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Review Article

# Prognostic Value of Circulating Tumor DNA (ctDNA) in Diffuse Large B-Cell Lymphoma (DLBCL): Systemic Review and Meta-Analysis.

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## Abstract

Circulating cell-free DNA (ccf-DNA) and circulating tumor DNA (ctDNA) provide a minimally invasive method for cancer detection and measurement. However, their diagnostic and prognostic significance in hematological malignancies remains ambiguous. This meta-analysis aims to evaluate the prognostic value of ccf-DNA or ctDNA in patients with diffuse large B-cell lymphoma (DLBCL). All relevant literature was retrieved through a systematic search of electronic databases, including PubMed, Embase, Scopus, and the Cochrane Library. Eight eligible studies were selected for the analysis of prognostic value of ccf-DNA or ctDNA. Statistical analyses were performed using R software. The results indicate significant associations with both PFS (HR = 2.14; 95% CI: 1.31–3.40; $p < 0.01$) and OS (HR = 2.51; 95% CI: 1.84–3.40) for patients with elevated ccf-DNA or ctDNA levels. The results of this meta-analysis strongly suggest that elevated levels of ccfDNA or ctDNA are indicative of poor prognosis in patients with DLBCL.

**Keywords** : *Circulating cell-free DNA , circulating tumor DNA , DLBCL, Prognosis, Hazard Ratio, Meta-analysis.*

## INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of lymphoma, currently, the International Prognostic Index (IPI) is used for risk stratification of DLBCL which is an old prognostic score with limitations and there is an unmet need for a more refined prognostic tool with better representation of tumor biology (1). Over the past decade, liquid biopsy techniques utilizing circulating cell-free DNA (ccf-DNA) or circulating tumor DNA (ctDNA) from peripheral blood have emerged as robust diagnostic and prognostic tools for various cancers, including lung, prostate, and gastrointestinal cancers (2-4).

The role of ccf-DNA or ctDNA as a tool for risk stratification in patients with aggressive lymphoma has gained attention in recent years. However, most studies in this area are small, single-center experiences, and their findings are varied (5-12). There is limited systematic evidence demonstrating the prognostic value of ccf-DNA or ctDNA for DLBCL patients treated with R-CHOP regimen (13-15).

To address this gap, we conducted a systematic review and meta-analysis of studies that utilized ccf-DNA or ctDNA for prognostication of DLBCL, either at the time of diagnosis or during the course of treatment.

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## MATERIALS AND METHODS

This meta-analysis was conducted according to the guidelines provided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). The study was also registered with the International Prospective Register of Systematic Reviews (PROSPERO) with registration number CRD42024520358.

### Inclusion criteria

1. Patients with treatment naïve DLBCL
2. Studies reporting ccf-DNA or ctDNA in plasma/serum at one or more than one time points
3. Studies with information on patients' survival outcomes such as OS, PFS and EFS
4. Studies mentioning the technique for ccf-DNA or ctDNA detection or quantification.

### Exclusion criteria

1. All case reports, case series, review articles, editorials, letters or comments, and conference abstracts were excluded
2. Studies without survival data/Hazard ratio (HR) required to perform meta-analysis were excluded
3. Duplicate studies/ abstracts were excluded
4. Studies published in non-English language were excluded

### Search Strategy

Literature searches were conducted by searching electronic databases of Pubmed, Embase, Scopus and Cochrane Library for relevant papers published up to 31 May 2024. The following terms were used for searching: "ctDNA" or "cell-free DNA" or "cell-free tumor DNA" or "Circulating Nucleic Acids" or "Cell-Free Deoxyribonucleic acid" or "Cell-Free Nucleic Acid" or "circulating tumour DNA" or "tumor DNA" and "non-Hodgkin lymphoma" or "NHL" or "lymphoma". The Mesh terms and detailed search strategy is provided in the supplement file.

### Study Screening and Selection

Relevant articles were selected based on predefined eligibility criteria, and studies not meeting these criteria were excluded from the analysis. Two independent reviewers thoroughly analyzed and assessed the texts of the selected articles to confirm their eligibility before extracting data. Additionally, a manual search of the reference lists of the selected articles was conducted to ensure the inclusion of any relevant studies that were not initially identified through the search

### Data extraction and quality assessment

Two independent reviewers performed data extraction. The following variables was extracted from each study: first author, year of publication, country, study design, number of participants, age, sex, disease stage, bulky/non-bulky disease, IPI, PS, LDH, ccf-DNA or ctDNA measurement method, target gene(s), time point(s) of ccf-DNA or ctDNA measurement, follow up period of the study and HR value of prognostic index with 95% confidence intervals (CI) for survival outcomes. Extracted data was entered in an excel sheet and analyzed for outcomes. The Newcastle-Ottawa Scale (NOS) checklist was used to evaluate the quality of the eligible studies.

### Data synthesis and Statistical analysis

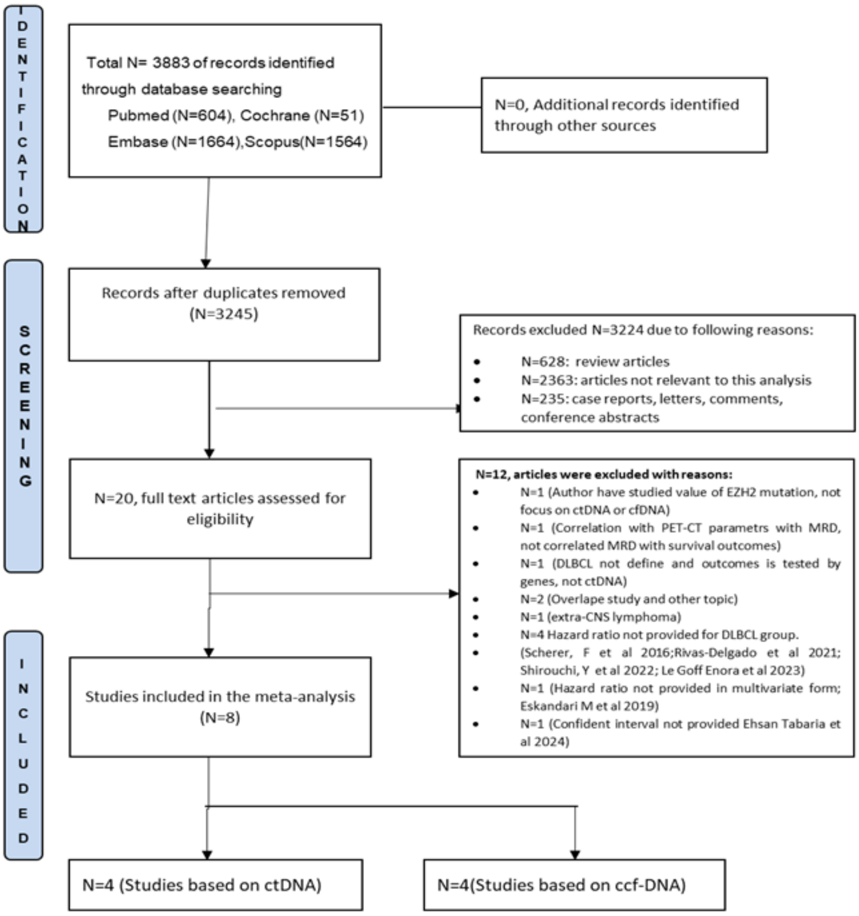
The total HR was computed with the addition of each study. Heterogeneity among the studies was assessed using the $I^2$ statistic. An $I^2$ value exceeding 40% indicated significant heterogeneity. In cases of significant heterogeneity, a random-effects (RE) model was employed; otherwise, a fixed-effects (FE) model was used. A pooled HR of $\geq 1$ indicated a worse survival outcome for patients with detectable ccf-DNA or ctDNA. Subgroup analyses were conducted to explore sources of heterogeneity between studies. Publication bias was assessed by examining the asymmetry of the funnel plot, and the Egger test was used to detect publication bias among the studies. Statistical analyses were performed using R software, with a P-value of $< 0.05$ considered statistically significant.

## RESULTS

### Literature screening and selection of study

In the process of literature screening and study selection, 3883 potentially relevant articles were identified and after removing duplicates, 3245 records were selected for further study. Three thousand, two hundred, twenty four records were excluded because they were reviews, case reports, letters to the editor, non-English articles, or conference abstracts. This screening process ensured that only relevant studies, according to the inclusion criteria, were included for subsequent analysis. Full text of 20 studies was reviewed, and 12 studies were excluded due to reasons of lack of focus on ccf-DNA or ctDNA or absence of HR for outcome. Finally, 8 eligible studies were selected for meta-analysis, encompassing 427 patients with baseline ccf-DNA or ctDNA values and 287 patients with ccf-DNA or ctDNA values measured after starting anticancer therapy. The PRISMA flow chart presenting the steps of the study selection in detail is shown in **Figure (1)**.

**Figure 1.** PRISMA flow diagram of the study selection process.



## Data extraction and quality management

Data extraction and quality assessment were conducted across all selected studies, which were published between 2016 and 2024. Each of the eight studies evaluated ccf-DNA or ctDNA in plasma (5-6,8-12) with one study assessing ctDNA both in serum and plasma ( 7) (**Table 1**). Out of eight selected studies, four studies focused on ctDNA and another four studies focused on ccf-DNA values (**Table 2**). Two studies provided ccf-DNA values, measured in ng/ml, one study utilized methylation levels for ccf-DNA quantification, and one study used a 29-gene-based weighted prognostic score (wp-score) to measure ctDNA levels. Four studies used a threshold based on log haploid genome equivalent per ml (hGE/mL) or Variant Allele Frequency (VAF) (**Table 2**).

The hazard ratio for outcomes, accompanied by their 95% confidence intervals (95% CI), were directly extracted from selected studies. Five studies examined prognostic value of ccf-DNA or ctDNA on the basis of baseline values ( 5-9) while, two studies utilized ctDNA values after starting anticancer therapy (but before completion of treatment) (10, 12) , one study has estimate ctDNA both at baseline and after initiation of therapy (11) (**Table 2**).

On the Newcastle-Ottawa Scale (NOS), notably, all studies achieved scores exceeding seven, indicating robust methodological quality and reliant ability. Detailed are available in **Table 3**.

**Table 1.** Characteristics of included studies for quantitative synthesis of meta-analysis.

| S.No. | Author | Country | Type of study | Patients | Patients number | Type of treatment | Source of sample | Time point of plasma / serum collection | Technique | Gene Symbol | Follow-up time period |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Kristensen, L S et al., 2016 | Denmark | Retro | DLBCL | 71 | 59 received RCHOP-like chemotherapy FOR others not reported | Plasma | Baseline | PCR: Pyrosequencing | DAPK1 | NR |
| 2 | Li M et al., 2017 | China | Pros | DLBCL | 98 | Chemotherapy type not mentioned | Plasma | Baseline | Fluorometer, qPCR | APP Gene | 13.5 ( 1 - 36 months) |
| 3 | Kurtz, D. M et al., 2018 | North America and Europe | Retro | DLBCL | 108 | RCHOP-45%; EPOCH R 34%;Others-22% | Plasma/ Serum | Baseline | CAPP-Seq NGS | TP53,BCL2, BCL6,CARD11 etc. | 31.2 months |
| 4 | Chiu, B.C.-H. et al., 2018 | America | Pros | DLBCL | 48 | RCHOP- 66.6%; EPOCH R- 16.7% | Plasma | Baseline | Illumina NextSeq 500 platform (NGS) | 5hmC | NR |
| 5 | Hur, J Y et al.,2020 | Korea | Pros | DLBCL | 51 | Chemotherapy type not mentioned | Plasma | Baseline | Qubit 2.0 Fluorometer;2200 TapeStation Instrument | NA | NR |
| 6a | Guan, T et al., 2022 | China | Pros | DLBCL-Training | 85 | RCHOP | Plasma | within 1week of receiving anticancer treatment and | NGS: Illumina NovaSeq 5000 | 59 gene Panel | NR |
| 6b | Guan, T et al., 2022 | China | Pros | DLBCL-Validation | 84 | RCHOP | Plasma | within 1week of receiving anticancer treatment and | NGS: Illumina NovaSeq 5000 | 60 gene Panel | NR |
| 7a | Li, M et al., 2022 | China | Pros | DLBCL | 51 | R-CHOP or R-CHOP like | Plasma | Baseline | NovaSeq6000 NGS platform (Illumina) | 188 Gene Panel | 30.3 (range, 3.8–101.2) months |
| 7b | Li, M et al., 2022 | China | Pros | DLBCL | 37 | R-CHOP or R-CHOP like | Plasma | After 2 cycles of therapy | NovaSeq6000 NGS platform (Illumina) | | |
| 7c | Li, M et al., 2022 | China | Pros | DLBCL | 38 | R-CHOP or R-CHOP like | Plasma | After 4 cycles of therapy | NovaSeq6000 NGS platform (Illumina) | | |
| 8 | Miguel Alcoceba et al., 2022 | Spian | Pros | DLBCL | 43 | R-CHOP or R-CHOP like | Plasma | After 2 cycles of therapy | NGS:NextSeq 500 (Illumina | Panel | 3 year (range 0.2–4.8) |

**Table 2.** Survival Outcomes of included studies for quantitative synthesis of the meta-analysis.

| S.No. | Author | Time point of plasma /serum collection | Survival Outcome | Defination of cfDNA positive | ctDNA +ve | PFS/EFS(HR) | OS (HR) |
|---|---|---|---|---|---|---|---|
| 1 | Kristensen, L S et al., 2016 | Baseline | OS | Aberrant methylation level; The cutoffs were 5.5% for DAPK1. | 14 (19%) | | 8.90 (2.70–29.30), p=0.0007 |
| 2 | Li M et al., 2017 | Baseline | PFS | 1586 ng/ml cfDNA | NA | 1.45 (0.490–4.263), p=0.504 | |
| 3 | Kurtz, D. M et al., 2018 | Baseline | OS;EFS | >2.5 log hGE/mL threshold | 212 (98%) | 1.90 (1.12-3.23) | 1.30 (0.65-2.59), p=0.46 |
| 4 | Chiu, B.C.-H. et al., 2018 | Baseline | EFS | 29 gene based weighted prognostic score (wp-score) High/Low | 23 High wp score ,<br>23 Low wp score | 9.17 (2.01–41.89), p=0.04 | |
| 5 | Hur, J Y et al.,2020 | Baseline | OS;EFS | > 16.8 ng/ml cfDNA for EFS, and >30.3 ng/ml cfDNA for OS | 19 High cfDNA; 32 Low cfDNA | 5.33 (1.72–16.52), p=0.003 | 4.51 (1.35-15.05), p=0.014 |
| 6a | Guan, T et al., 2022 | within 1 week of receiving anticancer treatment and | PFS;OS | a mean VAF value ≥ 4.94%, | ctDNA positive in 64 (75.3%) patients | 2.5 (1.1-5.8), p=0.04 | 2.6 (1.1-6.4), p=0.04 |
| 6b | Guan, T et al., 2022 | within 1 week of receiving anticancer treatment and | PFS;OS | a mean VAF value ≥ 4.94%, | ctDNA positive in 67 (79.8%) patients | 1.8 (0.8-4.0), p=0.014 | 1.9 (0.8-4.3), p=0.093 |
| 7a | Li, M et al., 2022 | Baseline | PFS;OS | .>2.44 log hGE/ ml(Concentrations of ctDNA were expressed in log hGE/ mL, | High=26 ; Low=25 | 2.47 (1.35-4.5), p=0.004 | 2.49 (1.238-5), p=0.011 |
| 7b | Li, M et al., 2022 | After 2 cycles of therapy | PFS;OS | .>2.44 log hGE/ ml(Concentrations of ctDNA were expressed in log hGE/ mL, | High=21 ; Low=16 | 2.22 (1.14-4.3), p=0.019 | 2.79 (1.20-6.5), p=0.017 |
| 7c | Li, M et al., 2022 | After 4 cycles of therapy | PFS;OS | ctDNA negative or positive | 28=ctDNA negative<br>10= ctDNA postive | 3.65 (1.452-9.178, p=0.0003 | 3.56 (1.192-10.49), p=0.016 |
| 8 | Miguel Alcoceba et al., 2022 | After 2 cycles of therapy | PFS | 2.5 log hGE/mL | NA | 0.193 [0.058–0.639], p=0.007 | |

**Table3.** Quality assessment of all included studies.

| S.No. | Study ID | Selection (Maximum 4 points) | | | | Comparability (Maximum 2 points) | Outcome (Maximum 3 points) | | | Total points (Maximum 9 points) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Representative of exposed cohort | Selection of the non-exposed cohort | Ascertainment of exposure | Outcomes not presented at start of study | Impact of bias | Assessment of outcome | Was follow-up long enough for outcomes occur | Adequacy of follow-up | |
| 1 | Kristensen, L S et al., 2016 | * | * | * | * | * | * | * | * | 8 |
| 2 | Li M et al., 2017 | * | * | * | * | * | * | * | * | 8 |
| 3 | Kurtz, D. M et al., 2018 | * | * | * | * | * | * | * | * | 8 |
| 4 | Chiu, B.C.H. et al.,2019 | * | * | * | * | * | * | * | * | 8 |
| 5 | Hur, J Y et al. 2020 | * | * | * | * | * | * | * | * | 8 |
| 6 | Guan, T et al., 2022 | * | * | * | * | * | * | * | * | 8 |
| 7 | Li, M et al. 2022 | * | * | * | * | * | * | * | * | 8 |
| 8 | Miguel Alcoceba et al., 2024 | * | * | * | * | * | * | * | * | 8 |

### Techniques used for Detection of ctDNA or ccf-DNA

Detection of ccf-DNA or ctDNA in the reviewed studies predominantly utilized Polymerase Chain Reaction (PCR) and Next-Generation Sequencing (NGS) techniques. Among the 8 studies included, NGS was employed in 5 studies ( 7-8, 10-12), PCR in one study ( 5) and fluorometry in two studies ( 6, 9) . Specifically, out of the 5 studies using NGS technology, one study employed Cancer Personalized Profiling by Deep Sequencing (CAPP-Seq) to measure ctDNA levels (7) , while the other 4 studies utilized the Illumina platform for sequencing (8,10-12) . The 8 studies investigated PFS, EFS, and OS as outcome indicators using multivariate analysis. These data were pooled for meta-analysis to examine the association between ccf-DNA or ctDNA levels and the prognosis of patients with DLBCL. The results of this meta-analysis are illustrated in **Figure 2, 3 and 4** depicting synthesized evidence and statistical outcomes derived from the combined analysis of the selected studies. These figures typically present key metrics such as HR with 95% confidence intervals (CIs) and forest plots to visualize the pooled effect sizes and their variability across studies.

### Prognostic role of ccf-DNA or ctDNA

Prognostic value of ccf-DNA or ctDNA of all eight studies was analyzed. The results indicate significant associations with both PFS and OS. Specifically, for patients with high ccf-DNA or ctDNA, HR for PFS was 2.14 (95% CI, 1.31–3.40) (Figure 2C), and for OS 2.51 (95% CI, 1.84–3.40) (Figure 3C). Significant heterogeneity was observed in data analysis for PFS (I2 = 62%, $p < 0.01$) (Figure 2C), indicating variability across the studies included in the meta-analysis. Therefore, a random-effects model was employed to account for this heterogeneity and provide a more conservative estimation of the pooled effect size. For OS, no significant heterogeneity was observed (I2 = 27%, $p = 0.21$) thus a fixed effect model was applied (Figure 3C).

**Figure 2.** Forest plot of HR for ccf-DNA/ctDNA associated with PFS in DLBCL: A) the original HRs of PFS for ccf-DNA/ctDNA before initiation of therapy. B) The original HRs of PFS for ccf-DNA/ctDNA after starting anticancer therapy. C) The original HRs of PFS for ccf-DNA/ctDNA for all studies.

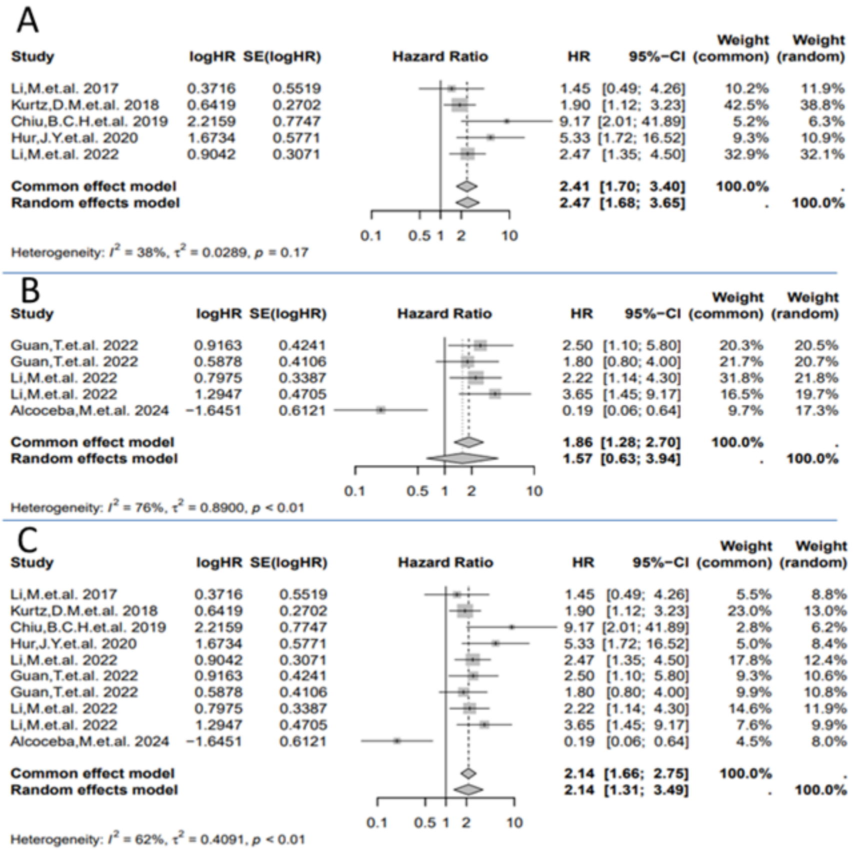


**Figure 3.** Forest plot of HR for ccf-DNA/ctDNA associated with OS in DLBCL: A) the original HRs of OS for ccf-DNA/ctDNA at baseline. B) The original HRs of OS for ccf-DNA/ctDNA after starting anticancer therapy. C) The original HRs of OS for ccf-DNA/ctDNA for all studies.

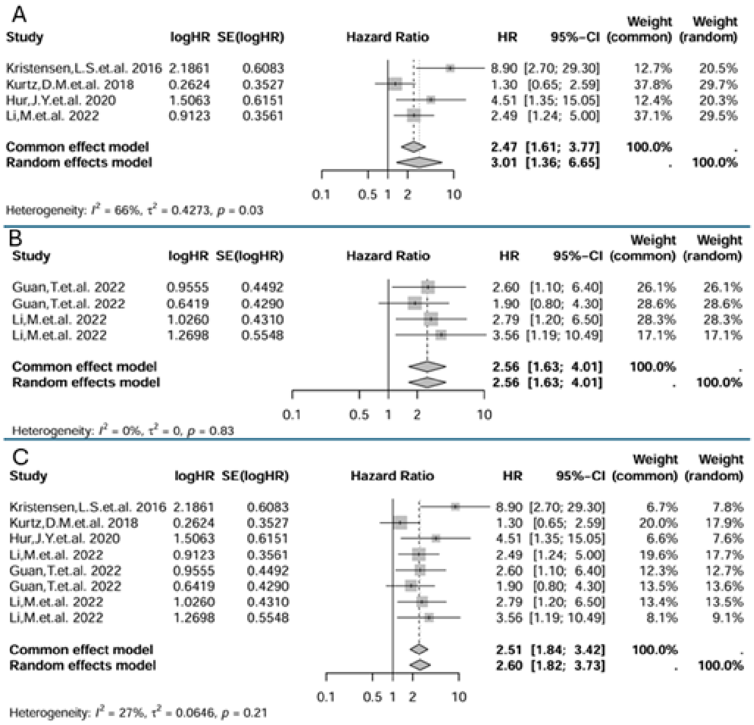
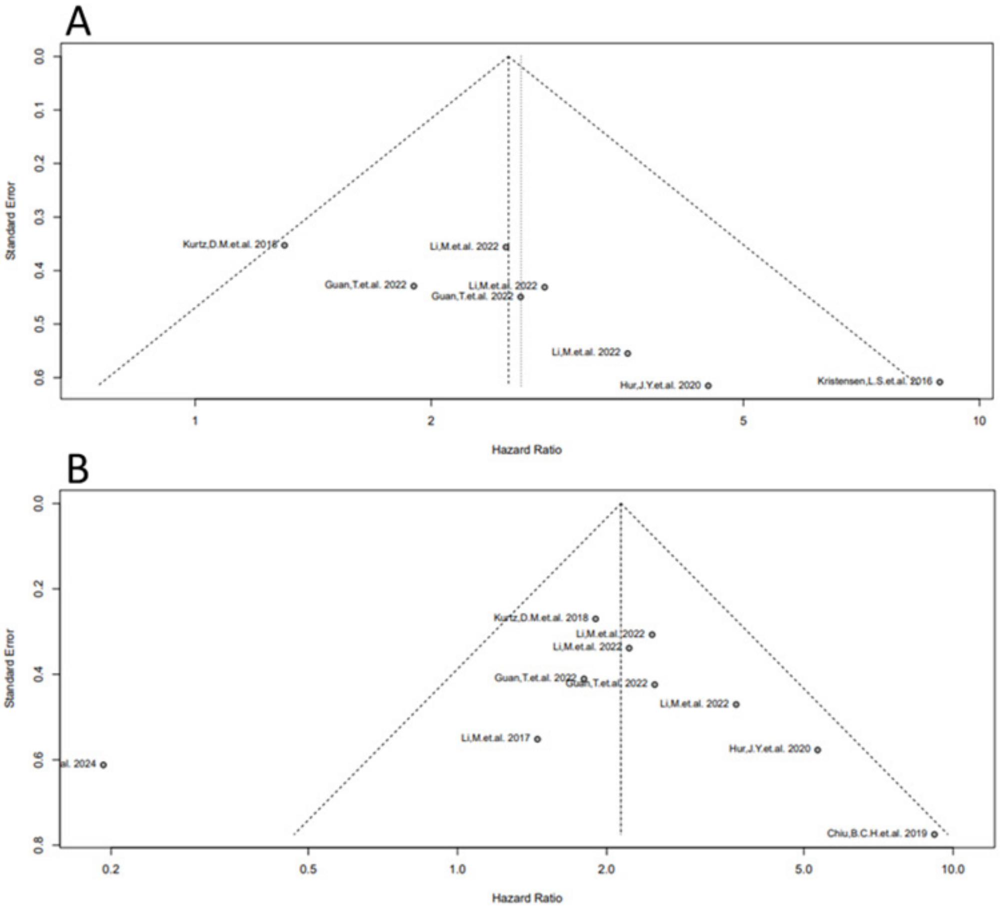


**Figure 4.** Funnel plot for the assessment of publication bias of the included studies. A) Funnel plot of OS for all included studies B) Funnel plot of PFS for all included studies.



### Subgroup analysis

Subgroup analysis was performed to study difference in prognostic value of ccf-DNA or ctDNA according to pateints' ethnicity, method of ccf-DNA or ctDNA estimation, time of ccf-DNA or ctDNA sampling with respect to administration of chemotherapy, and type of DNA studied (ccf-DNA vs ctDNA) (supplement file).

### Prognostic role of ccf-DNA or ctDNA at baseline

Five studies have assessed baseline levels of ccf-DNA or ctDNA, revealing that patients with elevated levels prior to therapy had significantly worse OS (HR = 3.01; 95% CI, 1.36–6.65, $p = 0.03$) and PFS (HR = 2.41; 95% CI, 1.70–3.40, $p = 0.17$).

### Prognostic role of only ccf-DNA at Baseline

In three studies that utilized PFS as the outcome measure, elevated levels of ccf-DNA were associated with poor PFS (HR = 3.87; 95% CI: 1.30-11.48). Furthermore, in two studies, high levels of ccf-DNA were correlated with poor OS (HR = 6.36; 95% CI: 2.72-14.85). No studies were found which measured ccf-DNA during or after therapy.

To further analyze the relationship between ctDNA and survival in patients with DLBCL, we conducted additional subgroup analyses focusing exclusively on studies that evaluated ctDNA.

### Prognostic role of ctDNA at Baseline

In two studies, elevated levels of ctDNA were associated with poor PFS (HR = 2.13; 95% CI: 1.43-3.17) and poor OS (HR = 1.80; 95% CI: 0.95-3.40).

### Prognostic role of ctDNA at ongoing therapy and after therapy

Several studies assessed ctDNA following therapy, revealing that high ctDNA levels were linked to poor PFS according to three studies (HR = 1.57; 95% CI: 0.63-3.94; $I^2 = 76\%$, $p < 0.01$) and poor OS according to two studies (HR = 2.56; 95% CI: 1.63-4.01; $I^2 = 0\%$, $p = 0.83$).

### Publication bias

To assess publication bias, funnel plots were examined for both OS and PFS, revealing a roughly symmetrical shape, as illustrated in Figures 4A and 4B. Additionally, the P value from Egger's test was 0.96. This indicates that there was no significant evidence of publication bias.

## DISCUSSION

Most hematologic malignancies benefit from the availability of blood or bone-marrow samples for diagnosis, risk stratification, and monitoring treatment response (16).

However, due to the organ- or tissue-specific presentation of most lymphomas, a blood-based test for diagnosis or monitoring depth-of-response is not currently available. Recently, the use of ccf-DNA or ctDNA as a non-invasive biomarker has gained significant attention for many solid tumors. (17). Developing ccf-DNA or ctDNA as a robust prognostic tool, similar to its use in solid tumors, would be a valuable addition to the management of patients with lymphoma.

We conducted a comprehensive meta-analysis to evaluate the potential significance of ccf-DNA or ctDNA in patients with DLBCL who were treated with combination chemotherapy (mainly R-CHOP or R-CHOP-like therapies) After screening many studies met the inclusion criteria, however they were excluded due to limitations in data availability (18-30) and finally total 8 eligible studies comprising of 714 patients were analyzed (5-12).

The optimal time point for detection of ccf-DNA or ctDNA is not yet defined. We found that five studies analyzed ccf-DNA or ctDNA exclusively at baseline (5-9) , two studies only after starting chemotherapy (10,12) , and one study assessed ctDNA both at baseline and after initiation of therapy (11). As a result we grouped the studies according to the time of estimation of ccf-DNA or ctDNA.

In five of the selected studies, NGS was used to detect ccf-DNA or ctDNA, (7-8,10-12) whereas in three studies PCR with fluorometric techniques was used for estimation of ccf-DNA (5-6,9). PCR-based methods are both rapid and cost-effective but are limited in their ability to analyze only specific loci at a time. In contrast, NGS-based methods can assess a larger number of loci and even perform whole-exome sequencing, though they generally have lower sensitivity compared to PCR-based methods and are more expensive.

After synthesizing the composite evidence, we found that higher ccf-DNA or ctDNA values before starting treatment were significantly associated with PFS and OS, with HR of 2.41 (1.70-3.40) and 3.01 (1.36-6.65), respectively (31-32) . However, testing of ctDNA after initiating antilymphoma therapy did not prognosticate for PFS but was significantly associated with poor OS, with HRs of 1.57 (0.63-3.94) for PFS and 2.56 (1.63-4.01) for OS.

When we combined the data for ccf-DNA or ctDNA assessed at any time point during treatment, higher values were found to be associated with poor PFS and OS, with HRs of 2.14 (1.31-3.49) for PFS and 2.51 (1.84-3.41) for OS (Figure 2,3) (33)

Kurtz et al. (2018) investigated the clinical utility of ctDNA profiling with CAPP-Seq in DLBCL patients, focusing on mutational genotyping and disease burden assessment. Their objective was to evaluate whether ctDNA quantification before and during treatment could predict EFS and OS. The study determined a threshold level of 2.5 log hGE/mL to stratify patients based on EFS. It was found that pretreatment ctDNA levels had a strong correlation with the IPI and Total Metabolic Tumor Volume (TMTV), indicating that ctDNA could serve as an independent surrogate marker for disease burden beyond these established factors (7).

Miaomiao Li et al. (2022) used a lymphoma-specific sequencing panel and similarly categorized ctDNA levels as log hGE/mL to evaluate the prognostic and predictive value of ctDNA measurements before, during, and after first-line therapy in 73 Chinese DLBCL patients (11) Miguel Alcoceba et al. (2023) also reported ctDNA levels as log hGE/mL, establishing various cutoff points (ranging from 2.0 to 3.5 log hGE/mL) to define the pretreatment ctDNA threshold for predicting PFS (12). These studies collectively highlight the emerging role of ctDNA as a promising biomarker in the management of DLBCL. Incorporating ctDNA analysis into clinical practice could enhance risk stratification, tailor treatment strategies, and ultimately improve patient outcomes in this aggressive form of lymphoma.

Li M et al. (2017) employed PCR and fluorometric technology to evaluate ccf-DNA as a prognostic biomarker and found that high levels of ccfDNA and an elevated integrity index were linked to poor prognosis (6). Similarly, Kristensen et al. (2016) suggested that DAPK1 methylation in ccf-DNA from plasma could serve as a useful biomarker for assessing treatment response in DLBCL (5). Hur et al. (2020) found that high ccf-DNA levels independently predicted poorer EFS in DLBCL patients (9). There is another study conducted by Chiu BC et al., 2019 developed a 29-gene weighted prognostic score (wp-score) using elastic net regularization in a Cox model to predict EFS and OS. Patients with high wp-scores had significantly worse EFS, compared to low-risk patients (8).

The cumulative findings from these studies indicate that ccf-DNA and its methylation status may also substantially improve prognostic accuracy and inform treatment strategies, thereby enhancing patient outcomes. Additionally, these studies underscore the potential of ccf-DNA as a valuable prognostic biomarker in the management of aggressive lymphomas, particularly DLBCL.

Our meta-analysis revealed that patients with higher ccf-DNA or ctDNA at baseline and after the initiation of therapy experienced worse PFS and OS. These results indicate the consistent prognostic value of ctDNA at various time points in DLBCL patients, underscoring its potential as a reliable biomarker for predicting clinical outcomes.

In the three studies that evaluated ccf-DNA at baseline using PFS as the outcome measure, high levels of ccf-DNA were associated with poor PFS (HR = 3.87; 95% CI:1.30-11.48) (6,8-9). Additionally, two studies indicated that elevated ccf-DNA levels correlated with poor OS (HR = 6.36; 95% CI: 2.72-14.85) (5,9) Similarly, in two studies that focused on ctDNA at baseline, high ctDNA levels were associated with poor PFS (HR = 2.13; 95% CI: 1.43-3.17) and poor OS(HR = 1.80; 95% CI:0.95-

3.40) (7,11). The lower HR for survival outcomes at baseline for ctDNA compared to ccf-DNA observed in this meta-analysis could be due to the fewer number of studies available that assessed ctDNA at baseline; despite the fact that theoretically ctDNA is more specific representation of tumor activity as it is less likely to be affected by DNA from other cells/tissue.

Further, there are many challenges that need to be resolved to use ctDNA as a prognostic biomarker to monitor disease. Since, different genes and methods have been used by the researchers to measure the level of ctDNA. Hence, there is need to establish a common method and a similar set of genes or hot spot mutations for future prognosis, diagnosis, and analysis of ctDNA in DLBCL patients.

In our current meta-analysis, there are few limitations. The methodologies for ccf-DNA or ctDNA estimation of the included studies were not entirely uniform, which may contribute to heterogeneity in the results. The differences in patient characteristics could also be a source of variability. The sample sizes of included studies were small. There are no uniform threshold for cfDNA or ctDNA provided in the include studies. These limitations reduced the reliability of our findings. Our meta-analysis was limited to studies published in English.

## CONCLUSION

The results of this meta-analysis strongly suggest that elevated levels of ccf-DNA or ctDNA are predictive of poor overall and progression free survival in patients with DLBCL. Additionally, these biomarkers have a strong potential to emerge as a valuable tool for monitoring disease status, such as MRD in DLBCL patients. As there are tumor heteroginity in DLBCL patients

### Highlights

1. Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma (NHL).
2. Relapses in DLBCL frequently arise from MRD that is not detectable by imaging techniques, highlighting the need for a sensitive and accurate biomarker to enhance the prediction of therapy response.
3. The use of ccf-DNA or ctDNA as a non-invasive biomarker has gained significant attention for the management of patients with DLBCL in the recent years.
4. Many studies have explored ccf-DNA or ctDNA as a prognostic biomarker for patients with DLBCL, but they are small, single center experience with inconsistent results.
5. This meta-analysis was conducted to evaluate the potential role of ccf-DNA or ctDNA in patients with DLBCL who were treated with combination chemotherapy (mainly R-CHOP or R-CHOP-like therapies)
6. This study could be a stepping stone toward utilizing ccfDNA or ctDNA as prognostic biomarker for DLBCL patients.

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### Declarations of Interest statement

**Availability of data and material:** Yes

**Competing interests:** None

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### Authors Contribution

AK: Conceptualization, Data extraction, analysis and writing
AK: Conceptualization, writing, supervision,
CS: Data extraction
AG: Software, Data analysis
PS: Review, editing, supervision
AJ: Review, editing, supervision
SS: Review, editing, supervision
AB: Review, editing, supervision
ARK: Review, editing, supervision
PM: Review, editing, supervision
GP: Conceptualization, Data extraction, writing, supervision

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