off | Dysregulated miRNA/Total | Total# (and the most) over-expressed miRNAs | Total # (and the most) under-expressed-miRNAs |
|---|---|---|---|---|---|---|---|
| **1**-Zanette et al., 2007 (Zanette et al., 2007) | 7 ALL vs 6 controls ($CD19^+$-cells) (Brazilian) | Trizol LS(BM/PB) | TaqMan® MicroRNA Assays Human Panel. ***Validation***: Real-time PCR | Fold change by 2ˆ-ΔΔCt method *Reference*: **miR-30b** | 46/164 | $NR (***miR-128b***, miR-204, miR-218, ***miR-331***) | $NR (miR-135b, miR- 132, miR-199 s, miR-139, miR-150) |
| **2**-Ju et al., 2009 (Ju et al., 2009) | 40 pre-B-ALL vs 6 N L controls(Chinese) | TRIZOL assay (BM&PB/BM) | Microarray analysis. ***Validation***: Stem-Loop qRT-PCR | Fold change $>2$ or $<0.5$ compared to the mean of normal samples. | 11/328 | 6 (miR-519e; miR-487b; miR-361; ***miR-142}3p***; ***miR-222***; ***miR-339***) | 5 (***miR-451***; ***miR-373;*** miR-296; miR-485-3p-MM1; miR-483) |
| **3**-Zhang et al., 2009 (Zhang et al., 2009) | 27 ALL vs 5 controls (Chinese) | TRIZOL assay (BM/BM) | Sequencing-by-synthesis (SBS) strategy ***Validation:*** qRT-PCR | fold changes $>2.0$ ($P < 0.001$) | 144/847 | 77(miR-9*,miR-9, miR-122,miR-181a, miR-128, miR-92a-1*,miR-181a*,miR-181a-1*miR-181a-2*,miR-181d, miR-25*,miR-181b, miR-130b,miR363, miR-598, miR-181c, let-7e,miR-499-5p, miR342-3p, miR-17*) | 67 (miR-486-5p,miR374a*-miR-424,miR-30a, miR-582-5p,miR-451, miR-143*,miR-199b-3p, miR-199b-5p miR126, miR-335, miR-144, miR10a,miR-144*, ***miR-223***, miR-143, miR-126, miR-618,miR-1277, miR-145) |
| **4**-Schotte et al., 2011 (Schotte et al., 2011) | 81 ALL subtypes vs 17 control/ (Caucasian) | TRIZOL assay(BM &PB/BM) | Stem-loop RT-qPCR ***Validation***:None | Fold change, $P_{FDR} < 0.05$ | 89/397 | NR (miR-196a, miR-383, miR-542-5p, miR-133a) | NR (miR-708, miR-511, miR-708, let-7b) |
| **5**-Li et al., 2013a (X. Li et al., 2013b) | 34 precursor B-cell ALL vs 5 control (Chinese) | TRIZOL assay(BM &PB/BM) | Microarray assay, ***Validation***: stem-loop RT-PCR | twofold difference and a q-value $< 0.05$ | 11/NR | 8 (***miR-708, miR-210, mi-181b, miR-345, miR-324-5p, and miR-125b***) | 3 (***miR-23a, miR-27a, and miR-23b***) |
| **6**-Li et al, 2013 (X. J. Li et al., 2013c) | 111 ALL vs 10 controls (Chinese) | TRIzol reagent (BM/BM) | RT-PCR ***Validation***:None | NR | 2/2 | 0 | 2 (MiR-100 and miR-99) |
| **7**-Duyu et. al., 2014 (Duyu et al., 2014) | 43 ALL vs 14 control (Turkish) | Qiazol(BM/BM) | Microarray ***Validation***: qRT-PCR | Fold change ± 2-fold, $P_{FDR} < 0.05$ | 15/1136 | 13 (***miR-548i, miR-708, miR-181b, miR-449a, miR-146a, miR-155, miR-181a, miR-3121, miR-128, miR-1323, miR-195, miR-587, miR-640***) | 2 ***(miR-640, miR-145***) |
| **8**-Organista-Nava et. al. (Organista-Nava et al., 2015), 2015 | 111 ALL vs 100 control (Mexican) | TRIZOL assay (BM/PB) | RT-PCR ***Validation***: None | Median Expression level with $P < 0.05$ | 1/1 | 0 | miR-24 |
| **9**-Cao *et. al.*, 2015 (Cao et al., 2016) | 42 ALL vs 20 AML vs 11 MLL + vs 20 Control (Chinese) | TRIZOL assay (BM/BM) | RT-qPCR ***validation***:None | Mean of expression level with $P < 0.05$ | 1/1 | 1 (miR-34b) | 0 |
| **10**-Ramani *et. al.*, (Ramani et al., 2017) 2017 | 60 Pre-B ALL vs 17 Controls (Caucasian) | TRIZOL assay(BM or PB in both) | Stem-loop RT-qPCR ***validation***:None | ($P_{FDR}$) $< 0.05$. | 136/365 | NR | NR |

$NR= not reported, we adopted the conventional cut-off either $< 0.5$ or $> 1.00$ in case of 2ˆ-ΔΔCt method.
-All reported dysregulated miRNA in this table were the outcome of the first quantification analysis step, miRNAs are present in ***bold and italics font*** means they underwent validation process.
BM: Bone Marrow, PB: peripheral Blood, $P_{FDR=}$ False discovery rate- *P* values.

**Table 2**
Baseline characteristics of studies presenting frequency data (sensitivity and specificity) of microRNA in childhood ALL.

| Study | Sample size (Population) | RNA extraction (sample source: case/control) | Quantification method | Cut-off | miRNA | Sensitivity %, | Specificity | DOR[95% CI] |
|---|---|---|---|---|---|---|---|---|
| (Mi et al., 2007) | 54 ALL and 44 AML vs 10 controls(USA) | bead-based expression assay | genome-wide microRNA expression profiling assay ***Validation***: TaqMan PCR | cross-validated probabilities (CV probabilities) = 2.0 | (+)MiR-128a/(+)128b | 96% | 89% | 202.8[39.362-1100.793] |
| | | | | | (+)MiR-128a and(-) let-7b | 91% | 82% | 44.1[13.318-146.025] |
| | | | | | (+)MiR-128b and (-)let-7b | 91% | 86% | 62.067[17.603-218-848] |
| | | | | | (+)MiR-128a and(-) miR-223 | 96% | 95% | 546[73.768-4041.282] |
| (Swellam et al., 2018) | 43 ALL vs 23 control **(Egyptians)** | miRNeasy Mini kit (Qiagen)(PB/PB) | qRT-PCR | Construct ROC curve. (AUC > 0.5)miR-203$_{cutoff=}$ 0.973 miR-125b-1$_{cutoff=}$3.209 | (-) miR-203 | 97.67% | 86.96% | 280[27.38-2863.382] |
| | | | | | (+)miR-125b-1 | 83.72% | 100% | FP = 0 |
| | | | | | miR-125b-1+ miR-203 | 100% | 87% | FN= 0 |
| (Swellam and El-Khazragy, 2016) | 85 ALL vs 25 control/ **(Egyptians)** | miRNeasy serum/plasma cell lysates kit (Qiagen) | qRT-PCR | miR-100$_{cutoff=}$3.029 | (+)miR-100 | 82.76% | 100 | FP = 0 |
| | | | | miR-196a$_{cutoff=}$0.973 | (-)miR-196a | 46.55% | 100 | FP = 0 |
| | | | | miR-146a$_{cutoff=}$3.727 | (+)miR-146a | 100 | 100 | FP = 0 |
| (Luna-Aguirre et al., 2015) | 39 B-AL vs 7 control **(Mexican)** | miRNeasy Mini kit (PB) | TaqMan low density array (TLDA) plates, ***Validation***: qRT-PCR | **miR-511,** ΔCq cutoff = 9.458 **miR-34a**, ΔCq cutoff = 7.179**miR-222,** ΔCq cutoff= −0.1325**miR-26a,** ΔCq cutoff = 2.073**miR-221,** ΔCq cutoff = 0.1861**miR-223,** ΔCq cutoff=-4.309 | (+) miR-511 | 100 | 100 | FP = 0 |
| | | | | | (+) miR-34a | 92 | 100 | FP = 0 |
| | | | | | (+) miR-222 | 79 | 100 | FP = 0 |
| | | | | | (-) miR-26a | 79 | 100 | FP = 0 |
| | | | | | (-) miR-221 | 83 | 100 | FP = 0 |
| | | | | | (-) miR-223 | 89 | 100 | FP = 0 |
| (Nabhan et al., 2017) | 30 ALL vs 30 healthy control **(Egyptians)** | miRNeasy serum/plasma kit (Qiagen) | qRT-PCR | Cut-off value was 0.97, (AUC) = 0.93 | (+) MiR-181a | 86.5% | 93.3% | 89.226 [22.59 - 352.426] |
| (Labib et al., 2017) | 75 Pre-B ALL vs 50 control **(Egyptians)** | miRNeasy serum/plasma kit (Qiagen) | qRT-PCR | Cut-off value was 3.23; Expression cut-off level = 9.83 | (+) miR-21 | 88.7 | 71.8 | 18.857[7.435-47.825] |
| (Ghodousi and Rahgozar, 2018) | 46 ALL vs 16 control(**Iranian**) | TRizol reagent (BM/BM) | qRT-PCR | **miR-326**$_{cutoff=}$0.29**miR-200c**$_{cutoff=}$0.42 | (-) miR-326 | 0.83 | 0.708 | 11.445 [3.47-37.8] |
| | | | | | (-) miR-200c | 0.66 | 0.714 | 4.84[1.39-16.77] |

**BH**= Benjamini-Hochberg, **NR**= not reported, FP= false-positive, FN= false negative, **ROC**=Receiver operating characteristic, **AUC**=Area under the ROC curve, MLL+: AL patients with mixed lineage leukemia rearrangement. (+) over-expressed, (-) under-expressed. PB= peripheral blood, BM= bone marrow.

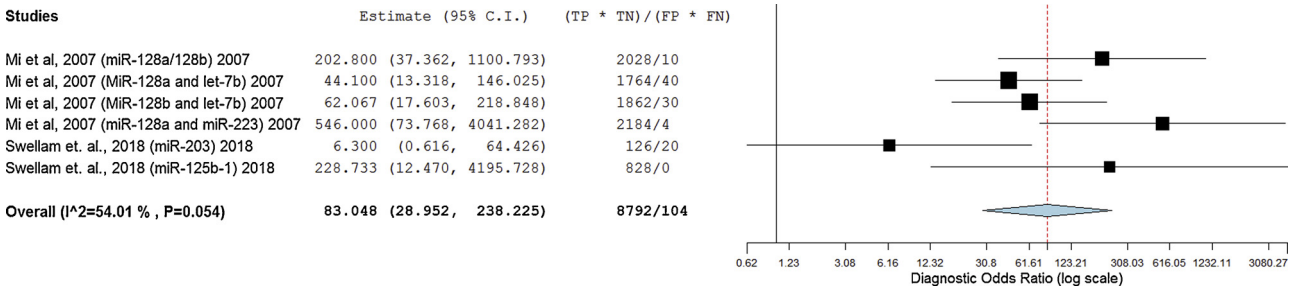


**Fig. 1.** Forest plot of the pooled diagnostic odds ratio (DOR) of the six microRNAs.

Another Brazilian study in the same year found five highly expressed miRNAs including miR-128b, miR-204, miR-218, miR-331, and miR-181b-1 (Zanette et al., 2007). Great advances in technology enabled both Mi et al. and Zhang et al. to report the same findings in ALL regarding the upregulation of miR-128, miR-130b and miR-210 and the downregulation of miR-424, miR-223, miR-23a, miR-27a, although they have used two different miRNA expression analysis platforms (Mi et al., 2007; Zhang et al., 2009). The same findings from other studies were reported for both miR-223 (Luna-Aguirre et al., 2015) and miR-128 (Duyu et al., 2014; Zanette et al., 2007).

Great advances in bioinformatics have enabled researchers to establish multiple free online database as a valuable source for microRNA target prediction. Supplementary Table (S1) summarized all target prediction database for miRNA used in most studies mentioned here. These microRNA target prediction databases are considered a complementary step after expression profile analysis of miRNA diagnostic biomarker in childhood ALL. These databases enable researchers to put hypothetical correlations between the aberrant expression of miRNA in ALL and the underlying disease etiology. These hypotheses open gates for more future confirmatory studies. Using target prediction databases, many putative targets for up/down-regulated miRNAs were reported (Table 3).

MiR-128 is an intronic miRNA (Ching and Ahmad-Annuar, 2015) that expressed by two distinct genes; miR-128-1 (2q21.3) and miR-128-2 (3p22.3). Both are translated to produce the same mature miR-128. It is extensively reported by many studies regarding its role in cancer, in general, and in childhood ALL in particular (Li et al., 2013a). Our study highlighted its role as a diagnostic discriminatory biomarker between ALL and AML and as a member of the diagnostic panel for childhood ALL. Also, it has been reported its association with certain ALL immunophenotype (T-ALL) (de Oliveira et al., 2012; Drobna et al., 2018), and certain crucial gene (*IKFZ1* deletion that affect ALL prognosis) (Krzanowski et al., 2017). The association between miR-128b dysregulated expression and ALL treatment response specifically glucocorticoid (Kotani et al., 2010, 2009) and survival (Nemes et al., 2015) has also been reported.

miR-223 gene is located on chromosome Xq12 (Luan et al., 2015). miR-223 has potential targets in lymphoid cells which consequently affects the regulation of cell cycle or different signaling mechanism (Fazi et al., 2005; Liu et al., 2010). In a cohort of 70 newly diagnosed childhood ALL patients, downregulation of miR-223 proved its association with the individual relapse-free survival (Han et al., 2011).

miR-203 gene is within a fragile region on chromosome region 14q32.33 (Calin et al., 2004). Downregulation of miR-203 in childhood

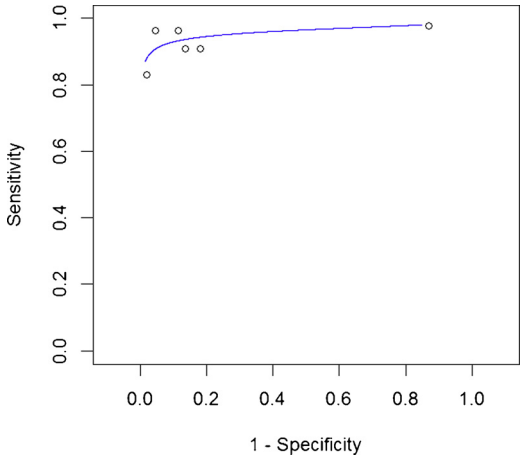


**Fig. 3.** The summary receiver operator curve (ROC) of the six microRNAs.

ALL has been investigated to be a diagnostic molecular biomarker in childhood ALL (Swellam et al., 2018). Also, miR-203 was reported to be associated with poor prognosis in pediatric ALL (Wang et al., 2013). Dysregulation of miR-203 is associated with genetic and epigenetic mechanisms in hematological malignancy (Bueno et al., 2008; Chim et al., 2011).

MiR-181a and miR-181b were reported to be highly expressed in childhood ALL by many studies (Duyu et al., 2014; Li et al., 2013b; Nabhan et al., 2017; Ramani et al., 2017; Zanette et al., 2007; Zhang et al., 2009). The sensitivity & specificity of miR-181a were 86.5% & 93.3%, respectively.

MiR-146a had 100% specificity and 100% sensitivity in childhood ALL (Swellam and El-Khazragy, 2016). Duyu et al. study reported the association of miR-181a, miR-146a as well as miR-155 overexpression with down-regulation of genes included in the innate immunity and inflammation (Duyu et al., 2014). So, their overexpression may play role in leukmogenesis via dysregulation of the normal immune function.

In mi-RNA members of let-7 family, let-7e is significantly upregulated in childhood ALL (Ramani et al., 2017; Zhang et al., 2009). Let-7b gene is located on chromosome 22q13.31. Both let-7b and let-7c are significantly upregulated in *MLL* rearranged-ALL, while let-7c is upregulated in *ETV-RUNX1* patients (Schotte et al., 2011). Using one of target prediction databases, Schotte et al. has reported target genes for most discriminative miRNAs per some subtypes of childhood ALL. The

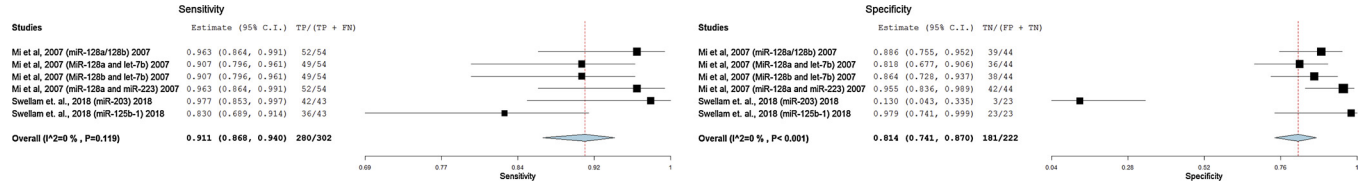


**Fig. 2.** Forest plot of the pooled estimated sensitivity and specificity.

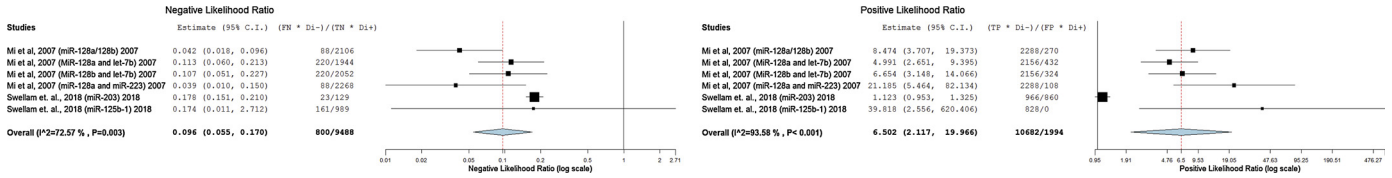


**Fig. 4.** Forest plot of the pooled estimates of positive and negative likelihood ratio of the six microRNAs.

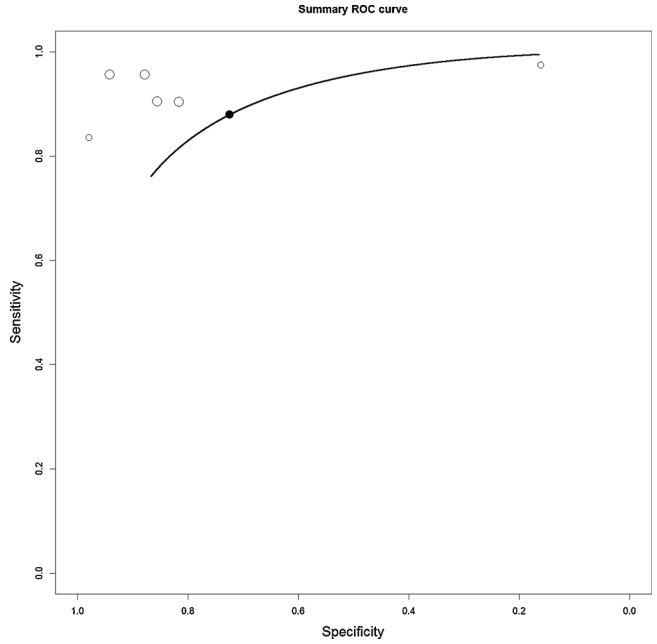


**Fig. 5.** Summary curve from the ROC model of the six microRNAs indicating the summary operating point, the circle bullet is the summary value for sensitivity and specificity, the empty bullets are the studies estimates.

let-7 family has two documented targets (RAS and c-MYC). Although *MLL*-rearranged ALL patients have 3- to 5-fold up-regulated levels of mRNA for both RAS and c-MYC ($P < 0.0001$), but only *c-Myc* protein showed a 2-fold up-regulation ($P < 0.05$).

MiR-21 gene is located on chromosome 17q23.1 (Lagos-Quintana et al., 2001). The upregulation of miR-21 has been reported to play an important role in the biology of T-acute lymphoblastic leukemia. It affects JAK/STAT pathway as it inhibits STAT3 protein expression, promotes both proliferation and invasion and decreases apoptosis (Shi et al., 2016). It also controls survival, in part, through repression of the tumor suppressor gene (*PDCD4*) (Junker et al., 2015). Also, miR-21 plays an important role as a prognostic biomarker in childhood B-ALL (Labib et al., 2017).

MiR-326 and miR-200c genes are located on chromosome 11q13.4 and 12p13.31, respectively. The DORs of both miR-326 and miR-200c were the lowest in our study. The inverse relation between miR-326 and the multidrug resistance in pediatric ALL via ABCA2 transporter adds a prognostic significance to its diagnostic impact (Ghodousi and Rahgozar, 2018).

All these data along with our results should get attention of scientists to the importance of these miRNAs (miR-128a, miR-128b, miR-203, miR-223, let-7b, miR-21, miR-326 and miR-200c) as potential diagnostic biomarkers to be incorporated in future large studies. In addition, the prognostic impact of some of them in childhood ALL is recommended to be investigated in large cohort studies.

Schotte et al. study was the only study that was able to highlight the role of miRNAs in different ALL subtypes (Schotte et al., 2011):

- In *BCR-ABL*-positive ALL samples, the heterogeneity nature of miRNA subtype in addition to the low number with low fold changes of significantly expressed mi-RNA indicated that miRNA profile in this subtype plays a minor role.
- In hyperdiploid ALL subtype, miR-223, miR-222/222*, miR-98 and miR-511 have relatively high expressions and genes encoding these miRNAs were located on two chromosomes (10 or X) whose extra copies are commonly found in hyperdiploid patients. The sensitivity and specificity of miR-511, miR-222 and miR-223 were reported by a Mexican study, in Table 2, in which miR-511 had 100% sensitivity and 100% specificity (Luna-Aguirre et al., 2015).
- Also, In *ETV-RUNX1*-positive ALL subtype samples, various miRNAs including miR-99a, miR-100, miR-125b, miR-383 and let-7c showed 5- to 1700-fold up-regulation ($P_{FDR} < 0.001$) (Schotte et al., 2011).
- MiR-196b is normally promoting proliferation and survival of hematopoietic cells. miR-196b is highly expressed in both *MLL* rearranged-ALL and T-ALL which may be *HOXA* genes-driven activation. Also, this high expression in *MLL* rearranged-ALL can be explained based on DNA hypo-methylation of the miR-196b-embedded *HOXA* area (Schotte et al., 2011).
- The miR-708-5p is the most significantly dysregulated miRNA in childhood precursor B-cell ALL reported by many studies (Duyu et al., 2014; Li et al., 2013b; Schotte et al., 2011). Also, it was reported to have a role in glucocorticoids response and ALL risk classification (Han et al., 2011). In addition, high miR-708 expression was reported to be associated with *ETV6 deletion* (Krzanowski et al., 2017). Worthy of note, miR-708-5p has no association with clinical features (de Oliveira et al., 2015). Recently, miR-708 was studied as a candidate immunotherapy (anti-CD47) in T-cell (Huang et al., 2018).

Actually, we highly recommend Schotte et al. study design as it optimized the utilization of miRNA to diagnose different subtypes of childhood ALL. Consequently, microRNA will be a valuable diagnostic tool in the risk stratification and treatment allocation of pediatric ALL patients.

We have noticed the controversy in reporting dysregulation of some miRNAs. Both miR-100 and miR-99a whose expressions were significantly lower in ALL in comparison to AML and healthy control (Li et al., 2013c). Hesowever, both of them were reported to be overexpressed in childhood ALL by another study (Schotte et al., 2011). Large studies should be done to assess the expression level of both miR-100 and miR-99a and their role as a diagnostic biomarker in childhood ALL.

Our study has certain limitations that include: (1)- Many studies had different sources of samples either cases or controls (BM and peripheral blood" PB"). It was reported that there is a difference between miRNA withdrawn from PB to that from BM in leukemic cases (Duyu et al., 2014). Recently, it is recommended to study miRNAs from PB due to its stability (Glinge et al., 2017; Grasedieck et al., 2013). (2)- Not all studies included healthy control. (3)- Some studies didn't have certain cut-off value of leukemic blasts for recruited cases. (4) some studies didn't do validation for predicted miRNAs. (5)- One study have used Gene Ontology (GO) enrichment analysis and reported the role of m**iR-1986** in CNS relapse in ALL based on its putative target *sox11* gene that is related to nervous system development (Zhang et al., 2009). Due to observed annotation bias in the GO annotations (Tomczak et al., 2018), caution should be taken for studies that use GO enrichment analyses for

**Table 3**
Some of putative targets and implicated signaling pathways for certain miRNAs in childhood ALL.

| Study | miRNA | Relative Expression | Locus | Effect on Putative targets | Function |
|---|---|---|---|---|---|
| (Zanette et al., 2007) | miR-331 | 74.03[a] | 12q22 | SOCS1 down-regulation | Promotes STAT activation with increased cell proliferation. |
| | miR-17-92 cluster:<br>-miR-17-3p,<br>-miR-17-5p, -miR-19a,<br>-miR-20,<br>-miR-92. | In order[a] :<br>−2.56<br>−6.36<br>−6.99<br>−9.06<br>−5.70 | 13q31.3 | Regulated by c-MYC | -Apoptosis suppression.<br>-Transformation of B-cell progenitor. |
| (Ju et al., 2009) | miR-142{3p | 2.47 | 17q23.2 | BCLAF1, LIFR, BCL2L1 downregulation | Apoptosis, lineage commitment |
| | miR-222 | 3.22 | Xp11.3 | Inhibit c-KIT | Proto-oncogene |
| | miR-373 | 0.39 | 19q13.42 | BCL11 A, BTG1 | Regulates cell proliferation |
| | miR-339 | 3.44 | 7p22.3 | BCL-6 gene | Cell survival and proliferation |
| | miR-451 | 0.12 | 17q11.2 | Target AKTIP, FBXO33, calcium binding protein 39. | Cell proliferation |
| (Han et al., 2011; Piatopoulou et al., 2018; Schotte et al., 2011; Shen et al., 2014; Zhang et al., 2009) | miR-143 | 0.04 | 5q32 | Target BCL-2 gene (anti-apoptotic) and PI3K/Akt/mTOR pathway. | Leukemogenesis |
| (Li et al., 2013c) | MiR-708 | 9.02 | 11q14.1 | Regulate CNTFR, NNAT, and GNG12 genes | Leukemogenesis |
| (Li et al., 2013b) | MiR-100 and miR-99a | NA | 11q24.1 and 21q21.1 | Target both FKBP51 and IGF1R/mTOR pathway | Promotes the response to Dex- induced apoptosis |
| Cao et al., 2016 | miR-34b | 1.65 ± 0.69 | 11q23.1 | Regulated by CpG island hypermethylation | Inhibits leukemia-cell proliferation |
| (Swellam et al., 2018) | miR-203 | 0.03 | 14q32.33 | -ABL1<br>-novel miR-203/survivin/Bmi-1 | -Tumor suppressor<br>-regulation of biological properties of leukemia stem cells. |

**Abbreviations: AKTIP**: AKT interacting protein, **BCLAF1**: BCL2-associated transcription factor 1; **BCL2L1**: BCL2-like 1; **FBXO33**: F-box protein 33; **FKBP51**: FK506-binding protein 51: **LIFR**: leukemia inhibitory factor receptor alpha; **SOCS1**: suppressor of cytokine signaling.

[a] These numbers are relative fold values to that obtained from $CD19^{+}$ normal (control) subjects pool.

interpreting the high throughput molecular data. Also, researchers must revise previous results with the most updated versions of GO.

On the other hand, our study has certain advantages: (1). We concluded some miRNAs that can be used as a diagnostic discriminatory biomarker between ALL and AML. (2). Pooling data of some studies enable us to calculate DOR but further large cohort studies are needed to confirm our results.

## 5. Recommendations

Reproducibility of data-based biomedical research gets attention as an important topic for discussion in favor of the feasibility of clinical translation. Following a certain strategy or methodology, as the below-mentioned recommendations, allows researchers to do systematic review and/or meta-analysis studies with optimized conclusion. These studies may help to get final conclusion regarding the everyday publications regarding the use of miRNA as a diagnostic biomarker in childhood ALL. The presence of consensus publication guidelines/checklist that each researcher should take in consideration while setting up miRNA-based research study can lead to optimization of these published data.

1 **Study Design:** Case-control study design is thought to be a valid and optimum design to study the usefulness of miRNA as a diagnostic biomarker in childhood ALL. It is recommended to choose cases of certain ALL subtype (B or T lineage) and control from healthy population. It is valuable to include AML patients to test the index miRNA as diagnostic discriminatory biomarker between ALL and AML.

2 **Sample size:** It will be valuable to study miRNA as a diagnostic biomarker in childhood ALL using a large sample size to get a proper statistical power. Large sample size can be easily achieved through collaboration between specialized centers.

3 **Sample source:** It was reported that there is a difference between miRNA withdrawn from PB to that from BM in leukemic cases (Duyu et al., 2014). Circulating miRNAs show significant stability, making them potential biomarkers (Grasedieck et al., 2013; Mitchell et al., 2008). So, using non-invasive approach of blood-based detection of tumor-derived miRNAs (serum or plasma) is recommended in the hematology field (Glinge et al., 2017).

4 **Leukemic blast:** It is recommended to put cut-off value for the percentage of leukemia blasts in the initial samples taken from the study participants. In addition, it is recommended to use enrichment method (Ramani et al., 2017) in case of samples with leukemic blast percentage less than the cut-off value predetermined for inclusion in the study (if possible), otherwise, it should be excluded from the study.

5 **Validating the expression of predicted miRNA:** In general, prediction followed by validation of miRNAs expression is carried out according to pre-determined algorithm (Bentwich, 2005). Also, factors that may affect techniques used in expression validation should be considered (Morey et al., 2006). It should be carried out in the same population as microRNA expression may be population specific.

6 **Gene Ontology (GO):** GO is a valuable tool for interpretation of the high throughput gene expression data. Recently, it was reported to take **c**aution for studies that used Gene Ontology (GO) enrichment analyses for interpretations of their results to avoid annotation bias in the GO annotations (Tomczak et al., 2018). It is important to use the most updated version of GO annotation.

7 **Target prediction database:** Supplementary Table (S1) shows many free online databases for miRNA target prediction that can be used.

8 **Validation of predicted target genes:** Target prediction of miRNA followed by biological validation techniques are the core steps in most research studies for novel miRNA discovery. Researchers can use Venn diagrams (Venny[2.1], http://bioinfogp.cnb.csic.es/tools/venny/) to compare lists of the predicted target genes then select the highest related to the index miRNA. In general, it is recommended that validation of predicted target genes should be done on the same population/ethnicity as gene expression can be population-specific.

## Conflict of interests

No potential conflicts of interest were disclosed by the authors.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.critrevonc.2019.02.008.

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