MicroRNAs as novel biomarkers in diffuse large B-cell lymphoma – a systematic review

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ABSTRACT

INTRODUCTION: MicroRNAs (miRNAs) are short non-coding RNAs that have the ability to regulate gene expression at the post-transcriptional level. MiRNAs are deregulated in many cancer types, and several miRNAs have been suggested as novel diagnostic and prognostic biomarkers in diffuse large B-cell lymphoma (DLBCL). The objective of this study was to systematically collect and evaluate current knowledge of miRNAs functioning as diagnostic and prognostic biomarkers within DLBCL.

METHODS: This review was conducted according to the Preferred Reporting for Systematic Reviews and Meta-analyses guidelines. A systematic search of literature in PubMed and Embase was made and supplemented by screening of reference lists. Only original peer-reviewed studies written in English were included and screened based on miRNA expression, molecular subtypes of DLBCL and patient outcome.

RESULTS: Out of 277 candidate records, a total of 20 studies qualified for inclusion in this review. In all, 11 studies reported a total of 48 miRNAs with expression patterns associated with specific molecular DLBCL subtypes, and 14 studies reported a total of 30 miRNAs associated with patient outcome. However, only few miRNAs showed significant results in more than one study.

CONCLUSION: MiRNAs qualify as potential diagnostic and prognostic biomarkers in DLBCL. However, more clinical validation including prospective and cross-centre studies are required before specific miRNAs can be integrated into the daily practice as biomarkers in DLBCL, which would contribute to an era of more personalised medicine.

Diffuse large B-cell lymphoma (DLBCL) is a highly aggressive disease, and it is the most common form of non-Hodgkin's lymphoma [1]. In Denmark, the incidence of BLBCL is approximately 400 new cases per year according to the annual reports from the Danish Lymphoma Group (DLG). Currently, the diagnosis and classification of DLBCL is based on morphology, histology and clinical findings as well as on cytogenetics, immunophenotyping and molecular genetic assessments [2, 3]. Gene expression profiling (GEP) and immunohistochemical studies have identified two molecular subtypes with different clinical outcomes; the germinal centre B-cell-like (GCB) DLBCL and the activated B-cell-like (ABC) DLBCL [4]. This classification is widely recognised, but not used in routine clinical settings due to the rather high cost of global gene expression profiling and the poor cross-centre performance of immunohistochemical surrogate markers [5]. GCB patients have a higher five-year survival rate (range: 69-79%) than patients with ABC DLBCL (52-53%) when patients are treated with a standard anthracycline-based multidrug chemotherapy regimen consisting of rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone; a regimen known as R-CHOP [6]. To date, the International Prognostic Index (IPI) is the only clinically used prognostic tool for the estimation of the prognosis of the DLBCL disease following therapy. IPI is an estimate based on survival statistics [7]. Although it is a good prognostic tool in routine clinical practice, the IPI is only based on clinical values and provide only little insight into the biology of the disease or mechanisms of resistance. Hence, identification of novel biological markers that can guide diagnostic sub-classification or improve the current prognostic evaluation has the potential to refine the stratification of DLBCL patients and facilitate a shift to a more personalised subtype-adjusted choice of therapy.

Biomarkers are defined by the National Institutes of Health (NIH) as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention", and can be used as both diagnostic and prognostic tools [8]. During the past decade, several novel molecular and biological candidates with diagnostic and prognostic potential in DLBCL have been suggested, and microRNAs (miRNAs) have frequently been among them. MiRNAs are short non-coding RNAs of 20-24 nucleotides that control gene expression mainly at the post-transcriptional level [9]. Typically, this is done by inhibition of mRNA translation or by direct mRNA degradation [10]. They play a crucial role in many biological processes such as cell differentiation and homeostasis; this role extends to cancer initiation, progression, pathogenesis, and treatment [11]. MiRNAs are deregulated in many cancer types, including DLBCL, and it was shown that some of them can function as oncogenes or tumour suppressors depending on the cell type or the microenvironment [9, 12]. Given their

SYSTEMATIC REVIEW

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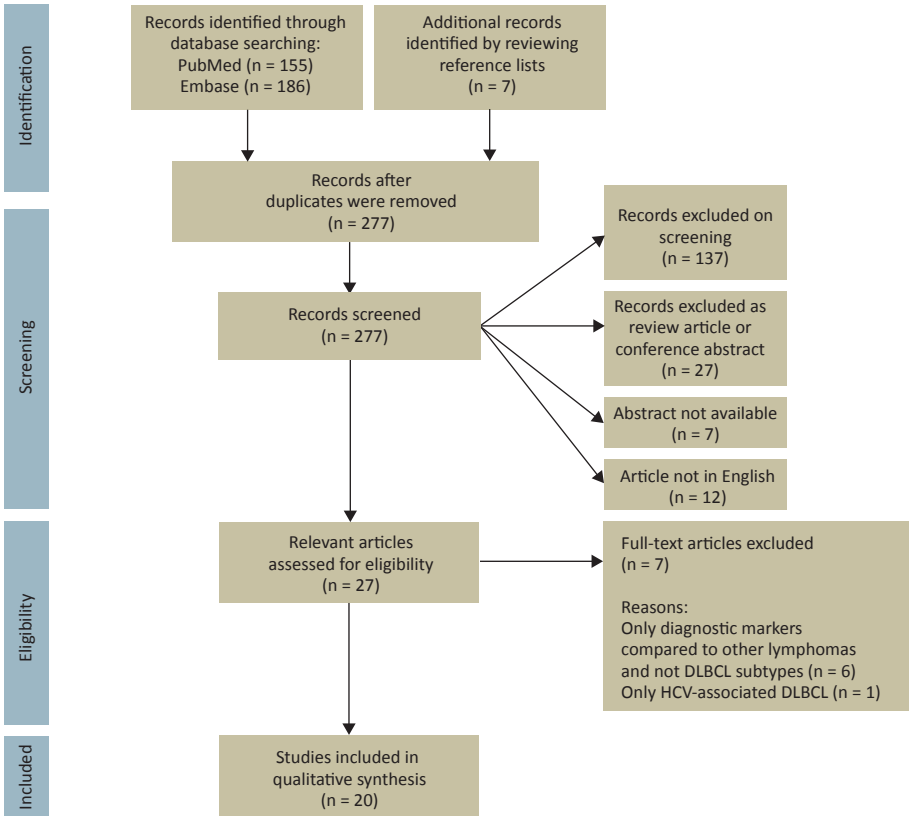
FIGURE 1

PRISMA flow diagram of literature search.



DLBCL = diffuse large B-cell lymphoma; HCV = hepatitis C virus; PRISMA = Preferred Reporting for Systematic Reviews and Meta-analyses.

important roles in normal development and their extensive deregulation in many cancers, they seem to be ideal diagnostic and prognostic biological markers. Furthermore, they are relatively stable both in vitro and in vivo; they can be detected in blood, urine and solid tissues; and they can be purified from formalin-fixed, paraffin-embedded (FFPE) tissue [13]. It is suggested that miRNA profiling can provide diagnostic information; helping to distinguish between ABC and GCB subtypes of DLBCL; it is also suggested that they can provide prognostic information such as clinical outcome in R-CHOP-treated DLBCL patients. However, this is not yet applicable in routine clinical work.

The aim of this systematic review was to collect current information of miRNAs functioning as novel diagnostic and prognostic biomarkers in DLBCL. Furthermore, we aimed to discuss their potential and limitations as clinical tools.

METHODS

This review was completed according to the Preferred Reporting for Systematic Reviews and Meta-analyses (PRISMA) Guidelines [14].

Search strategy

We systematically searched the MEDLINE (PubMed) and Embase databases for current literature on miRNAs as diagnostic and prognostic biomarkers in DLBCL. Using the problem-intervention-comparison-outcome (PICO) search approach, we combined the Mesh terms/Entry terms “Diffuse Large B-cell Lymphoma”, “microRNA”, “Biological Markers”, “Prognosis”, “Diagnosis”, and “Individualized Medicine”. For each of these search terms, free-text combinations were also included. No additional limits were set. The last search was performed on 9 July 2014 and identified 270 different publications. An additional 7 articles were added through screening of reference lists.

Study selection and data handling

The inclusion criteria were original peer-reviewed articles only, publication language English, available abstract, and papers addressing the subject miRNAs as diagnostic and prognostic biomarkers in DLBCL. In this review, we focussed on the potential of miRNAs as biomarkers only within DLBCL and its subtypes. Therefore, papers on miRNAs in the pathogenesis of DLBCL or mi-

RNA signatures as diagnostic classifiers comparing DLBCL with other lymphomas were excluded. Exclusion of 137 irrelevant articles (**Figure 1**) was based on evaluation of title and abstract. Hence, 27 articles fulfilled the inclusion criteria and were assessed for eligibility. Of these, seven articles were excluded, mainly because they did not encompass miRNA deregulation as a diagnostic tool of DLBCL subtypes. One was excluded as it only investigated hepatitis C virus-associated DLBCL.

Consequently, a total of 20 articles were included in this review (Figure 1). Data from the included articles were extracted and only significant results were reported.

The seven articles identified by reviewing reference lists were included in the final qualitative synthesis.

RESUTS

Study characteristics of included studies

Table 1 outlines important methodology and study characteristics of the 20 included studies. A total of 11 studies address miRNAs as diagnostic biomarkers, whereas 14 of the studies investigated the prognostic abilities.

Most of the diagnostic studies on miRNA expression patterns and ABC/GCB subtype classification of DLBCL are based on cell lines and/or FFPE clinical tumour samples using microarray or quantitative real-time polymerase chain reaction (qRT-PCR) as the method of detection. However, Chen et al found higher miR-21 levels in serum of ABC DLBCL patients (n = 32) than in GCB DLBCL patients (n = 30) [23]. Only three of the diagnostic studies were primarily done by global microarray screening on clinical patient samples [18, 20, 24]. Most of the studies were based on selection of a few miRNAs from cell line studies.

Regarding the prognostic studies, five of them were primarily done by global screening on clinical samples by either microarray or qRT-PCR [6, 24, 26, 28, 29]. The remaining based the initial selection of miRNAs on cell line studies or previous research. All prognostic studies used qRT-PCR for determination of miRNA expression level and survival curves with the Kaplan-Meier method as their primary statistical method. Most of the studies used FFPE clinical samples as sample types, whereas three of the 14 articles used serum and one study measured miRNA levels in plasma from DLBCL patients. The majority of the prognostic studies used dichotomous analysis stratifying the test group into low versus high expression levels. Two studies used healthy controls as comparison [30, 33]. A single study by Montes-Moreno et al defined an actual validation group [24].

MicroRNA expression as a diagnostic tool in diffuse large B-cell lymphoma

Through the systematic search, we found 11 studies focussing on the expression of miRNAs on the differentiation of the ABC and GCB subtypes of DLBCL. **Table 2** summarises the significant findings of 48 miRNAs and their expression levels. Of the 48 miRNAs associated with either ABC or GCB subtypes, the expression levels of eight miRNAs were confirmed in ≥ 2 studies. Thus, miR-155, miR-21, miR-221, miR-222, miR-146a, miR-363 and miR-518a are more highly expressed in the ABC subtype; and miR-421 is more highly expressed in the GCB subtype. Lawrie et al [22] performed microarray analysis on four DLBCL cell lines and demonstrated that miR-155, miR-21 and miR-221 were upregulated in ABC as compared with GCB DLBCL. This was confirmed by qRT-PCR in 35 de novo DLBCL clinical samples [22]. Subsequently, the association of these three miRNAs with the molecular ABC subtype of DLBCL has been verified in several studies, and the high expression of miR-155 in the ABC subtype has actually been documented in eight different studies [16-22, 25]. Malumbres et al [21] performed microarray analysis on eight cell lines (five ABC and three GCB DLBCL cell lines) using 217 miRNA probes and found nine miRNAs with the ability to differentiate DLBCL into ABC and GCB subtype, namely miR-146b-5p, miR-146a, miR-21, miR-155, miR-500, miR-222, miR-363, miR-574-3p and miR-574-5p [21]. Culpin et al [15] also established a series of nine differentially expressed miRNAs between the two subtypes using cell lines. However, none of the above-mentioned miRNAs were among the nine found by Culpin et al [23].

Together these reports confirm that specific miRNAs have the potential to become useful diagnostic tools in defining DLBCL subtypes. However, it should be taken into account that most of the studies include only small numbers of tumour samples, they used different methods of detection are used, and very often they

! KEY POINTS

MicroRNAs (MiRNAs) are short non-coding RNAs and have the ability to regulate gene expression, mainly at the post-transcriptional level.

MiRNAs are deregulated in many cancer types, and several miRNAs have been suggested as novel diagnostic and prognostic biomarkers in diffuse large B-cell lymphoma (DLBCL).

A total of 11 studies (reviewed here) have found that specific miRNA profiles can be used to differentiate activated B-cell-like (ABC) and germinal centre B-cell-like (GCB) DLBCL subtypes.

A total of 14 studies have found that the expression level of a total of 30 miRNAs is associated with patient prognosis.

MiRNA profiles as diagnostic and prognostic biomarkers have the potential of being an integrated part of daily practice in combination with other clinical factors. Their use as diagnostic and prognostic biomarkers seems able to improve the information provided by current clinical factors such as the International Prognostic Index (IPI).

TABLE 1

Study characteristics of included diagnostic and prognostic biomarker studies.

Reference	Study type	Initial miRNA-selection	Study size
Culpin et al, 2010 [15]	Diagnostic	Global screening by microarray	8 DLBCL cell lines: 4 ABC-like 4 GCB-like
Rai et al, 2008 [16]	Diagnostic	Selection based on cell line study 22 cell lines	119 DLBCL pt. (training set): 34 ABC DLBCL 85 GCB DLBCL
Eis et al, 2005 [17]	Diagnostic	Selection based on cell line study 3 DLBCL cell lines: 1 GCB DLBCL 2 ABC DLBCL	23 DLBCL pt.: 19 ABC DLBCL 4 GCB DLBCL
Caramuta et al, 2013 [18]	Diagnostic	Global screening by microarray on 45 CS	54 DLBCL pt.: 20 GCB DLBCL 34 ABC DLBCL
Lawrie et al, 2008 [19]	Diagnostic	Global screening by microarray	8 DLBCL cell lines: 4 GCB DLBCL 4 ABC DLBCL
Lawrie et al, 2009 [20]	Diagnostic	Global screening by microarray	60 DLBCL pt.: 32 GCB DLBCL 28 ABC DLBCL
Malumbres et al, 2009 [21]	Diagnostic and prognostic	Selection based on cell line study 8 DLBCL cell lines: 3 GCB DLBCL 5 ABC DLBCL	106 DLBCL pt. Stratified in low versus high expression levels
Lawrie et al, 2007 [22]	Diagnostic and prognostic	Selection based on cell line study 5 DLBCL cell lines: 3 GCB DLBCL 2 ABC DLBCL	35 DLBCL pt. Stratified in low versus high expression levels: 17 GCB DLBCL 18 ABC DLBCL
Chen et al, 2014 [23]	Diagnostic and prognostic	Selection based on cell line study 9 DLBCL cell lines: 6 GCB DLBCL 3 ABC DLBCL	62 DLBCL pt. Stratified in low versus high expression levels: 32 GCB DLBCL 30 ABC DLBCL
Montes-Moreno et al, 2011 [24]	Diagnostic and prognostic	Global screening by microarray on 36 CS	240 DLBCL pt. Including test and validation group Stratified in low versus high expression levels: 106 GCB DLBCL 126 ABC DLBCL
Zhong et al, 2012 [25]	Diagnostic and prognostic	Previous research (2 miRNAs selected)	90 DLBCL pt. Stratified in low versus high expression levels: 21 GCB DLBCL 69 ABC DLBCL
Wu et al, 2014 [26]	Prognostic	Global screening by microarray comparing 6 CS from DLBCL pt. with good prognosis and 6 DLBCL pt. with bad prognosis 29 miRNAs were selected for qRT-PCR	106 DLBCL pt. Stratified in low versus high expression levels
Song et al, 2014 [6]	Prognostic	Screening of 736 miRNAs by qRT-PCR on a complete remission group (n = 20) versus a primary refractory group (n = 20) 5 miRNA were chosen for further analysis	133 DLBCL pt. Stratified in low versus high expression levels

ABC = activated B-cell; CS = clinical samples (lymph node biopsies from DLBCL patients); DLBCL = diffuse large B-cell lymphoma; FFPE = formalin-fixed paraffin-embedded; GCB = germinal-centre B-

studied a selection of miRNAs as opposed to a global miRNA expression analysis. Additionally, the definition of molecular subtype by GEP is not always used, and this may explain why some studies fail to identify differentially expressed miRNAs between ABC and GCB DLBCL [29].

MicroRNA expression as a prognostic tool in diffuse large B-cell lymphoma

The potential clinical utility of miRNAs as biomarkers is not limited to diagnostic subtyping, but may also have direct prognostic value. In this systematic review, we found 14 articles that together identified a total of 30

Sample type	Data measurement	Significant miRNAs	Statistical methods
Cell lines	Microarray and qRT-PCR validation	miR-17, miR-19b, miR-20a, miR-29a, miR-92a, miR-106a, miR-720, miR-1260, miR-1280	1-way ANOVA test Spearman's correlation test
FFPE CS	Microarray	miR-155	Kruskal-Wallis test
Frozen CS	Invader assay (copies/cell)	miR-155	Unpaired t-test
Frozen CS	Microarray qRT-PCR validation	miR-155, miR-146a	1-way ANOVA Unpaired t-test
Cell lines	Microarray	miR-155, miR-21, miR-221, miR-222, miR-518a, miR-363 and miR-421, miR-324, miR-590, miR-181a	1-way ANOVA test Unpaired t-test
FFPE CS	Microarray	miR-158a, miR-363, miR-21, miR-132, miR-340, miR-301, miR-30d, miR-221, miR-422b, miR-146b, miR-155, miR-190, miR-194, miR-660, miR-213, miR-222, miR-186, miR-620, miR-616, miR-199b miR-421, miR-569, miR-653, miR-138, miR-520h, miR-129	1-way ANOVA test p-value adjustment by Benjamini-Hodgberg correction method
FFPE CS	Microarray qRT-PCR	miR-222,miR-155, miR-21, miR-146a, miR-363, miR-146b-5p, miR-500, miR-574-3p, miR-574-5p	1-way ANOVA test Survival curves with Kaplan-Meier method and log rank test
FFPE CS	Microarray qRT-PCR	miR-21, miR-155, miR-221	Mann-Whitney independent t-test Survival curves with Kaplan-Meier method and log rank test
Serum	qRT-PCR	miR-21	Unpaired t-test Survival curves with Kaplan-Meier method and log rank test
FFPE CS	qRT-PCR	miR-221, miR-222, miR-144, miR-451, miR-331, miR-151, miR-28, miR-454-3p, miR-148a, miR-93, miR-491	Chi-squared test Survival curves with Kaplan-Meier method and log rank test COX regression analysis ROC curves
FFPE CS	qRT-PCR	miR-155, miR-146a	Chi-squared test Mann-Whitney U test Survival curves with Kaplan-Meier method and log rank test ROC curves
FFPE CS	qRT-PCR	miR-320d, miR-146b-5p	COX proportional hazard ratio Survival curves with Kaplan-Meier method and log rank test Unpaired t-test
Serum	qRT-PCR	miR-33a, miR-445-3p, miR-224, miR-1236, miR-520d-3p	ROC curves COX proportional hazard ratio Survival curves with Kaplan-Meier method and log rank test

-cell; miRNA = microRNA; pt. = patients; qRT-PCR = quantitative real-time polymerase chain reaction; ROC = receiver operating characteristic.

CONTINUES

miRNAs with expression patterns significantly associated with patient outcome (**Table 3**).

Three miRNAs showed a significant association between expression level and outcome in more than one study. Thus, high expression levels of miR-21 are demonstrated in three different studies to be associated with improved relapse-free survival (RFS). Serum is analysed in two of the studies [23, 33] and tumour tissue in FFPE in one study [22]. High expression levels of miR-222 correlate with poor progression-free survival (PFS) in three different studies [21, 24, 27]. Together, these three studies cover a cohort of 522 FFPE clinical DLBCL

TABLE 1, CONTINUED

Study characteristics of included diagnostic and prognostic biomarker studies.

Reference	Study type	Initial miRNA-selection	Study size
Alencar et al, 2011 [27]	Prognostic	Previous research (11 miRNAs selected)	176 DLBCL pt.
Ohyashiki et al, 2011 [28]	Prognostic	Global screening by microarray on 4 DLBCL CS compared to CS from healthy controls	34 DLBCL pt. Stratified in low versus normal expression levels
Roehle et al, 2008 [29]	Prognostic	qRT-PCR screening of 157 miRNAs on pt. samples	58 DLBCL pt. 0 controls
Wang et al, 2014 [30]	Prognostic	Previous research (1 miRNA selected)	104 DLBCL pt. 28 controls with reactive lymph nodes
Berglund et al, 2013 [31]	Prognostic	Previous research (1 miRNA selected)	61 DLBCL pt. Stratified in low versus high expression levels
Hedström et al, 2013 [32]	Prognostic	Previous research (1 miRNA selected)	61 DLBCL pt. Stratified in low versus high expression levels
Lawrie et al. (2008) [33]	Prognostic	Previous research (3 miRNAs selected)	61 DLBCL pt. 43 healthy controls

ABC = activated B-cell; CS = clinical samples (lymph node biopsies from DLBCL patients); DLBCL = diffuse large B-cell lymphoma; FFPE = formalin-fixed paraffin-embedded; GCB = germinal-centre B-

samples, and the patients were all treated uniformly with the R-CHOP regimen.

The results for miR-23a are incongruent. For this miRNA, both high and low expression levels in clinical samples were reported to be associated with poor overall survival (OS), in [30] and [29], respectively. However, one of the studies looked into a remarkably larger cohort than the others; this cohort consisted of 104 FFPE clinical samples [30] as opposed to 58 FFPE clinical samples [29].

Alencar et al identified three miRNAs (miR-18a, miR-181a, and miR-222) in a cohort of 176 FFPE clinical samples independently associated with outcome in DLBCL [27]. Simultaneously, Montes-Moreno et al applied a miRNA expression-based model to predict OS and PFS, evaluated in 240 clinical samples [24]. They found that low expression levels of nine miRNAs (miR-221, miR-222, miR-331, miR-451, miR-28, miR-151, miR-148, miR-93 and miR-491) were significantly associated with better OS and PFS. The authors suggested that a combination of the miRNA-based model and the IPI score could improve the accuracy of prognostic evaluation. In a recent study, serum levels of miR-33a and miR-445-3p above the mean level were associated with a higher possibility of remission; and serum levels of miR-224, miR-1236 and miR-520d-3p below the mean were associated with a lower rate of remission in R-CHOP-treated DLBCL patients [6]. Thus, miRNA expression profiles of tumour samples and/or body fluids as serum or plasma at time of diagnosis appear to hold prognostic information.

DISCUSSION

This systematic review identified a total of 20 studies addressing the role of miRNAs as diagnostic or prognostic biomarkers. The results, outlined in Table 2 and Table 3, present evidence that miRNA expression may function as a valuable tool in both diagnosis of DLBCL patients and in their stratification according to prognosis. The validity of our systematic search is improved by the fact that the search strategy encompassed Mesh terms/Entry terms as well as free-text words and by the fact that it meets the PRISMA guidelines.

Although it is suggested that miRNAs can be used to distinguish DLBCL from other lymphoma types [34, 29] the focus of our review was to decipher miRNA expression patterns in DLBCL subtype diagnostics and prognostics.

We present evidence that miRNA profiles are associated with DLBCL molecular subtypes. Associations of 8 important miRNAs were found in more than one study; miR-155, miR-21, miR-221, miR-222, miR-146a, miR-363 and miR-518a were found to be more highly expressed in the ABC subtype, and miR-421 was more highly expressed in the GCB subtype. Furthermore, data presented in Table 3 suggest that specific miRNA expression

Sample type	Data measurement	Significant miRNAs	Statistical methods
FFPE CS	qRT-PCR	miR-18a, miR-181a, miR-222	Survival curves with Kaplan-Meier method and log rank test miR-18a and 181a as continuous variables, miR-222 as dichotomous variable
Plasma	qRT-PCR	miR-92a	Chi-squared test
FFPE CS	qRT-PCR	miR-195, miR-23a, miR-19a, miR-27a, miR-34a, miR-127	Survival curves with Kaplan-Meier method and log rank test
FFPE CS	qRT-PCR	miR-23a	Chi-squared test, unpaired t-test Survival curves with Kaplan-Meier method and log rank test ROC curves
FFPE CS	qRT-PCR	miR-200c	Survival curves with Kaplan-Meier method and log rank test Fisher's exact test Unpaired t-test
FFPE CS	qRT-PCR	miR-129-5p	Survival curves with Kaplan-Meier method and log rank test Fisher's exact test Unpaired t-test
Serum	qRT-PCR	miR-21	Survival curves with Kaplan-Meier method and log rank test Unpaired t-test

cell; miRNA = microRNA; pt. = patients; qRT-PCR = quantitative real-time polymerase chain reaction; ROC = receiver operating characteristic.

patterns are associated with outcome in DLBCL patients. However, only a limited number of miRNAs (miR-21, miR-23a, and miR-222) were found with results showing consistency across more studies. The number of samples used in the studies also varies considerably. Naturally, the larger the cohort is the more valid are the results. Therefore, the results found in the studies by Montes-Moreno et al [24], Alencar et al [27] and Song et al [6] stand out with large cohorts of 240, 176 and 133 samples, respectively.

Another important matter that makes the studies less comparable is the fact that patients did not receive the same treatment in the different studies reviewed. In the majority of the studies, the patients received the R-CHOP regimen; however, this was not verified in all studies. This is a potential bias because the uniqueness of prognostic biomarkers is dependent on the effectiveness of the treatment [35]. When analysing the results of the studies, it is also important to consider the differences in methodology (Table 1). The initial microarray screening that was performed in several of the studies was not based on the same platforms and the microarray screening therefore does not contain probes for the same miRNAs. This also applies qRT-PCR, where different primer probes and platforms were used. The accuracy of the qRT-PCR therefore cannot be compared across studies.

There are examples of disagreements between the studies. For miR-23a, inconsistency was observed, as both high and low expression levels were associated with a poor OS [29, 30]. Considering the fact that patients with the ABC subtype have a poorer prognosis than patients with the GCB DLBCL subtype, it was remarkable that only a few miRNAs were highly expressed in the ABC subtype also associated with the overall prognosis of the disease.

The only consistent results in Table 2 and Table 3 are for miR-221, miR-222, miR-146a and miR-155. Interestingly, a high expression of miR-21 is associated with the ABC subtype in 5 different studies [19-23], which is in contradiction to the observation that a high miR-21 expression is associated with improved RFS which is found in three different studies [22, 23, 33]. Considering the fact that miR-21 is most often reported to behave as an oncogene, the finding of it being a positive prognostic biomarker is noteworthy. Chen et al suggest that the different findings may be caused by different methods or sample quantity. Perhaps the different sample materials (serum versus FFPE clinical samples) may cause conflicting results. There is not necessarily any coherence between what is identified in tumour biopsies and in serum. Another factor that may influence the conflicting results is that in clinical tumour samples, the miRNA expression levels may come from both tumour tissue and the microenvironment, which was shown for miR-21 [36].

TABLE 2

MicroRNAs and diffuse large B-cell lymphoma subtypes. ABC = activated B-cell; DLBCL = diffuse large B-cell lymphoma; GCB = germinal-centre B-cell; miR = microRNA.
a) Associated with 1 subtype in ≥ 2 studies.

High expression in	References
ABC DLBCL	
miR-155[a]	[16-22, 25]
miR-21[a]	[19-23]
miR-221[a]	[19, 20, 22, 24]
miR-222[a]	[19-21, 24]
miR-146a[a]	[18, 20, 21, 25]
miR-363[a]	[19-21]
miR-518a[a]	[19, 20]
miR-146b-5p	[21]
miR-500	[21]
miR-574-3p	[21]
miR-574-5p	[21]
miR-144	[24]
miR-451	[24]
miR-17	[15]
miR-19b	[15]
miR-20a	[15]
miR-29a	[15]
miR-92a	[15]
miR-106a	[15]
miR-720	[15]
miR-1260	[15]
miR-1280	[15]
miR-132	[20]
miR-340	[20]
miR-301	[20]
miR-30d	[20]
miR-442b	[20]
miR-190	[20]
miR-194	[20]
miR-660	[20]
miR-213	[20]
miR-186	[20]
GCB DLBCL	
miR-421[a]	[19, 20]
miR-331	[24]
miR-151	[24]
miR-28	[24]
miR-454-3p	[24]
miR-620	[20]
miR-616	[20]
miR-199b	[20]
miR-569	[20]
miR-653	[20]
miR-138	[20]
miR-520h	[20]
miR-129	[20]
miR-181a	[19]
miR-590	[19]
miR-324	[19]

MiRNAs show promise as biomarkers. Besides being very stable and easy to measure in body fluids or tissue samples, an ideal prognostic biomarker should be both sensitive and specific [37]. Furthermore, it should a have direct association with disease progression or outcome, and therefore should be able to predict long-term changes in disease prognosis based on short-term changes in its expression [37]. MiRNAs hold the potential to fulfil all of these criteria.

To guide the process of diagnostic biomarker development and evaluation, a formal screening structure consisting of 5 phases has been developed by the Early Detection Research Network (EDRN) [38]. By using the ERDN strategy, the development of biomarkers is systematised, and high standards may be achieved before implementation of biomarkers as a routine clinical tool. The results and methods presented in this review concerning miRNAs as biomarkers are all performed in the early phases of the EDRN biomarker development, and few miRNAs were validated in cross-centre studies. Therefore, further research is required, following the ERDN validation stages, before miRNA biomarkers can be used in daily practice.

New methods for measurement of miRNA expression levels should be considered. In recent years, next-generation sequencing (NGS), also known as high-throughput sequencing, has been used extensively in genetic research. This has become a relatively inexpensive and effective method of genome-wide DNA/RNA analyses [39] enabling a new method in miRNA research called miRNA sequencing [40, 41]. MiRNA sequencing has a number of advantages compared to conventional methods of measuring miRNA expression, such as qRT-PCR and microarray, because it can identify by sequence and quantify by counting events in hundreds of miRNAs at the same time [41]. It is therefore not necessary to choose a specific miRNA of interest beforehand as in qRT-PCR or to select complementary probes for known miRNAs as with microarrays. This also allows identification of new miRNAs [39, 41]. However, miRNA sequencing also has its limitations because a large sample amount is needed, and it remains more expensive and time-consuming than qRT-PCR and microarrays analysis [41]. To date, only few miRNA sequencing studies on DLBCL have been conducted [42, 43].

The fact that differentially expressed miRNAs have the ability to distinguish molecular subtypes of DLBCL and may contribute to diagnostic accuracy means that their expression levels may assist in early and rapid diagnosis and prognostic evaluation. Therefore, miRNA biomarkers as screened by the EDRN strategy can contribute to an era of more personalised medicine and enable a shift in diagnostic and treatment guidance strategy towards more efficient ones.

TABLE 3

MicroRNAs associated with patient prognosis in diffuse large B-cell lymphoma.

miRNA	Prognosis	Expression	Outcome measurement	Treatment	Samples	Reference
miR-33a	Improved	High	RSR	R-CHOP	Serum from 133 DLBCL pt.	[6]
miR-445-3p	Improved	High	RSR	R-CHOP	Serum from 133 DLBCL pt.	[6]
miR-181a	Improved	High	PFS	R-CHOP	176 FFPE CS	[27]
miR-21[a]	Improved	High	RFS	-	Serum from 61 DLBCL pt.	[33]
	Improved	High	RFS	-	Serum from 62 DLBCL pt.	[23]
	Improved	High	RFS	-	35 FFPE CS	[22]
miR-146a	Improved	Low	RSR, OS and PFS	R-CHOP/ CHOP	90 FFPE CS	[25]
miR-155	Improved	Low	RSR, OS and PFS	R-CHOP/ CHOP	90 FFPE CS	[25]
miR-195	Improved	Low	OS	CHOP	58 FFPE CS	[29]
miR-200c	Poor	High	OS	R-CHOP	61 FFPE CS	[31]
miR-18a	Poor	High	OS	R-CHOP	176 FFPE CS	[27]
miR-23a[a]	Poor	High	OS	-	104 FFPE CS	[30]
	Poor	Low	OS	CHOP	58 FFPE CS	[29]
miR-222[a]	Poor	High	PFS	R-CHOP	176 FFPE CS	[27]
	Poor	High	OS and PFS	R-CHOP	106 FFPE CS	[21]
	Poor	High	OS and PFS	R-CHOP	240 FFPE CS	[24]
miR-221	Poor	High	OS and PFS	R-CHOP	240 FFPE CS	[24]
miR-331	Poor	High	OS and PFS	R-CHOP	240 FFPE CS	[24]
miR-451	Poor	High	OS and PFS	R-CHOP	240 FFPE CS	[24]
miR-28	Poor	High	OS and PFS	R-CHOP	240 FFPE CS	[24]
miR-151	Poor	High	OS and PFS	R-CHOP	240 FFPE CS	[24]
miR-148a	Poor	High	OS and PFS	R-CHOP	240 FFPE CS	[24]
miR-93	Poor	High	OS and PFS	R-CHOP	240 FFPE CS	[24]
miR-491	Poor	High	OS and PFS	R-CHOP	240 FFPE CS	[24]
miR-224	Poor	High	RSR	R-CHOP	Serum from 133 DLBCL pt.	[6]
miR-1236	Poor	High	RSR	R-CHOP	Serum from 133 DLBCL pt.	[6]
miR-520d-3p	Poor	High	RSR	R-CHOP	Serum from 133 DLBCL pt.	[6]
miR-19a	Poor	Low	PFS	CHOP	58 FFPE CS	[29]
miR-27a	Poor	Low	OS	CHOP	58 FFPE CS	[29]
miR-34a	Poor	Low	OS	CHOP	58 FFPE CS	[29]
miR-127	Poor	Low	OS and PFS	CHOP	58 FFPE CS	[29]
miR-92a	Poor	Low	Increased RR	CHOP	Plasma from 34 DLBCL pt.	[28]
miR-320d	Poor	Low	OS and PFS	CHOP	106 FFPE CS	[26]
miR-146b-5p	Poor	Low	PFS	CHOP	106 FFPE CS	[26]
miR-129-5p	Poor	Low	OS	R-CHOP	61 FFPE CS	[32]

CS = clinical samples (lymph node biopsies from DLBCL patients); DLBCL = diffuse large B-cell lymphoma; FFPE = formalin-fixed paraffin-embedded; miRNA = microRNA; OS = overall survival; PFS = progression-free survival; pt. = patients; (R-)CHOP = (rituximab-) cyclophosphamide doxorubicin vincristine prednisone; RFS = relapse-free survival; RR = relapse rate; RSR = remission rate.
a) Associated with 1 subtype in ≥ 2 studies.

CONCLUSION

In conclusion, this systematic review has collected current literature on miRNA expression as diagnostic and prognostic biomarkers in DLBCL. We have presented the results, advantages and limitations of these studies, and we have summarised the steps yet to be taken before miRNAs can be used clinically as biomarkers.

Based on the results presented in this review, it is clear that the potential of miRNAs as diagnostic and prognostic biomarkers is promising, and it is possible that the use of specific miRNA signature models can be integrated into daily practice and used in combination with clinical factors such as IPI. We believe that miRNAs as biomarkers can contribute to a shift towards a more individualised and patient-tailored treatment of DLBCL patients. However, additional research is needed before miRNA biomarkers become a reality in a routine clinical setting.

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LITERATURE

1. Lenz G, Staudt LM. Aggressive lymphomas. N Engl J Med 2010;362:1417-29.
2. Campo E, Swerdlow SH, Harris NL et al. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. Blood 2011;117:5019-32.
3. Tilly H, Vitolo U, Walewski J et al. Diffuse large B-cell lymphoma (DLBCL): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2012;23(suppl 7):vii78-82.
4. Alizadeh AA, Eisen MB, Davis RE et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 2000;403:503-11.
5. Gutierrez-Garcia G, Cardesa-Salzmann T, Climent F et al. Gene-expression profiling and not immunophenotypic algorithms predicts prognosis in patients with diffuse large B-cell lymphoma treated with immunochemotherapy. Blood 2011;117:4836-43.
6. Song G, Gu L, Li J et al. Serum microRNA expression profiling predict response to R-CHOP treatment in diffuse large B cell lymphoma patients. Ann Hematol 2014;93:1735-43.
7. A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. N Engl J Med 1993;329:987-94.
8. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 2001;69:89-95.
9. Jansson MD, Lund AH. MicroRNA and cancer. Mol Oncol 2012;6:590-610.
10. Macfarlane LA, Murphy PR. MicroRNA: biogenesis, function and role in cancer. Curr Genomics 2010;11:537-61.
11. Jardin F, Figeac M. MicroRNAs in lymphoma, from diagnosis to targeted therapy. Curr Opin Oncol 2013;25:480-6.
12. Mazan-Mamczarz K, Gartenhaus RB. Role of microRNA deregulation in the pathogenesis of diffuse large B-cell lymphoma (DLBCL). Leuk Res 2013;37:1420-8.
13. Scholer N, Langer C, Dohner H et al. Serum microRNAs as a novel class of biomarkers: a comprehensive review of the literature. Exp Hematol 2010;38:1126-30.
14. Moher D, Liberati A, Tetzlaff J et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Int J Surg 2010;8:336-41.
15. Culpin RE, Proctor SJ, Angus B et al. A 9 series microRNA signature differentiates between germinal centre and activated B-cell-like diffuse large B-cell lymphoma cell lines. Int J Oncol 2010;37:367-76.
16. Rai D, Karanti S, Jung I et al. Coordinated expression of microRNA-155 and predicted target genes in diffuse large B-cell lymphoma. Cancer Genet Cytogenet 2008;181:8-15.
17. Eis PS, Tam W, Sun L et al. Accumulation of miR-155 and BIC RNA in human B cell lymphomas. Proc Natl Acad Sci U S A 2005;102:3627-32.
18. Caramuta S, Lee L, Ozata DM et al. Role of microRNAs and microRNA machinery in the pathogenesis of diffuse large B-cell lymphoma. Blood Cancer J 2013;3:e152.
19. Lawrie CH, Saunders NJ, Soneji S et al. MicroRNA expression in lymphocyte development and malignancy. Leukemia 2008;22:1440-6.
20. Lawrie CH, Chi J, Taylor S et al. Expression of microRNAs in diffuse large B cell lymphoma is associated with immunophenotype, survival and transformation from follicular lymphoma. J Cell Mol Med 2009;13:1248-60.
21. Malumbres R, Sarosiek KA, Cubedo E et al. Differentiation stage-specific expression of microRNAs in B lymphocytes and diffuse large B-cell lymphomas. Blood 2009;113:3754-64.
22. 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;121:1156-61.
23. Chen W, Wang H, Chen H et al. Clinical significance and detection of microRNA-21 in serum of patients with diffuse large B-cell lymphoma in Chinese population. Eur J Haematol 2014;92:407-12.
24. Montes-Moreno S, Martinez N, Sanchez-Espiridion B et al. miRNA expression in diffuse large B-cell lymphoma treated with chemoimmunotherapy. Blood 2011;118:1034-40.
25. Zhong H, Xu L, Zhong JH et al. Clinical and prognostic significance of miR-155 and miR-146a expression levels in formalin-fixed/paraffin-embedded tissue of patients with diffuse large B-cell lymphoma. Exp Ther Med 2012;3:763-70.
26. Wu PY, Zhang XD, Zhu J et al. Low expression of microRNA-146b-5p and microRNA-320d predicts poor outcome of large B-cell lymphoma treated with cyclophosphamide, doxorubicin, vincristine, and prednisone. Hum Pathol 2014;45:1664-73.
27. Alencar AJ, Malumbres R, Kozloski GA et al. MicroRNAs are independent predictors of outcome in diffuse large B-cell lymphoma patients treated with R-CHOP. Clin Cancer Res 2011;17:4125-35.
28. Ohyashiki K, Umezu T, Yoshizawa S et al. Clinical impact of down-regulated plasma miR-92a levels in non-Hodgkin's lymphoma. PLoS One 2011;6:e16408.
29. Roehle A, Hoefig KP, Repsilber D et al. MicroRNA signatures characterize diffuse large B-cell lymphomas and follicular lymphomas. Br J Haematol 2008;142:732-44.
30. Wang WL, Yang C, Han XL et al. MicroRNA-23a expression in paraffin-embedded specimen correlates with overall survival of diffuse large B-cell lymphoma. Med Oncol 2014;31:919,014-0919-2.
31. Berglund M, Hedstrom G, Amini RM et al. High expression of microRNA-200c predicts poor clinical outcome in diffuse large B-cell lymphoma. Oncol Rep 2013;29:720-4.
32. Hedstrom G, Thunberg U, Berglund M et al. Low expression of microRNA-129-5p predicts poor clinical outcome in diffuse large B cell lymphoma (DLBCL). Int J Hematol 2013;97:465-71.
33. Lawrie CH, Gal S, Dunlop HM et al. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br J Haematol 2008;141:672-5.
34. Di Lisio L, Sanchez-Beato M, Gomez-Lopez G et al. MicroRNA signatures in B-cell lymphomas. Blood Cancer J 2012;2:e57.
35. Frank R, Hargreaves R. Clinical biomarkers in drug discovery and development. Nat Rev Drug Discov 2003;2:566-80.
36. Munch-Petersen HD, Ralfkiaer U, Sjo LD et al. Differential expression of miR-155 and miR-21 in tumor and stroma cells in diffuse large B-cell lymphoma. Appl Immunohistochem Mol Morphol 2014;22:635-41.
37. Pepe MS, Janes H, Longton G et al. Limitations of the odds ratio in gauging the performance of a diagnostic, prognostic, or screening marker. Am J Epidemiol 2004;159:882-90.
38. Pepe MS, Etzioni R, Feng Z et al. Phases of biomarker development for early detection of cancer. J Natl Cancer Inst 2001;93:1054-61.
39. van Dijk EL, Auger H, Jaszczyszyn Y et al. Ten years of next-generation sequencing technology. Trends Genet 2014;30:418-26.
40. Dogini DB, Pascoal VD, Avansini SH et al. The new world of RNAs. Genet Mol Biol 2014;37:285-93.
41. Baker M. MicroRNA profiling: separating signal from noise. Nat Methods 2010;7:687-92.
42. Jima DD, Zhang J, Jacobs C et al. Deep sequencing of the small RNA transcriptome of normal and malignant human B cells identifies hundreds of novel microRNAs. Blood 2010;116:e118-27.
43. Koens L, Qin Y, Leung WY et al. MicroRNA profiling of primary cutaneous large B-cell lymphomas. PLoS One 2013;8:e82471.