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Clinical significance of aberrant microRNAs expression in predicting disease relapse/refractoriness to treatment in diffuse large B-cell lymphoma: A meta-analysis

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ABSTRACT

The clinical significance of aberrantly expressed microRNAs in predicting treatment response to chemotherapy in diffuse large B-cell lymphoma patients (DLBCL) remains uncertain. Feasibility of microRNA testing to predict treatment outcome was evaluated. Twenty-two types of aberrantly expressed microRNAs were associated with poor treatment response; pooled hazard ratio (HR) was 2.14 [95%CI:1.78-2.57, P < 0.00001]. DLBCL patients with aberrant expression of miR-155, miR-17/92 clusters, miR-21, miR-224, or miR-146b-5p had a higher risk of treatment resistance or shorter period of disease relapse/progression free survival, with HR = 2.71 (95%CI:1.66-4.42, P < 0.0001), HR = 2.70 (95%CI:1.50-4.85, P = 0.0010), HR = 2.20 (95%CI:1.31-3.69, P = 0.003), HR = 2.07 (95%CI:1.50-2.86, P < 0.00001), HR = 2.26 (95%CI:1.40-3.65, P = 0.0009), respectively. The association between aberrant expression of microRNAs and treatment response appears to be stronger in formalin-fixed-paraffin-embedded tissue (HR = 2.41, 95%CI:1.79-3.25, P < 0.00001) than in fresh-frozen samples (HR = 1.94, 95%CI: 1.22–3.08, P = 0.005) and peripheral blood samples (HR = 1.94, 95%CI:1.53-2.46, P < 0.00001). Mir-155, miR-17/92 clusters, miR-21, miR-224, and mir-146b-5p have value in predicting treatment response to chemotherapy in DLBCL.

1. Introduction

Incorporation of rituximab to the frontline chemotherapy regimens cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) for DLBCL has significantly improved the survival of DLBCL patients (Younes, 2013). Although the majority of patients achieved complete response from this chemotherapy regimen, about 30% of patients relapsed or were refractory to treatment within 24 months after diagnosis. The prognosis of this group of patients is usually poor and their median survival period is reported to be only 10 months (Maurer et al., 2016).

Treatment resistance is a complex phenomenon. It is driven by tumor microenvironment, genetic alteration such as single nucleotide polymorphism (which reduces drug effectiveness), expression of tumor suppressor genes, and the presence of oncogenes (Senthebane et al., 2017; Zheng, 2017). Dysregulation of microRNA expression has been postulated to be one of the factors contributing to treatment failure. MicroRNAs affect the efficacy of drugs in a few ways. These include aberrant expression of microRNAs which alters the production of target mRNA and downstream proteins expression and hence reduces drug function (Leskelä et al., 2011); single nucleotide polymorphisms on microRNA disrupting the binding between microRNA and target mRNA and restraining drug mechanism of action (Mishra et al., 2007) and aberrant expression microRNAs which suppress expression of pro-apoptosis proteins and restrict drug-induced apoptosis (Shi et al., 2010). A number of target genes (mRNAs) have been found to be

Abbreviations: MiR, microRNA; DLBCL, diffuse large B-cell lymphoma; PFS, progression free survival; DR/PFS, disease relapse/progression free survival period; HR, hazard ratio; CI, confidence interval; FFPE, formalin-fixed-paraffin-embedded; RCHOP, rituximab cyclophosphamide, doxorubicin, vincristine and prednisone; PI3K/Akt, phosphoinositide-3-kinase–protein kinase B/Akt signaling pathway

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involved in drug metabolism. For instance, BCL2 gene showed counter-regulation effects on vincristine, etoposide, adriamycin and cisplatin. Therefore, down-regulation of miR-15b and miR-16 (the regulators of BCL2 gene) could up-regulate BCL2 gene and contribute to resistance to these drugs (Xia et al., 2008). Aberrant expression of miR-21 was also shown to affect the tumor cell sensitivity to RCHOP chemotherapy. Up-regulation of miR-21 can induce chemoresistance through suppression of the PTEN gene (Bai et al., 2013).

Besides having direct impact on efficacy, alteration in microRNAs expression also affects molecular signaling pathways that mediate chemoresistance. Aberrant expression of microRNAs causes defects in cell cycle and apoptotic machinery (Cittelly et al., 2010), inducing autophagy instead of cell apoptosis (Xu et al., 2016) and may alter drug targets and the process of DNA repair (Valeri et al., 2010). These studies have suggested that aberrant expression of microRNAs have a profound impact on treatment outcome.

In order to clarify the prognostic value of aberrant expression of microRNAs, a comprehensive meta-analysis on the association between treatment resistance and aberrant expression of microRNAs was conducted. The aim of this review was to investigate the literature on the microRNAs for prediction of treatment resistance, disease relapse and good response to chemotherapy in patients with diffuse large B-cell lymphoma. The pooled effect size and the pattern of aberrant expression of each type of microRNA was determined. The feasibility of performing microRNA assays on peripheral blood, formalin-fixed paraffin embedded tissue, and fresh tissue samples was also evaluated in order to find out the most appropriate sample for microRNA testing.

2. Materials and methods

This systematic review and meta-analysis were carried out based on the guidelines provided by PRISMA (Moher et al., 2009), AMSTAR (Shea et al., 2017), and COCHRANE (Furlan et al., 2009).

2.1. Search strategy

The following electronic medical literature databases were searched from inception January 2000 to August 2018: PUBMED, Embase, Cochrane database of Systematic Review (CDSR), Cochrane Central Register of Controlled Trials (CENTRAL). PROSPERO (International Prospective Register of Systematic Reviews) and ClinicalTrials.gov (U.S. National Library of Medicine Clinical Trials Registry) were also searched for articles that fit the criteria. The Medical Subject Headings text-words terms for the biomarker “microRNA”, “miR” and disease “lymphoma, large B-cell, diffuse” were combined in the search strategy (Table 1).

2.1.1. Inclusion criteria

All published and unpublished prospective studies, retrospective studies, case control studies, cohort studies, clinical trials were eligible for inclusion if the following criteria were fulfilled:

i MicroRNAs studies on diffuse large B-cell lymphoma disease (DLBCL)
ii Limited to human subjects
iii No restriction on the language of published manuscript

Table 1
Text-words terms used in the literature search.

No	MeSH terms/text words used
1	MicroRNA (MeSH) **AND** Lymphoma, Large B-cell, Diffuse (MeSH)
2	MicroRNA **AND** Lymphoma **AND** Large B-cell **AND** Diffuse
3	MiR **AND** Lymphoma, Large B-cell, Diffuse
4	MiR **AND** Lymphoma **AND** Large B-cell AND Diffuse
5	MicroRNA **AND** Lymphoma* **AND** Large B-cell* **AND** Diffuse
6	MiR **AND** Lymphoma* **AND** Large B-cell* **AND** Diffuse

2.1.2. Exclusion criteria

We excluded studies on animals, DLBCL cell lines and DLBCL xenografts. Studies on other type of lymphomas besides DLBCL and DLBCL treated with stem cell transplantation were excluded. Review articles reporting duplicate data were also excluded.

2.1.3. Outcome measures

Treatment response was assessed as good response to treatment or disease relapse/refractory. Other outcomes used in the studies were duration of disease to relapse or progression free survival (shorter or longer period of survival with aberrant expression of microRNAs).

2.2. Data extraction

References were imported into EndNote X8, then duplicates were removed in Mendeley citation manager and finally exported to Rayyan QCRI software for screening. All articles were independently screened and reviewed by two reviewers (TCY and BPC). Initially, article titles and abstracts were screened to exclude studies irrelevant to inclusion criteria. Then, independent reviews of the full text of the articles were conducted to identify and select studies that met the requirements of inclusion criteria. Disagreement on article selection was resolved through discussion between the two reviewers. The third reviewer (LSM) determined the eligibility of articles where consensus could not be reached between TCY and BPC.

2.3. Quality assessment of the studies

Two reviewers independently performed quality assessment on the articles retrieved from the databases. Quality was assessed on six areas of potential biases on prognosis studies using criteria set by Hayden C et al. 2006 (Hayden et al., 2006).

a) **Study participants were clearly defined**: Patients with diffuse large B-cell lymphoma disease
b) **Adequate follow up**: Minimum follow up period of 24 months or completion of standard chemotherapy treatment with loss to follow up cases limited to less than 5% of initial data.
c) **The prognostic factor of interest is adequately measured**: Quantitative Polymerase Chain Reaction or microarray, preliminary micoRNAs findings must be validated by qRT-PCR.
d) **The outcome of interest is adequately measured**:
e) Resistant or good response to treatment or
f) Duration of disease relapse or progression free survival.
g) **Important potential confounders are appropriately accounted for**: No statistical differences between study cohort and controls. Patients treated with stem cell transplantation were excluded.
h) **The statistical analysis is appropriate for the design of study**: All study participants’ results must be included in data analysis. There must not be any bias in calculations.

2.4. Statistical analysis

Statistical analysis was performed on RevMan software. The significance of each subtype of microRNA in predicting treatment response was compared across the studies. For microRNA subtypes with two or more studies, a meta-analysis was performed. The pooled hazard ratio was calculated by incorporating the weight of individual studies and their variances. A random effects model was applied for pooled hazard ratio and 95% confidence interval calculations. Two tailed statistical analysis was performed and a P value of < 0.05 was considered significant. Higgins test (I^2) was used to describe heterogeneity across studies. Low heterogeneity is defined as $I^2 < 25\%$, moderate heterogeneity I^2: 25%–75% and high heterogeneity $I^2 > 75\%$. Publication

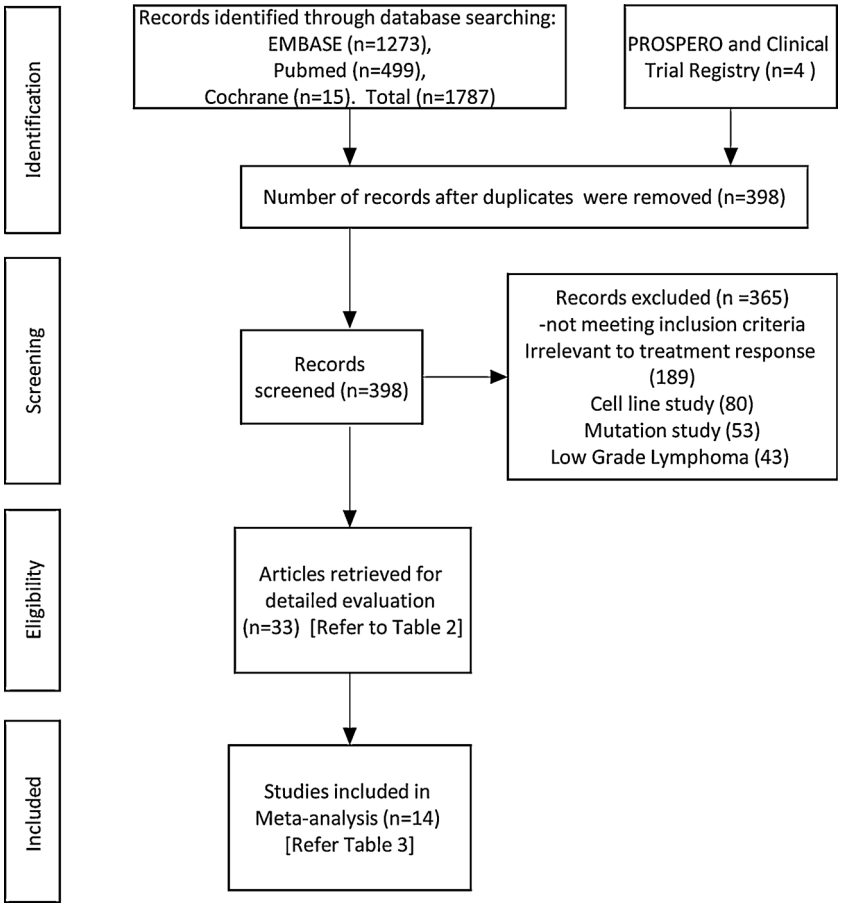


Fig. 1. Screening of the literature.

bias for each microRNA was assessed by funnel plot and Egger's regression asymmetry.

2.5. Subgroup analysis

As this review included all types of microRNAs aberrantly expressed in DLBCL disease, subgroup analysis was performed to improve the precision of estimated hazard effect of each type of microRNA on DLBCL patients. As for subgroup meta-analysis, pooled hazard ratio was calculated only if there were three or more publications for a specific microRNA.

3. Results

Fig. 1: Flowchart of the selection process of relevant studies for meta-analysis according to Moher D et al. 2009 guidelines.

3.1. Screening of the literature

A total of 1787 articles were identified from the initial electronic database search, and 1393 duplicates were removed. Four clinical trials were retrieved from PROSPERO (International Prospective Register of Systematic Reviews) and ClinicalTrials.gov. Titles and abstracts screening were performed on the 398 articles identified from electronic databases and only 33 articles addressed association between microRNAs expression and treatment responses in DLBCL patients. These 33 articles were further evaluated for their potential inclusion for meta-analysis. Of these, seven articles reported on miR-155, seven articles on miR-21, four articles on miR-222, two studies on miR-146b-5p, one on miR-146a, three studies on miR-224, two studies on miR-199a/miR-199b, two studies on miR-34a, two studies on miR-20a/miR-20b, two one study on miR-221, miR-222, miR-331, miR-451, miR-28, miR-151, miR-148a, miR-93, miR-491, one study on miR-127, miR-let7, miR-19a, one study on miR-22, one study on miR-200c, one study on miR-320d, and one study on miR-497. These 33 publications fulfilled the inclusion criteria and were critically appraised on their quality. Finally, 14 studies were combined in the current meta-analysis (Figs. 2).

3.2. Risk of selection bias

As shown on Table 2, risk of selection bias was low across the included studies in this review. Only 14 of 33 articles (42.4%) had no biases on all six parameters assessed (Hayden et al., 2006) and 48.5% (16 of 33 articles) fulfilled five of six parameters of bias assessment. The only parameter in which these primary studies failed to meet the requirement was "choosing appropriate statistical analysis tools for the study". Of the 33 articles, only 42.4% (14 articles) performed cox proportional hazard regression analysis and reported on hazard ratios of aberrantly expressed microRNAs, one article reported risk ratios, one reported odds ratio and the remaining 18 studies reported P values without confidence intervals.

3.3. Studies included for meta-analysis

Table 3 shows the findings on aberrant microRNA expression in DLBCL patients and their treatment outcomes for each primary study included in this meta-analysis.

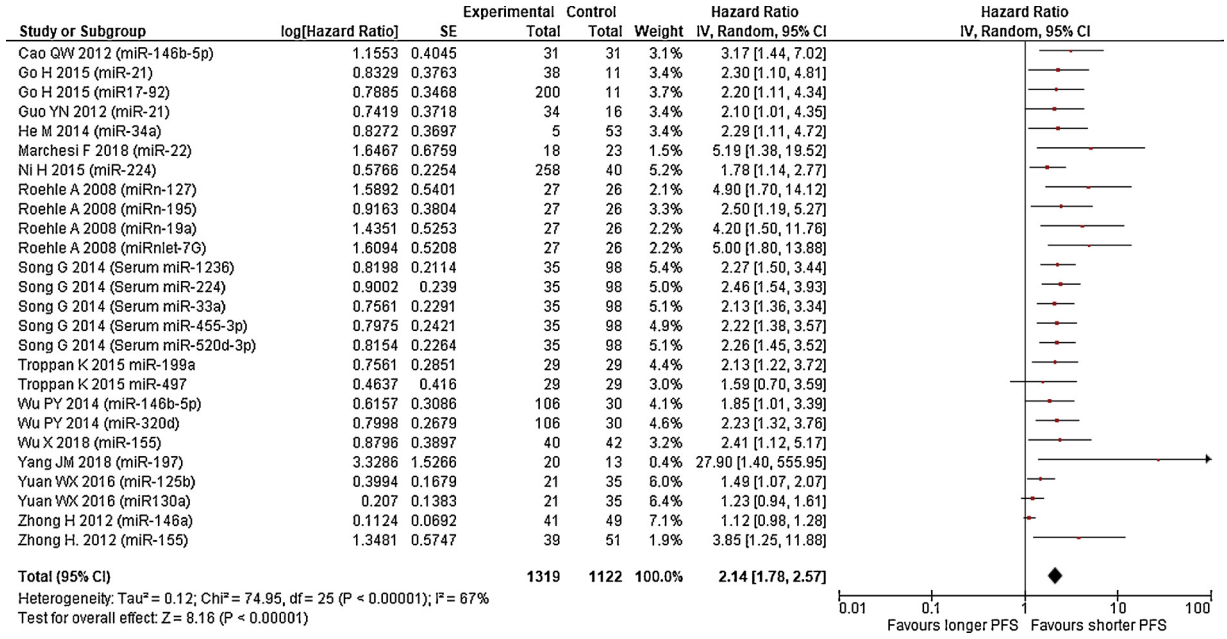


Fig. 2. Forest plot of meta-analysis of aberrant expression of microRNAs and treatment outcome. The pooled HR and 95% CI were calculated using a random effects model.

3.4. Included studies for meta-analysis

Fourteen articles met the quality appraisal requirements for inclusion in the meta-analysis as shown on Table 3. The year of publication ranged from 2008 to 2018. The duration of follow up ranged from 24 to 150 months. 11 studies were done in eastern countries while three studies were from the western countries. The number of studies performed on formalin fixed paraffin embedded tissue sample, peripheral blood/serum samples and fresh frozen tissue samples were 10 (71.4%), three (21.4%) and one (7.1%), respectively. In 14 of the publications,

Table 2
Quality appraisal for studies included in this systematic review and meta-analysis.

No	Journal	Year	Study Participation	Study Attrition	Prognostic factor	Outcome Measurement	Confounding measurement and account	Analysis
1	(Lawrie et al. (2008))	2008	+	+	+	+	+	?
2	(Roehle et al., 2008)	2008	+	+	+	+	+	+
3	(Jung and Aguiar, 2009)	2009	+	+	?	+	+	?
4	(Malumbres et al., 2009)	2009	+	+	+	+	?	?
5	(Montes-Moreno et al. (2011))	2011	+	?	+	+	+	?
6	(Alencar et al., 2011)	2011	+	+	+	+	+	?
7	(Yanan et al., 2012)	2012	+	+	+	+	+	+
8	(Zhong et al., 2012)	2012	+	+	+	+	?	+
9	(Cao et al., 2012)	2012	+	+	+	+	+	+
10	(Culpin et al., 2013)	2013	+	+	+	+	+	?
11	(Berglund et al., 2013)	2013	+	+	+	+	+	?
12	(Song et al., 2014a)	2014	+	+	+	+	+	+
13	(He et al., 2014)	2014	+	+	+	+	+	+
14	(Chen et al., 2014)	2014	+	+	+	+	+	?
15	(Wu et al., 2014)	2014	+	+	+	+	+	+
16	(Song et al., 2014b)	2014	+	+	+	+	+	?
17	(Go et al., 2015)	2015	+	+	+	+	+	+
18	(Troppan et al., 2015)	2015	+	+	+	+	+	+
19	(Lim et al., 2015)	2015	+	+	+	+	+	?
20	(Ni et al., 2015)	2015	+	+	+	+	+	+
21	(Shepshelovich et al., 2015)	2015	+	+	+	+	+	?
22	(Iqbal et al., 2015)	2015	+	+	+	+	+	?
23	(Battistella et al., 2015)	2015	+	+	+	+	+	?
24	(Marques et al., 2016)	2016	+	+	+	+	+	?
25	(Yuan et al., 2016)	2016	+	+	+	+	+	+
26	(El-Halawani et al., 2017)	2017	+	+	+	+	+	?
27	(Leivonen et al., 2017)	2017	+	+	+	+	+	?
28	(Bedewy et al. (2017))	2017	+	+	+	+	+	+
29	(Wu et al., 2017)	2017	+	?	+	+	+	+
30	(Zheng et al., 2017)	2017	+	+	+	+	+	?
31	(Marchesi et al., 2018)	2018	+	+	+	+	+	+
32	(Wu et al., 2018)	2018	+	+	+	+	+	+
33	(Yang et al., 2018)	2018	+	+	+	+	+	+

Symbols used + : Low risk of bias ? : Uncertain.

Table 3
Primary studies included in this meta-analysis.

	Journal	Country	Biomarker	Sample Type	Main Findings
1	(Roehle et al., 2008)	Germany, Hungary	miRn-19a, miRn-127, miRn-195, miRnlet-7G	FFPE	Down-regulation of miRn-19a and miR-127 were significantly associated with shorter event free survival, while down-regulation of miRn-Let-7 and miRn-195 contributed to longer event free survival.
2	(Yanan et al., 2012)	China	miR-21	FFPE	High miR-21 was associated with shorter survival (P = 0.046).
3	(Zhong et al., 2012)	China	miR-155 and miR-146a	FFPE	DLBCL with low levels of miR-155 and miR-146a had longer 5-year progression free survival (83.3 ± 5.9 vs. 44.3 ± 10.8%, P = 0.013; 92 ± 4.4 vs. 43.5 ± 9.7%, P < 0.001).
4	(Cao et al., 2012)	China	miR-146b-5p	FFPE	Down-regulation of miR-146b-5p was associated with shorter relapse-free survival (P = 0.005).
5	(He et al., 2014)	China	miR-34a	FFPE	Down-regulation of miR-34a positive is associated with shorter progression free survival (P = 0.009).
6	(Wu et al., 2014)	China	miR-146b-5p and miR-320d	FFPE	Down-regulation of miR-146b-5p and miR-320d were associated with shorter progression free survival (P = 0.046) and (P = 0.003).
7	(Song et al., 2014a)	China	miR-224, miR-1236, miR-520d-3p, miR-455-3p and miR-33a	Serum	Up-regulation of miR-224, miR-1236, and miR-520d-3p correlated with a higher rate of refractory (P < 0.001), whereas up-regulation of miR-455-3p and miR-33a correlated with a higher possibility of complete remission (P < 0.001).
8	(Go et al., 2015)	Korea	miR-21 and miR-17-92	FFPE	Up-regulation of miR-21 and miR-17-92 was associated with shorter progression-free survival (P = 0.003 and P = 0.014).
9	(Troppan et al., 2015)	Austria	miR-199a or miR-497	Fresh frozen tissue	Up-regulation of miR-199a and miR-497 levels are associated with improved survival and increase chemosensitivity (P = 0.008) and (P = 0.265) respectively.
10	(Ni et al., 2015)	China	miR-224	FFPE	DLBCL patients with down-regulation of miR-224 level had shorter progression free survival compared to those with high levels of miR-224 (P < 0.05).
11	(Yuan et al., 2016)	China	miR-130a and miR125b	FFPE	MiR-130a and miR-125b were upregulated in the drug- resistant group compared with the chemotherapy- sensitive group (P = 0.028 and P = 0.005).
12	(Marchesi et al., 2018)	Italy	miR-22	Serum	High expression level of serum miR-22 in DLBCL at diagnosis is associated with a shorter progression free survival and is an independent prognostic factor.
13	(Wu et al., 2018)	China	miR-155	FFPE	High miR-155 expression was associated with a shorter poor progression-free survival (P = 0.026).
14	(Yang et al., 2018)	Korea	miR-197	FFPE	Low miR-197 was associated with shorter period of progression-free survival in the R-CHOP treated DLBCL (P = 0.031).

polymerase chain reaction technique was applied for quantification of aberrantly expressed microRNAs.

Approximate 71.4% of studies (10/14) measured treatment outcomes by quantifying the period of disease relapse free or progression free survival (PFS), 21.4% (3/14) measured both PFS and the number of patients achieving complete remission and 7.2% (1/14) measured the number of patients who experienced treatment resistance.

3.5. Association between treatment outcome in DLBCL patients and aberrant expression of microRNAs

The current meta-analysis comprised of 865 DLBCL patients with aberrant expression of microRNAs and 498 DLBCL patients DLBCL without aberrant expression of microRNAs. Overall, aberrant expression of 14 types of microRNAs in DLBCL disease were found to be associated with a higher risk of treatment resistance or shorter period of disease relapse/progression free survival, with a pooled hazard ratio of 2.14 (95% CI: 1.78–2.57), P < 0.00001. The overall heterogeneity across the 14 studies with 22 types aberrantly expressed microRNA-s was moderate (I^2 = 67%). The random effects model was applied in the pooled meta-analysis as several types of microRNAs were analyzed in this meta-analysis.

3.6. Significant association between poor treatment outcome and aberrant expression of microRNAs irrespective of specimen types [formalin fixed paraffin embedded tissue (FFPE), peripheral blood/serum and fresh frozen tissue]

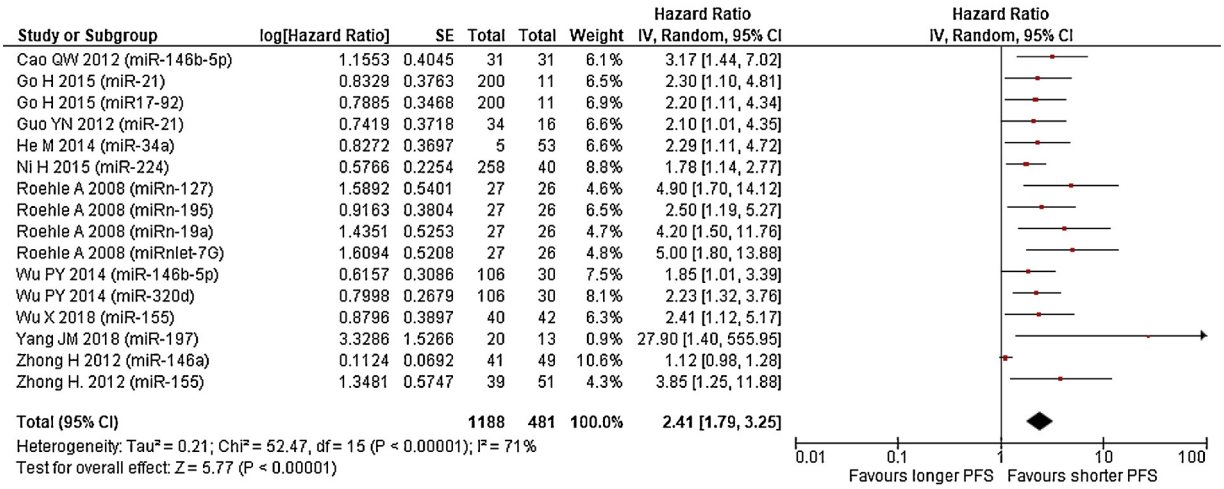
(Fig. 3) Analysis was stratified by sample type, in which studies were divided into three categories, FFPE samples (n = 10 studies, 762 DLBCL with aberrant microRNAs expression and 313 DLBCL without aberrant microRNAs expression), peripheral blood/serum (n = 3 studies, 74 DLBCL with aberrant microRNAs expression and 156 DLBCL without aberrant microRNAs expression) and fresh frozen tissue (n = 1 study, 29 DLBCL with aberrant microRNAs expression and 29 DLBCL without aberrant microRNAs expression). Meta-analysis showed consistent positive association between poor treatment outcome and aberrant expression of microRNAs in FFPE samples (HR: 2.41, 95% CI: 1.79–3.25, P < 0.00001); in peripheral blood/serum samples (HR: 1.94, 95% CI: 1.53–2.46, P < 0.00001) and in fresh frozen samples (HR: 1.94, 95%CI: 1.22–3.08, P = 0.005). The hazard effect was highest in FFPE tissue (HR: 2.41), while both fresh frozen tissue and peripheral blood/serum samples showed comparable hazard ratios (HR:1.94). Compared to peripheral blood/serum samples (I^2: 58%) and fresh frozen samples (I^2: 0%), heterogeneity was highest in FFPE samples (I^2: 71%) as it integrated 10 studies with 13 types of microRNAs.

3.7. Association between up-regulation of miR-155 and treatment outcome

(Fig. 4) MiR-155 has been reported as a regulator of Phosphatidylinositol-3 kinases/AKT signaling pathway which promotes proliferation of cancer cells (Jablonska et al., 2015). Three studies found positive association between aberrant miR-155 and poor treatment outcomes. The duration of follow up ranged from 60 to 96 months. Meta-analysis of these three studies with a total of 161 DLBCL with aberrant microRNAs expression and 93 DLBCL without aberrant microRNAs expression showed that up-regulation expression of miR-155 was associated with higher risk of treatment resistance or shorter period of disease relapse/progression free survival (HR = 2.71, 95%CI: 1.66 to 4.42, P < 0.0001). Heterogeneity was low (I^2: 0%) and there was no publication bias.

At the 24 month follow up, the rate of disease progression was high (80%) in DLBCL patients with aberrant expression of miR-155 group, but was much lower (20%) in DLBCL with normal level of miR-155 group (Wu et al., 2018). Similarly, Zhong H et al. 2012 (Zhong et al., 2012) reported that the five-year progression free survival rates for

(a)



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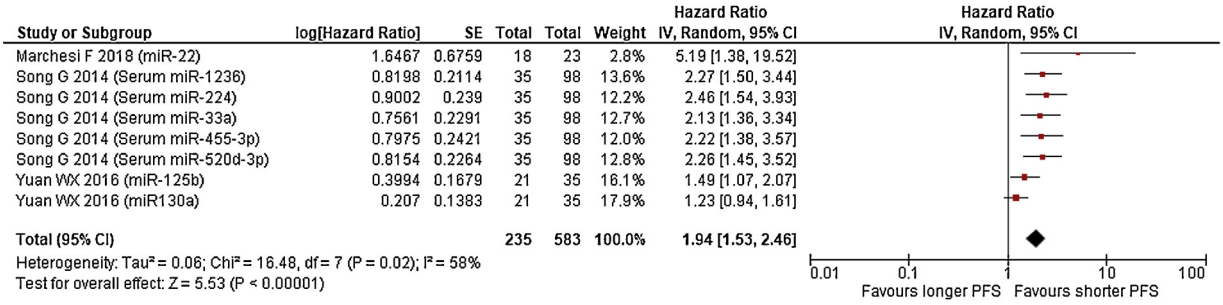

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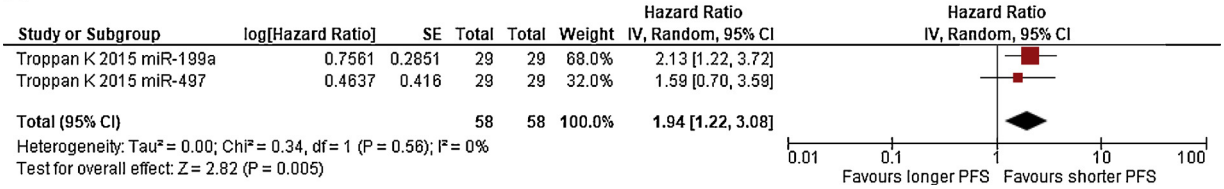

Fig. 3. Forest plot of stratified analysis by type of specimen (a) Formalin-fixed paraffin embedded tissue sample (n = 12 studies. (b) Peripheral blood/serum sample (n = 2 studies). (c) fresh frozen tissue sample (n = 1 study).

DLBCL patients without aberrant expression of miR-155 were better than DLBCL patients with aberrant expression of miR-155 (83.3 ± 5.9 vs. 44.3 ± 10.8%, P = 0.013). Another study (Wu et al., 2017) also concluded that miR-155 is a reliable biomarker to predict treatment response in DLBCL patients.

3.8. Association between aberrant expression of miR-17/92 cluster and treatment outcome

(Fig. 5) The members of miR-17/92 clusters include miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR-92a-1 (Mogilyansky and Rigoutsos, 2013). Two studies comprising 227 DLBCL patients with up-regulation of miR-17/92 and 37 DLBCL without up-regulation of miR-17/92 (controls) were included in this analysis. The duration of follow up ranged from 100 to 120 months (Roehle et al., 2008; Go et al., 2015). The risk of having short period of disease relapse/progression free survival was HR = 2.70 (95% CI: 1.50 to 4.85, P = 0.0010). Heterogeneity between these two studies was low (I^2 = 5%).

3.9. Association between up-regulation of miR-21 and treatment outcome

(Fig. 6) Up-regulation of miR-21 has been linked to various types of

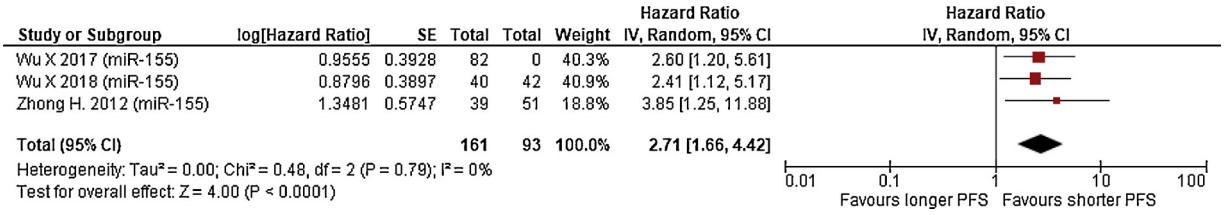

Fig. 4. Forest plot of meta-analysis of up-regulated miR-155 level and treatment outcome. The pooled HR and 95% CI were calculated using a random effects model.

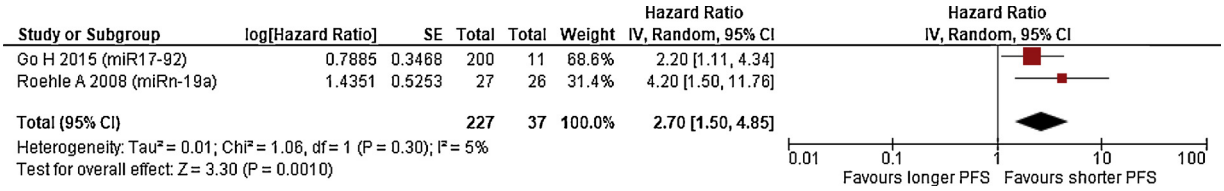

Fig. 5. Forest plot of meta-analysis of up-regulation of miR-17/92 cluster and treatment outcome. The pooled HR and 95% CI were calculated using a random effects model.

cancer which include non-small-cell lung cancer, gastric cancer, colorectal cancer and breast cancer. In this review, seven publications on miR-21 were retrieved, of which only two papers reported hazard ratios of aberrant expression of miR-21.

The total number of samples were 72 cases of DLBCL with up-regulated miR-21 and 27 cases of DLBCL without up-regulated miR-21 levels; with duration of follow up of 120 months. Overall analysis showed a significant association between up-regulation of miR-21 and a higher risk of treatment resistance or shorter period of disease relapse/progression free survival, with a pooled hazard ratio of 2.20 (95% CI: 1.31–3.69), P value was 0.003. Heterogeneity was low (I^2: 0%) and there was no publication bias.

At 120th month of follow up, the rate of disease relapse or disease progression was approximate 70% in DLBCL with up-regulated miR-21 and only 35% in DLBCL without up-regulated miR-21 group (Go et al., 2015). In addition, the survival rate of DLBCL with up-regulated miR-21 was significantly lower than DLBCL without up-regulated miR-21 levels (P = 0.038) (Yanan et al., 2012).

3.10. Association between aberrant expression of miR-224 and treatment outcome

(Fig. 7) Meta-analysis of these two studies demonstrated that aberrant expression of miR-224 was strongly associated with higher risk of treatment resistance or shorter periods of disease relapse/progression free survival (pooled HR = 2.07, 95%CI: 1.50–2.86, P < 0.00001). The total number of DLBCL down-regulation of miR-224 and DLBCL without down-regulation of miR-224 levels were 293 and 138, respectively. The patients were followed up for 60–120 months. Heterogeneity was low (I^2: 0%).

The percentage of refractory disease was much higher in the DLBCL group with aberrant expression of miR-224 as compared to the DLBCL without aberrant expression of miR-224 (46.7% versus 15.9%) (Song et al., 2014a).

DLBCL patients with resistant disease or disease relapses showed aberrant expression of miR-224 in both their tumor tissue and peripheral blood samples. MiR-224 levels were down-regulated in their tumor tissue samples (formalin-fixed-paraffin-embedded) (Ni et al., 2015), but they were up-regulated in their peripheral blood/serum samples (Song et al., 2014a).

3.11. Association between down-regulation of miR-146b-5p and treatment outcome

(Fig. 8) Two studies were pooled for meta-analysis to elucidate association between down-regulation of miR-146b-5p and treatment outcome. Overall analysis comprised of 137 DLBCL with down-regulated miR-146b-5p and 61 DLBCL without down-regulated miR-146b-5p, with duration of follow up ranged from 120 to 130 months. Significant association was found between down-regulation of miR-146b-5p and treatment outcomes with pooled HR of 2.26, 95% CI: 1.40 to 3.65, P = 0.0009. Heterogeneity was low (I^2: 11%) across these studies and there was no publication bias.

Both Cao QW et al. 2012 (Cao et al., 2012) and Wu PY et al. 2014 (Wu et al., 2014) report that patients with down-regulation of miR-146b-5p had significantly higher risk of treatment resistance or shorter period of disease relapse/progression free survival (HR:3.17, 95% CI: 1.44–7.02, P = 0.004) and (HR:1.85, 95% CI: 1.01–3.39, P = 0.046) respectively.

The incidence of disease relapse/progression was 72.5% in DLBCL with down-regulated miR-146b-5p group and 40% in DLBCL without down-regulated miR-146b-5p group (Cao et al., 2012). The median progression free survival period for DLBCL with down-regulated miR-146b-5p group was 9.4 months while it was 35.5 months for DLBCL without down-regulated miR-146b-5p level (Wu et al., 2014).

4. Discussion

4.1. Coverage of this meta-analysis

The current systematic review and meta-analysis incorporated 14 studies conducted in six countries, involving a total of 947 DLBCL patients with aberrant expression of microRNAs and 498 DLBCL patients without aberrant expression of microRNAs as controls. All studies (either in full text or conference abstract) focused on association between aberrant microRNAs expression and treatment outcomes in DLBCL disease were included in this meta-analysis.

4.2. Types of sample for microRNA assay

Data from these 14 studies provide evidence that aberrant expression of microRNAs could be detected not only on FFPE sample (ten studies), fresh frozen tissue sample (one study), but also on peripheral blood/serum sample (three studies). This finding is consistent with another systematic review study which concluded that liquid biopsy is a potential option for monitoring DLBCL disease relapse (Arzuaga-Mendez et al., 2019).

4.3. Clinical significance of aberrant microRNA expression in DLBCL patients

Overall results of this meta-analysis shows positive association

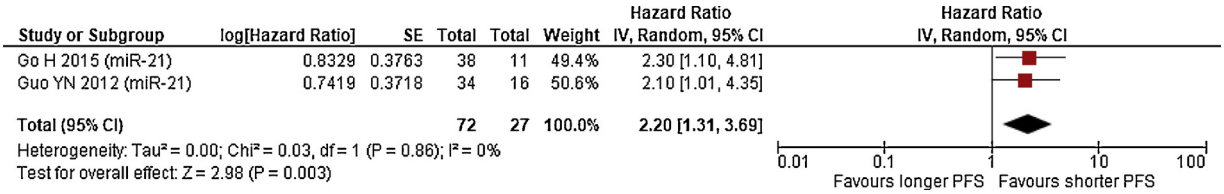

Fig. 6. Forest plot of meta-analysis of up-regulation of miR-21 and treatment outcome. The pooled HR and 95% CI were calculated using a random effects model.

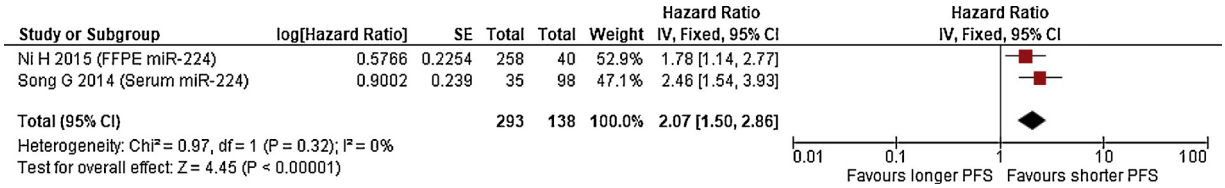

Fig. 7. Forest plot of meta-analysis of aberrant expression of miR-224 and treatment outcome. The pooled HR and 95% CI were calculated using a random effects model.

between aberrant expression of miR-155, miR-17/92 clusters, miR-21, miR-224 and miR-146b-5p and poor treatment outcomes. The risk of developing treatment resistance or having shorter period of disease relapse/progression free survival was 2.14 times higher in DLBCL patients with aberrant expression of microRNAs compared to DLBCL patients without aberrant microRNAs expression. Aberrant expression of miR-155, miR-17/92 clusters, miR-21, miR-224 and miR-146b-5p might be promising biomarkers to differentiate therapy resistant DLBCL patients from those who respond to treatment. The current meta-analysis shows that aberrant expression of these five microRNAs were significant predictors for poor treatment outcomes ($P = 0.0001$, $P = 0.0010$, $P = 0.003$, $P < 0.00001$ and $P = 0.0009$ respectively).

4.4. Association between aberrant expression of miR-155 and shorter period of disease relapse/progression free survival

The current meta-analysis shows that aberrant expression of miR-155 was associated with 2.71 times higher risk of treatment resistance or shorter period of disease relapse/progression free survival. Consistent findings were observed in acute myeloid leukemia [HR = 1.20 (95% CI: 0.89–1.61) P = 0.23] (Marcucci et al., 2013), B-chronic lymphocytic leukemia [HR = 1.18 (95% CI: 0.47–2.94) P = 0.14] (Ferrajoli et al., 2013), as well as non-small cell lung cancer [HR = 1.42 (95% CI: 1.10–1.83, P = 0.007)] (Yang et al., 2013).

MiR-155 involves in several cellular signaling pathways that affect the sensitivity of tumor cells to anticancer drugs. For example, aberrant expression of miR-155 could activate the PI3K-AKT signaling pathway and represses PTEN expression, resulting in resistance to rituximab therapy (Huang et al., 2012; Ma et al., 2015). In addition, altered expression of miR-155 dysregulates expression of PD-1/PD-L1, which facilitates the tumor cells by evading anti-tumor immunity, and results in disease progression (Zheng et al., 2019). Besides that, decreased level of miR-155 could induce production of Wee 1 protein (Visconti et al., 2015). Wee 1 is the target gene of miR-155 (Due et al., 2019). This protein encodes a kinase that inhibits phosphorylation of Cdk1 in tumor cells, weakens SAC-dependent mitotic arrest and results in survival of tumor cells (Visconti et al., 2015).

ABC subtype-DLBCL patients expresses higher level of miR-155 as compared to GCB subtype-DLBCL patients. A study had shown that up-regulation of miR-155 level improved the efficacy of vincristine therapy in GCB subtype-DLBCL patients, but not in ABC subtype-DLBCL patients (Due et al., 2019). Therefore, DLBCL study cohorts need to be categorized according to their subtypes (GCB versus ABC) before performing statistical analysis on clinical significance of aberrant microRNA expression.

4.5. Association between aberrant expression of miR-17/92 and shorter period of disease relapse/progression free survival

The effect size of aberrant expression of miR-17/92 clusters was similar to miR-155, with 2.70 times increased risk of treatment resistance, as compared to patients with normal levels of miR-17/92 clusters. Molecular signaling pathways of miR-17/92 leading to treatment resistance have been investigated in mantle cell lymphoma (Rao et al., 2012), gastric cancer (Wang et al., 2013a) and non-small cell lung cancer (Krysan et al., 2014). These studies demonstrated that miR-17/92 clusters suppress expression of tumor suppressor gene PTEN, activate PI3K/AKT pathway, and result in inhibition of chemotherapy-induced apoptosis of cancer cells (Pfeifer et al., 2013). The majority of the published review articles reported on recent diagnostic advancements, genetics, pathogenesis, and functions of miR-17/92 (Mogilyansky and Rigoutsos, 2013; Olive et al., 2010; Mendell, 2008; Petrocca et al., 2008). Meta-analysis on the risk of up-regulated miR-17/92 remains unreported.

4.6. Association between aberrant expression of miR-21 and shorter period of disease relapse/progression free survival

Positive association was found between up-regulated of miR-21 and poor treatment outcomes ($P = 0.003$). DLBCL patients with up-regulated miR-21 were 2.20 times at higher risk of experiencing treatment resistance or shorter period of disease relapse/progression free survival. We found a hazard ratio of 2.2 in DLBCL with up-regulated miR-21, similar to non-small-cell lung cancer with HR = 2.00 (95% CI: 1.38–2.89, P = 0.0001, $I^2 = 84.9\%$) (Wang et al., 2013b); and gastric cancer with HR = 2.00 (95% CI: 1.39–2.88, $P < 0.01$) (Wang et al., 2014a). This is slightly higher than that in colorectal cancer with HR = 1.76 (95% CI: 1.34–2.32, P = 0.000) (Xia et al., 2013), and breast cancer with HR = 1.49, (95%CI, 1.17–1.90; $P < 0.01$) (Tang et al., 2015). Up-regulation of miR-21 has been shown to inhibit expression of PTEN gene and activate PI3K/Akt pathway and is implicated in treatment resistance (Bai et al., 2011). Myristoylated alanine-rich protein kinase c substrate is another target of miR-21 which inhibits chemotherapy induced-apoptosis of cancer cells and results in treatment resistance (Li et al., 2009).

4.7. Association between aberrant expression of miR-224 and shorter period of disease relapse/progression free survival

Aberrant expression of miR-224 was also shown to be significantly linked to refractory disease, with 2.07 times higher risk of treatment

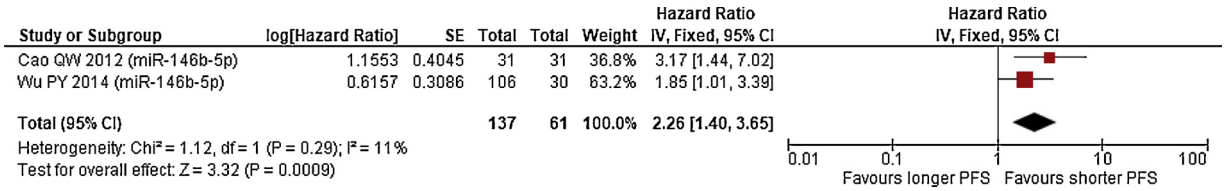

Fig. 8. Forest plot of meta-analysis of down-regulated miR-146b-5p and treatment outcome. The pooled HR and 95% CI were calculated using a random effects model.

resistance as compared to DLBCL patients without aberrant expression of miR-224 (95% CI: 1.50 to 2.86, P < 0.00001). MiR-224 alters expression of protein kinase C δ or PRKCD gene, hinders cancer cells from chemotherapy induced-apoptosis and results in chemoresistance (Zhao et al., 2014). Besides that, miR-224 could also affect the G1/S transition of cell cycles and regulate cellular apoptosis, and thus promoting chemoresistance among patients with lung adenocarcinoma (Wang et al., 2014b). In addition, the hazard risk for refractory disease in malignant brain tumor patients with down-regulation of miR-224 was 1.72 (P < 0.05) (Upraity et al., 2014); while HR = 2.054 (95%CI: 0.975 to 4.91, P = 0.022) for patients with aggressive prostate cancer (Mavridis et al., 2013).

4.8. Association between aberrant expression of miR-146b-5p and shorter period of disease relapse/progression free survival

This meta-analysis shows significant association between down-regulation of miR-146b-5p and poor treatment outcome. Mir-146b-5p had been shown to act as a tumor suppressor to promote cellular apoptosis. Down-regulation of miR-146b-5p enhances expression of TRAF6 oncogene, which activates TAK1 signaling pathway and suppresses cellular apoptosis (Liu et al., 2015). Besides that, TRAF6 is a direct E3 ligase for Akt (Yang et al., 2009), activates IκB kinase and NFκB leading to proliferation of cancer cells and contributes to chemoresistance (Paik et al., 2011; Jost and Ruland, 2007). Down-regulating of miR-146b-5p also enhances expression of epidermal growth factor receptor (EGFR) (Katakowski et al., 2010), deregulates downstream PTEN/PI3K/Akt/mTOR pathway and is implicated in treatment resistance (Gallardo et al., 2012).

4.9. Combination of microRNA assay with clinical, bioimaging and biological risk stratification tools for prognostication of DLBCL disease

Aberrant microRNA expression is a potential biological tool to predict disease relapse or refractoriness to therapy.in DLBCL patients. This new DLBCL disease prognostic tool could be incorporated with currently available clinical, bioimaging, and biological risk stratification tools for optimal personalized treatment approach for DLBCL patients. Comprehensive validation of the new model needs to be conducted and laboratory techniques need to be standardized before it could be translated in clinical setting (Jelicic et al., 2019).

4.10. Implications for clinical practice

This study has highlighted the clinical significance of up-regulated miR-155, miR-17/92 clusters, miR-21, as well as down-regulation of miR-224 and miR-146b-5p in predicting treatment resistance or shorter periods of disease relapse/progression free survival. Therefore, this panel of microRNA assay may be used along with the international prognostic score to risk stratify DLBCL patients prior to initiation of treatment. This microRNA profile may facilitate the selection of chemotherapy regimen and determine the need for upfront stem cell transplantation. In addition, serum microRNA assay may also be used together with CT/PET scan to monitor disease relapse or disease progression.

In the primary studies, microRNA assays were performed on formalin-fixed paraffin embedded tissue (FFPE), fresh frozen tissue and peripheral blood samples. Overall results showed that expression of microRNAs was higher on tissue sample (either FFPE or fresh frozen tissue) compared to peripheral blood samples, and this finding indicates that formaldehyde fixation of tumor tissue is a reliable technique to preserve microRNAs. Hence, FFPE is the preferred type of specimen for microRNA assays especially in diagnostic workup or prognostic or risk stratification of DLBCL patients. On the other hand, microRNA assays on peripheral blood samples could be used for disease relapse monitoring as it is less invasive and more applicable in routine clinical settings.

4.11. Limitations of this study

Lack of standardization in data reporting was observed in these studies. Of the 33 studies retrieved from electronic databases, only 14 studies performed cox proportional hazard regression analysis and reported hazard ratios of aberrant expression of microRNAs. Kaplan Meier survival analysis were used in the remaining 19 studies, and P values were reported to denote significance of risk impact of the aberrantly expressed microRNAs. P values from these individual studies could not be combined for pooled data meta-analysis. Despite these limitations, the findings of this meta-analysis demonstrate important biological markers that affect the relapse/refractory free survival period of DLBCL disease.

In addition to that, a validation study comprises of larger sample number should be carried out to verify clinical significance of aberrant miR-155 expression in DLBCL patients as some primary studies reported discordant findings (Troppan et al., 2014).

4.12. Recommendation for future microRNA studies

Therefore, a standard data reporting format should be established to enable integration of data from all studies to draw definite conclusions of the risks of the aberrant expressed microRNAs in the future.

5. Conclusion

DLBCL patients with aberrant expression of miR-155, miR-17/92 clusters, miR-21, miR-224 and miR-146b-5p were 2.14 times more likely to experience treatment resistance or a shorter period of disease relapse/progression free survival. Therefore, these five could be used to risk stratify DLBCL patients alongside routinely used international prognostic score. Expression of microRNA in tissue samples was noted to be higher than in corresponding peripheral blood samples.

Ethical approval

Not required.

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Authors' contributions

Ping-Chong Bee coordinated and led this study, screened and reviewed articles. Choo-Yuen Ting reviewed the primary study articles, created forest-plots and drafting this article. Su-May Liew designed the study, co-wrote this article and approved the final version of article for submission. Amy Price performed medical literature search on electronic databases, co-wrote this article and revised it critically for intellectual content. Gin-gin Gan, Soo-Yong Tan and Diana, Bee-Lan Ong critically revised this article and provided professional input.

Declaration of Competing Interest

None.

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