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# Cell-free DNA as a biomarker in diffuse large B-cell lymphoma: A systematic review

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ABSTRACT

Cell-free DNA (cfDNA), which is DNA released from cells into the circulation, is one of the most promising non-invasive biomarkers in cancer. This approach could be of interest for the management of Diffuse Large B-Cell Lymphoma (DLBCL) patients, which is the most common non-Hodgkin lymphoma. Then, the aim of this systematic review was to define the utility of cfDNA in this disease. Selected articles were classified in four groups, depending on the aspects of cfDNA studied, i.e. concentration, methylation, IgH gene rearrangements, and somatic mutations. While concentration and methylation of cfDNA need to be further analyzed, IgH gene rearrangements and somatic mutations seem to be the most promising biomarkers to date. Their detection has been shown to allow disease monitoring and early prediction of relapse. Although more efforts and standardization of techniques are needed, studying cfDNA in liquid biopsy may help improve the outcome of DLBCL patients.

## 1. Introduction

Diffuse Large B-Cell Lymphoma (DLBCL) accounts for 30–40% of all newly diagnosed cases of non-Hodgkin lymphoma (Swerdlow et al., 2016). Currently, standard first-line therapy for DLBCL is based on the combined chemotherapy of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP). This treatment leads to complete remission (CR) in 75–80% of patients. However, around 40% of patients are primarily refractory or relapse (Coiffier et al., 2010). It would be of great interest to identify those patients at an early stage in order to adjust the treatment and improve response. To date, several prognostic tools have been developed to predict the outcome of DLBCL patients, such as the International Prognostic Index (IPI) (International Non-Hodgkin's Lymphoma Prognostic Factors Project, 1993), cell-of-origin (COO) classification by gene expression profiling (Alizadeh et al., 2000; Rosenwald et al., 2002) or immunohistochemistry based algorithms (Scott, 2015), and MYC rearrangement status (Barrans et al., 2010; Horn et al., 2013). Among them, only MYC rearrangement status has been proven useful to identify patients that could benefit from a more intensive treatment (Howlett et al., 2015), but the prevalence of this rearrangement is low (Obermann et al., 2009). Although these tools have been useful to assess prognosis, they cannot accurately predict which patients would be refractory or relapse.

In recent years, the analysis of tumor biopsies by Next-Generation Sequencing (NGS) has allowed the identification of several mutations in DLBCL patients capable of predicting prognosis better than the above-mentioned approaches (Chapuy et al., 2018; Schmitz et al., 2018), e.g. those located in TP53, FOX01, and MLL3 genes (Guo et al., 2018; Lu et al., 2016). However, genotyping of tumor-derived samples presents some drawbacks. First, DLBCL usually presents a high number of subclonal mutations. Therefore, the biopsied region may be unrepresentative of all the mosaic of subclones present in a single DLBCL patient (Rossi et al., 2017). Second, serial biopsies cannot be obtained to follow-up the disease evolution over time, especially after achieving response, when there is not macroscopic evidence of the disease.

Currently, liquid biopsy, which refers to the study of tumor-derived biomarkers in bodily fluids, has emerged as a non-invasive diagnostic and prognostic tool to overcome those two problems (Crowley et al.,

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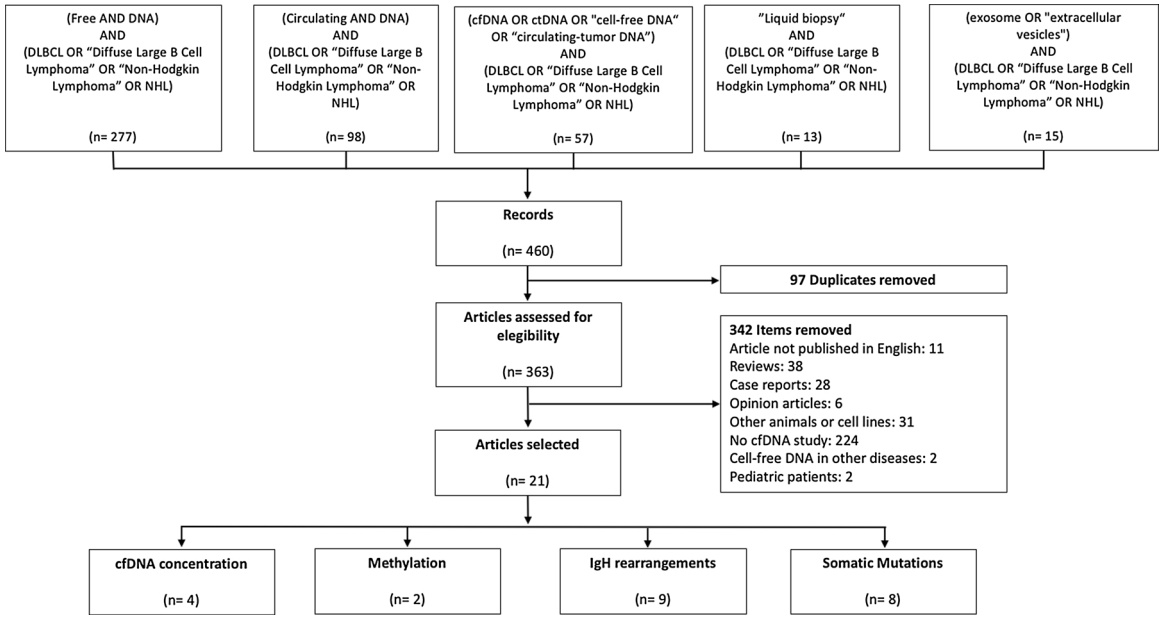


**Fig. 1.** Flowchart of study selection.

2013; Perakis and Speicher, 2017). Cell-free DNA (cfDNA) has arisen as a promising source of biomarkers in the liquid biopsy. cfDNA consists in single or double-stranded DNA that is released from cells into the circulation as a result of cell lysis, apoptosis or extracellular vesicle secretion (Jahr et al., 2001; Stroun et al., 2001). It has been described that cancer patients present higher concentrations of cfDNA than healthy individuals (Heitzer et al., 2015). A part of this cfDNA, known as circulating tumor DNA (ctDNA), is secreted directly by tumor cells and thus it may mirror the somatic mutations, immunoglobulin heavy chain (IgH) gene rearrangements and methylation patterns of the lymphoma (Andersen, 2018; Fleischhacker and Schmidt, 2007; Panagopoulou et al., 2019). Interestingly, cfDNA has already been shown useful for diagnosis, prognosis, and monitoring in several cancers, such as non-small cell lung cancer (Ansari et al., 2016), colon cancer (Siravegna and Bardelli, 2016), and pancreatic cancer (Chen et al., 2018). In DLBCL, some studies have investigated the potential role of cfDNA as a biomarker with interesting and promising results (Camus et al., 2017; Komatsubara and Sacher, 2017; Kwok et al., 2016; Melani and Roschewski, 2016). However, these studies had differences among subjects, phenotypes studied, and used a varying array of techniques, which make it difficult to compare the data Then, in this systematic review, we aimed to clarify the current state-of-the-art on the potential role of cfDNA in liquid biopsy for diagnosis, prognosis, and disease monitoring in patients with DLBCL.

## 2. Methods

We performed a systematic search in PubMed database to identify articles published between January 1960 and October 2018 using the following strategy: [(DLBCL OR "Diffuse Large B Cell Lymphoma" OR "Non-Hodgkin Lymphoma" OR NHL) AND ((Circulating AND DNA) OR (cfDNA OR ctDNA OR "cell-free DNA" OR "circulating-tumor DNA") OR ("liquid biopsy") OR (exosome OR "extracellular vesicles") OR (free AND DNA))]. Articles were considered if they presented original independent studies and evaluated the value of cfDNA in blood-derived fluids (plasma or serum) as a biomarker for diagnosis, prognosis, treatment response prediction or disease monitoring in adult patients with DLBCL. Articles not published in English, reviews, case reports, opinion articles, as well as those studies carried out in pediatric patients, other diseases (including other non-Hodgkin lymphoma or extranodal DLBCL), and animals or cell lines were directly excluded. All the references within the selected studies were also revised in search of additional matches. Each eligible manuscript was assessed independently by two researchers and disagreements were resolved by consensus. Data extracted from each study included: publication year, type of blood-based fluid analyzed (serum or plasma), number of DLBCL cases and controls in which cfDNA was studied, technical methodology, genetic variants studied, and relevant information about management of DLBCL patients (e.g. diagnostic and prognostic factors, as well as information about monitoring and response to treatment).

## 3. Results

A total of 460 records were initially identified, 363 remained after duplicates removal. Among them, 342 were excluded after abstract revision because they did not meet the inclusion criteria and 21 articles were included for further analysis (Alcaide et al., 2016; Armand et al., 2013; Assouline et al., 2016; Bo et al., 2016; Bohers et al., 2018, 2015; Camus et al., 2016; He et al., 2011; Herrera et al., 2016; Hohaus et al., 2009; Hossain et al., 2018; Kristensen et al., 2016; Kurtz et al., 2018, 2015; Li et al., 2017; Li and Xu, 2017; Roschewski et al., 2015; Rossi et al., 2017; Scherer et al., 2016; Wedge et al., 2017; Zhong and Huang, 2010). After full text review, we classified these articles into the following four groups, depending on the aspects of cfDNA studied: 1) cfDNA concentration, 2) cfDNA methylation, 3) detection of IgH gene rearrangements, and 4) detection of DLBCL-related somatic mutations in cfDNA (Fig. 1).

### 3.1. Cell-free DNA concentration

Four articles studied the value of cfDNA concentration in plasma as a biomarker in DLBCL (Hohaus et al., 2009; Kristensen et al., 2016; Li et al., 2017; Li and Xu, 2017). Although cfDNA concentration was highly variable among studies, total cfDNA concentration was consistently higher in DLBCL patients than in healthy controls in the three studies in which they were compared (Hohaus et al., 2009; Kristensen et al., 2016; Li et al., 2017). In fact, in all of them, mean cfDNA concentration was at least twice as high in DLBCL cases than in controls (Table 1).

Regarding prognosis, Li et al. and Hohaus et al. suggested that high cfDNA concentration predicted a worse outcome, as patients with high cfDNA concentration presented inferior 2-year progression free survival

**Table 1**
Cell-free DNA concentration as a prognostic biomarker in DLBCL.

| Reference | N | Plasma volume (ml) | DNA extraction method | DNA quantification method | Median (range) cfDNA concentration (ng/ml) | Prognosis |
|---|---|---|---|---|---|---|
| Li et al., 2017 | 98 DLBCL<br>80 controls | NA | MagMAX Cell-Free DNA Isolation Kit | Qubit dsDNA HS Assay Kit | DLBCL: 845 (100-14180)<br>Controls: 209 (100-456) p < 0.0001 | 2-year PFS: Elevated [cfDNA] (> 1586 ng/ml): 44%<br>Normal [cfDNA]: 78%<br>p = 0.001 |
| Hohaus et al., 2009 | 63 DLBCL<br>41 controls | 0.4-0.8 | QIAamp UltraSens Virus Kit | SYBR green-based RT-qPCR for the b-globin gene | DLBCL: 26.9 (4.0-940.5)<br>Controls: 12.1 (3.0-34.7) p < 0.004 | 2-year FFTF: Elevated [cfDNA] (> 29.4 ng/ml): 40%<br>Normal [cfDNA]: 88%<br>p = 0.030 |
| Kristensen et al., 2016 | 74 DLBCL<br>14 controls | NA | MagNa Pure LC Total Nucleic Acid Isolation Kit | Fluorometry (Qubit) | DLBCL: 7200<br>Controls: 1170 p < 0.0001 | 5-year OS: Elevated [cfDNA] (> 4700 ng/ml): 63.2%<br>Normal [cfDNA]: 63.6%<br>NS |
| Li and Xu, 2017 | 57 DLBCL (31 CR, 26 PR, 6 NR) | NA | NA | NA | DLBCL CR: 1206<br>DLBCL PR: 1212<br>DLBCL NR: 2027 | CR or PR: Reduction of cfDNA<br>Non-remission: stable cfDNA |

Abbreviations: CR: complete remission, FFTE: freedom from treatment failure; N: number of DLBCL cases/controls in which cfDNA was studied; NA: not addressed; NR: non-responding; NS: not significant; OS: overall survival; PFS: progression free survival; PR: partial remission.

(PFS) (44% and 40%, respectively) compared to those with normal cfDNA concentration (78% and 88%, respectively) (Hohaus et al., 2009; Li et al., 2017). Kristensen et al. did not find association between cfDNA concentration and 5-year overall survival (OS) (Kristensen et al., 2016). Li et al. observed higher cfDNA concentration at diagnosis in refractory patients if compared with CR patients (Li and Xu, 2017). In this study they also found an association between decrease in cfDNA levels after treatment and achievement of a complete or partial response (Table 1).

### 3.2. Cell-free DNA methylation

Two articles studied the prognostic value of aberrant methylation status in cfDNA (Kristensen et al., 2016; Wedge et al., 2017). Thus, Wedge et al. studied the methylation of LINE1 transposable elements, which has been considered a validated subrogate measure for global methylation. They observed that the 5-year OS in the normally methylated group was significantly higher than in the hypomethylated group (66.2% vs. 33.3%, respectively), suggesting a prognostic value of LINE1 methylation status in DLBCL cases (Wedge et al., 2017). Likewise, Kristensen et al. assessed the methylation of DAPK1, DBC1, MIR34 A and MIR34B/C, and found a significant correlation between the hypermethylation of DAPK1 and DBC1 and worse 5-year OS outcome (Kristensen et al., 2016) (Table 2).

### 3.3. Detection of IgH gene rearrangements in cfDNA

Nine articles demonstrated that clonotypic IgH gene rearrangements can be detected in cfDNA in DLBCL patients (Armand et al., 2013; Bo et al., 2016; He et al., 2011; Herrera et al., 2016; Hossain et al., 2018; Kurtz et al., 2015; Roschewski et al., 2015; Scherer et al., 2016; Zhong and Huang, 2010). Five articles among them studied the value of these rearrangements as non-invasive biomarkers for relapse prediction after achieving CR (Table 3). The proportion of patients with detectable IgH gene rearrangements before clinical relapse (i.e. sensitivity) was very variable among studies (33.3–88%). In those cases, the mean time between the molecular evidence and relapse (i.e. lead time) ranged between 33 and 170 days. Remarkably, IgH gene rearrangements were rarely found in those patients that did not relapse (specificity, > 98%). Moreover, Roschewski et al. demonstrated that IgH rearrangements measured after 2 cycles of treatment could also detect resistance and early relapse, with a sensitivity and specificity of 47% and 88%, respectively (Roschewski et al., 2015).

### 3.4. Detection of DLBCL-related somatic mutations in cfDNA

Eight articles looked for somatic mutations in cfDNA from DLBCL patients, six of them using different NGS panels and two using a targeted digital PCR approach for specific mutations (Alcaide et al., 2016; Assouline et al., 2016; Bohers et al., 2018, 2015; Camus et al., 2016; Kurtz et al., 2018; Rossi et al., 2017; Scherer et al., 2016) (Table 4).

On the one hand, without considering the mutations in the tumor site, all six NGS studies identified at least one cfDNA mutation in plasma or serum samples in 66.6% to 100% of patients. The mean number of mutations per patient at diagnosis ranged from 4 to 124, depending on the panel used (Table 4). Considering the four studies for which the mutations per patient were available (Assouline et al., 2016; Bohers et al., 2018, 2015; Camus et al., 2016), it can be observed that the mutations found were very different for each patient. Only 11 mutations were found in three or more individuals: EZHD Y646 F and MYD88 L265 P (frequency = 1.2%); EZH2 Y646 N, STAT6 D419 G, and PIM1 L184 F (frequency = 1%); MYD88 S219C and MYD88 *205R (frequency = 0.8%); and MEF2B E77 K, PIM1 E125 K, B2M p.13_13del, and TP53 R248Q (frequency = 0.6%). However, most of the mutations were concentrated in a few genes. In fact, mutations were only detected

**Table 2**
Cell-free DNA methylation as a diagnostic or prognostic biomarker in DLBCL patients.

| Reference | N | Genes | Diagnosis | 5-year OS | | |
|---|---|---|---|---|---|---|
| | | | Patients with altered methylation | Altered methylation | Normal methylation | P-value |
| Wedge et al., 2017 | 74 DLBCL | LINE1 | 8% hypomethylated | 33.3% | 66.2% | 0.009 |
| Kristensen et al., 2016 | 74 DLBCL | DAPK1 | 19% hypermethylated | 35.7% | 70.0% | 0.004 |
| | 14 controls | DBC1 | 16% hypermethylated | 41.7% | 67.8% | 0.044 |
| | | MIR34A | 8% hypermethylated | 83.3% | 60.9% | NS |
| | | MIR34B/C | 10% hypermethylated | 50.0% | 67.9% | NS |

Abbreviations: N: number of DLBCL cases/controls in which cfDNA was studied; NS: not significant; OS: overall survival.

**Table 3**
IgH gene rearrangements in the liquid biopsy-cfDNA of DLBCL patients.

| Reference | Source of cfDNA | Method | N | Relapse prediction during surveillance | | |
|---|---|---|---|---|---|---|
| | | | | Sensitivity[a] | Specificity[b] | Mean lead-time (days) |
| Scherer et al., 2016 | Plasma | IgHTS | 9 | 33.3% | NA | 33 |
| Roschewski et al., 2015 | Serum | IgHTS | 107 | 88% | 98% | 105 |
| Kurtz et al., 2015 | Plasma | IgHTS | 25 | 60% | 100% | 88 |
| Hossain et al., 2018 | Plasma | IgHTS | 6 | 75% | 100% | NA |
| Herrera et al., 2016 | Plasma | IgHTS | 6 | 75% | 100% | 170 |

[a] Sensitivity: proportion of relapsed patients with a previous IgHTS detection.
[b] Specificity: proportion of non-relapsing patients with negative IgHTS; Abbreviations: IgHTS: clonotypic immunoglobulin heavy chain gene rearrangements by High Throughput Sequencing; lead-time: time from positive biomarker result to diagnosis of relapse; N: number of DLBCL cases/controls in which cfDNA was studied.

in 23/34 genes (Bohers et al., 2015, 2018), 28/45 genes (Assouline et al., 2016), and 43/59 genes (Rossi et al., 2017) analyzed in each panel (Table S1). Mutations in a total of 13 common genes were identified in the above-mentioned panels, i.e. STAT6, CD79B, MYD88, CD58, GNA13, MYC, CREBBP, BCL2, PIM1, TP53, EZH2, MEF2B, and B2M (Figure S1). A panel containing only these 13 genes would have identified 79 out of the 91 individuals (86.8%) that were detected with each of the panels used in the different studies considered (Table S2).

On the other hand, a high concordance was found between the mutations identified in paired tumor and plasma samples. This correlation was detected regardless of the technique applied (Table 4).

Three out of the eight studies measured the prognostic value of the proportion of somatic mutations in cfDNA (i.e. ctDNA levels) (Table 4). They found that, high ctDNA levels were directly correlated with higher IPI, metabolic tumor volume (MTV) (Bohers et al., 2018; Kurtz et al., 2018; Scherer et al., 2016), serum lactate dehydrogenase (LDH) (Bohers et al., 2018; Scherer et al., 2016), and Ann Arbor staging (Scherer et al., 2016). Moreover, one study showed that patients with high levels of ctDNA had worse prognosis than those with low levels in terms of 2-year event-free survival (EFS) (Kurtz et al., 2015). Finally, Scherer et al. designed a COO classification algorithm based on the study of 32 genetic features. When this model was applied to cfDNA mutations, it stratified prognosis more accurately than Hans algorithm (Scherer et al., 2016).

Besides, four authors studied mutated ctDNA levels as a response assessing or monitoring biomarker during follow-up (Assouline et al., 2016; Kurtz et al., 2018; Rossi et al., 2017; Scherer et al., 2016) (Table 4). Scherer et al. found that ctDNA was detected in liquid biopsy before relapse in 73% of patients. The median time from detection to clinical relapse was of 188 days (Scherer et al., 2016). Rossi et al. found that ctDNA mutations were detectable after treatment in non-responding patients only and mutations found at relapse were not always the same ones identified at diagnosis (Rossi et al., 2017). Kurtz et al. suggested that a 2-log decrease in ctDNA concentration after one cycle of chemotherapy (i.e. Early Molecular Response, EMR) and a 2.5-log decrease after two cycles (i.e. Mayor Molecular Response, MMR) was associated with improved 2-year EFS (83% vs. 50% and 82% vs. 46%, respectively) (Kurtz et al., 2018). Finally, Assouline et al. detected that an increase in ctDNA concentration at day 15 of treatment predicted lack of response with 71.4% of sensitivity and 100% of specificity (Assouline et al., 2016).

## 4. Discussion

In this systematic review, we have performed a comprehensive analysis of the current literature in relation to the potential role of cfDNA as non-invasive biomarker for diagnosis, prognosis and disease monitoring in patients with DLBCL. In global, reviewed articles show that cfDNA is an interesting biomarker that could provide useful information to improve clinical management of DLBCL. Those articles were focused on different aspects of the cfDNA, such as concentration, methylation, IgH gene rearrangements detection, and identification of DLBCL-related somatic mutations.

We would like to remark that for all these four approaches cfDNA has been mainly obtained from blood plasma. In fact, although serum presents a higher concentration of total cfDNA, the proportion of ctDNA is lower than in plasma due to wild type DNA contamination from leukocyte lysis during serum acquisition (Kurtz et al., 2018; Sorber et al., 2019). In view of the above, plasma seems to be the most recommendable source of cfDNA for further studies in patients with DLBCL. However, cfDNA recovery might be also affected by pre-analytical variables. On the one hand, due to the short half-life of cfDNA (Sorber et al., 2019), it is important to minimize the time between plasma collection and laboratory processing (Li et al., 2017; Rossi et al., 2017). On the other hand, different plasma centrifugation (Sorber et al., 2019) and cfDNA extraction protocols (Cavallone et al., 2019) have different yields. Therefore, standardization is required before its clinical implementation.

### 4.1. Cell-free DNA concentration prognostic value is not clear

Reviewed articles reported that DLBCL patients presented elevated cfDNA concentration when compared to healthy controls (Hohaus et al., 2009; Kristensen et al., 2016; Li et al., 2017). These results are in accordance with data published regarding other types of cancer, such as colorectal, renal, and non-small cell lung cancer (Bhangu et al., 2017; Skrypkina et al., 2016; Szpechcinski et al., 2016). However, cfDNA concentration, in spite of being a promising biomarker, can be elevated not only in cancer, but also in other conditions such as sepsis, major trauma, liver injury, and inflammatory diseases (Ahmed et al., 2016; Karlas et al., 2017; Prakash et al., 2017; Truszewska et al., 2017). Thus, cfDNA concentration alone may not be an optimal biomarker for differential diagnosis in DLBCL.

There are conflicting results about cfDNA concentration value as a prognostic biomarker in DLBCL. In this regard, only three studies have evaluated cfDNA concentration and prognosis in DLBCL. Two studies found association between high cfDNA concentration and lower 2-year progression free survival (PFS) (Hohaus et al., 2009; Li et al., 2017) and the other one did not find any association between cfDNA concentration and 5-year OS (Kristensen et al., 2016). In DLBCL, 2-year PFS and 5-year OS present a high correlation, therefore, it could be said that those results are contradictory. Considering that a correlation between high cfDNA concentration and worse outcome has already been reported in other tumors, such as pancreatic (Chen et al., 2018) and lung cancers (Tissot et al., 2015), further studies are needed to clarify its applicability in DLBCL.

Finally, one study showed that patients that responded to treatment had a significant reduction in cfDNA concentration, while non-responding patients did not (Li and Xu, 2017). This suggests that cfDNA concertation may be useful as a response-assessing biomarker (Valpione et al., 2018; Wu et al., 2019) but this study requires replication before conclusions can be drawn about this topic.

**Table 4**
Somatic mutations in cfDNA in DLBCL.

| Reference | Source of cfDNA | Method | Genes | N | % patients with at least one mutation | Mutations per patient mean (range) | Concordance (vs. tumor) | Prognostic factor correlated with high ctDNA levels | ctDNA monitoring |
|---|---|---|---|---|---|---|---|---|---|
| Scherer et al., 2016 | Plasma | CAPP-Seq | 786 genes | 59 DLBCL<br>24 controls | 100%<br>0% | 124 (8–454) | Sensitivity [A]: 91%<br>Specificity [B]: 99.8% | Higher IPI, LDH, and Ann Arbor stage. Inferior PFS. | ctDNA detection, relapse. |
| Rossi et al., 2017 | Plasma | CAPP-Seq | 59 genes | 30 DLBCL 20 DLBCL<br>6 controls | 66.6% 85%<br>0% | 6 (1–13) | Sensitivity [A]: 82.8%<br>Specificity [B]: 99.9% | NA | ctDNA only in non-responding patients. |
| Kurtz et al., 2018 | Plasma/ serum | CAPP-Seq | 466 genes | 217 DLBCL<br>48 controls | 98%<br>0% | 117 (range NA) | NA | Inferior EFS (all patients), inferior OS (patients treated with salvage therapy). | Persistence of ctDNA levels, treatment failure and inferior OS. |
| Bohers et al., 2015 | Plasma | NGS ion torrent | 34 genes | 12 DLBCL | 83.3% | 6 (1–9) | Sensitivity [A]: 75.9% | NA | NA |
| Bohers et al., 2018 | Plasma | NGS ion torrent | 34 genes | 30 DLBCL | 86.7% | 6 (1–19) | NA | Higher IPI and LDH and tumor volume. | NA |
| Assouline et al., 2016 | Plasma | CAPP-Seq | 45 genes | 18 DLBCL | 100% | 4 (1–10) | NA | NA | Increasing or stable ctDNA, treatment failure. |
| Alcaide et al., 2016 | Plasma | Multiplexed dPCR | 6 genes | 7 DLBCL | NA[C] | NA[C] | Sensitivity [A]: 100% | NA | NA |
| Camus et al., 2016 | Plasma | dPCR | 3 genes | 14 DLBCL | NA[C] | NA[C] | Sensitivity [A]: 100% for EZH2 and XPO1, 88.9% for MYD88<br>Specificity [B]: 100% | NA | NA |

(A) Sensitivity: True positive rate or the proportion of tumor proven mutations that are also found in plasma cfDNA; (B) Specificity: True negative rate or the proportion of non-mutated genes in tumor that are also not mutated in plasma. (C) Only patients known to have target mutations were included. Abbreviations: DLBCL: diffuse large B-cell lymphoma; CAPP-Seq: CAncer Personalized Profiling by deep Sequencing; cfDNA: cell-free DNA; ctDNA: circulating-tumor DNA; dPCR: digital PCR; EFS: event-free survival; N: number of DLBCL cases/controls in which cfDNA was studied; IPI: International Prognostic Index; LDH: Serum Lactate Dehydrogenase; NA: not addressed; OS: overall survival; PFS: progression free survival.

### 4.2. Cell-free DNA methylation status in patients with DLBCL needs to be more deeply addressed

Two studies have evaluated cfDNA methylation in DLBCL (Kristensen et al., 2016; Wedge et al., 2017). Hypomethylation in LINE1 sequences, which is considered a surrogate for global hypomethylation (Lisanti et al., 2013), was associated with poorer 5-year OS in DLBCL patients (Wedge et al., 2017). LINE1 hypomethylation in cfDNA had not been previously studied in DLBCL, but it has already been shown as an adverse prognostic factor in other tumors, such as colorectal (Nagai et al., 2017) and breast cancers (Lee et al., 2019).

Kristensen et al. found a significant correlation between hypermethylation of DAPK1 and DBC1 and 5-year OS, indicating poor prognosis (Kristensen et al., 2016). These genes are tumor suppressor genes that have been shown to be methylated in DLBCL tumor biopsies (Amara et al., 2008; Grønbaek et al., 2008; Kristensen et al., 2014). This is in accord with other studies reporting association between hypermethylation of cfDNA and worse survival in other types of cancers, e.g. pancreatic (Chen et al., 2018), prostate (Hendriks et al., 2018), and lung cancers (Powrózek et al., 2016), among others.

These studies are promising, specially taking into account the fact that DNA methylation has been shown to play a major role in the pathogenesis of DLBCL (Jiang et al., 2016). For example, mutations in EZH2 and KMT2D genes, which are frequent in DLBCL, disrupt normal methylation in malignant DLBCL cells (Caganova et al., 2013; Zhang et al., 2015). However, cfDNA methylation in DLBCL should be more deeply studied to be able to draw conclusions about this topic.

### 4.3. IgH gene rearrangements in liquid biopsy as a biomarker of response to treatment in DLBCL

Given that DLBCL is a clonal neoplastic disease, all tumor cells share the same IgH VDJ sequence, which is formed during the IgH gene rearrangement phase of B-cell maturation. It has been shown that these rearrangements can be detected in tumor and plasma of DLBCL patients (Armand et al., 2013; Bo et al., 2016; Sebastián et al., 2012; Zhong and Huang, 2010). Hence, five studies have evaluated the performance of IgH gene rearrangements detection in plasma as a non-invasive disease monitoring biomarker during or after treatment (Herrera et al., 2016; Hossain et al., 2018; Kurtz et al., 2015; Roschewski et al., 2015; Scherer et al., 2016). However, the success rate in predicting relapse is variable among studies (between 33.3 and 88%).

These differences could not be attributable to the type of therapy used, i.e. R-CHOP, Chimeric Antigen Receptor T-cell therapy (CAR-T), and Allogenic Stem Cell Transplantation (Allo-SCT). However, those patients responding to CAR-T presented an initial elevation of ctDNA levels (Hossain et al., 2018), which could be explained by the excess of ctDNA released by tumor necrosis. As a consequence, only values obtained after this elevation should be considered to avoid false positives.

On the other hand, a plausible explanation for the discrepancies among studies may be that those with highest sensitivity rates (75–88%) were performed with patients in which the rearrangements had been previously detected at diagnosis in tumor and/or plasma (Herrera et al., 2016; Hossain et al., 2018; Roschewski et al., 2015). Therefore, it may be of relevance to analyze the presence of these rearrangements at diagnosis to identify which patients would benefit the most from this approach. Then, considering that 1) IgH gene rearrangements had shown to be detectable before relapse (mean lead-time, 33–170 days) and 2) almost all patients that presented positive IgH gene rearrangements after achieving CR relapse (specificity, > 98%), this approach could be useful for identifying those patients that could benefit from treatment intensification.

### 4.4. Detection of DLBCL related somatic mutations in cfDNA

On the one hand, eight articles have studied somatic mutations in cfDNA in patients with DLBCL. All of them were able to detect mutations in cfDNA at the time of diagnosis in a high percentage of patients (66.6%–100%). While the panel designed by Scherer et al. was able to detect the highest number of mutations in 100% of patients (Scherer et al., 2016), other panel was also able to detect mutations in all patients using a much lower number of genes, which could be more affordable (Assouline et al., 2016). Therefore, further studies with large panels would be needed to further define the most commonly mutated genes in cfDNA. This would allow the construction of more suitable panels analyzing a more limited number of genes. In fact, we have identified 13 genes that were mutated in the four studies in which the mutation profiles were available. We have observed that analyzing those 13 genes, the 86.8% of patients with identified mutations would be detected. This reinforces the idea that using an effective panel with a more reduced number of genes could obtain results robust enough to translate into the clinical practice.

On the other hand, the five studies that compared mutations identified in paired tumor and cfDNA samples found a high correlation. This concordance suggested that non-invasive diagnosis using cfDNA could become nearly as accurate as genotyping DNA obtained from biopsies. Moreover, it could help overcome the disadvantages of the tumor biopsy, including its invasive nature and its failure to comprehend the whole clonal heterogeneity of the lymphoma.

Taking all these into account, a possible cost-effective monitoring strategy could be based on detecting mutations at diagnosis using an efficient NGS panel, and then, performing targeted serial analyses of those specific mutations during follow-up. This strategy, which has not been exploited in DLBCL yet, has already been tested in other cancers with promising results (Iwama et al., 2017). However, it must be considered that some of the mutations detected at diagnosis in DLBCL patients may not be detectable at relapse (Rossi et al., 2017). Thus, this monitoring strategy should be based on a set of several mutations for each patient to avoid false negatives. In view of the above, despite significant advances in recent years, further studies are needed to establish the best strategy.

Regarding prognosis, three studies analyzed the association between ctDNA levels and different prognostic markers, such as IPI, LDH, Ann Arbor stage, tumor size or survival (Bohers et al., 2018; Kurtz et al., 2018; Scherer et al., 2016). These results point to a connection between high ctDNA levels and a poor prognosis. Therefore, ctDNA levels could help identifying patients that could benefit from a more intense treatment, complementing the current prognostic tools. On the other hand, specific genetic alterations with a prognostic value can be analyzed in the cfDNA, such as the COO genetic classification by Scherer et al. (Scherer et al., 2016), which could stratify prognosis better than the Hans algorithm. Additionally, the non-invasive nature of ctDNA genotyping eases the application of the new prognostic classifications based on somatic mutations that have recently been defined in DLBCL tumor biopsies (Schmitz et al., 2018).

Four authors have studied the connection between ctDNA levels and treatment response with different approaches (Assouline et al., 2016; Kurtz et al., 2018; Rossi et al., 2017; Scherer et al., 2016). All of them identified a correlation between ctDNA and a worse response to treatment. Some of these approaches could be useful for clinical practice. For instance, serial ctDNA monitoring was able to predict relapse in 73% with a median lead time from ctDNA detection and clinical relapse of 188 days (Scherer et al., 2016). On the other hand, Kurtz et al. found that patients achieving EMR or MMR in ctDNA concentration presented better 2-year PFS (Kurtz et al., 2018). As a result, early monitoring of ctDNA could provide an opportunity for early intervention with intensification treatments, such as autologous bone marrow transplantation. These results suggest that ctDNA could be used as a biomarker to predict relapse during surveillance, thus, clinical trials studying the effect of early interventions based on ctDNA monitoring are needed to be able to translate these promising approaches into response guided treatment strategies.

### 4.5. Limitations of the study

It must be noted that this review presented several limitations. First of all, the number of selected articles in each section was limited, which has reduced the ability to make comparisons among them. Moreover, the included studies presented heterogeneity in sample sources or methodology, which could lead to differences in results. Then, it would be of great relevance to reach a consensus and standardize the methodology for future research to facilitate data reproducibility. In addition, it is possible that some additional articles have been missed by our search strategy. Furthermore, there is a tendency to only publish statistically significant results, which may lead to a bias and an underrepresentation of non-significant results.

## 5. Conclusion

Cell-free DNA in the liquid biopsy constitutes a feasible non-invasive biomarker for studying DLBCL since it offers a surrogate to direct tumor biopsies. While concentration and methylation of cfDNA need to be further analyzed, IgH gene rearrangements and somatic mutations seem to be the most promising biomarkers to date. Their detection has been shown to allow disease monitoring and early prediction of relapse, which opens the door to the design of new strategies for early intervention or treatment intensification. In conclusion, although more efforts and standardization of techniques are needed before its translation into the clinic, cfDNA study in the liquid biopsy may help improve the management and outcome of DLBCL patients.

## Declarations of interest

None.

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## 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.2019.04.013.

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