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The Role of Cell-Free DNA as a Prognostic Marker in Diffuse Large B Cell Lymphoma with Hepatitis C

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In Loving Memory of Late Professor Doctor ""Mohamed Refaat Hussein Mahran""

Abstract

Background: Hepatitis C infection was a serious problem. Over 50 million individuals were successfully screened, and 4 million people were treated.
Hepatitis C Virus (HCV) also affects lymphocytes, which explains why non-Hodgkin lymphoma, primarily diffuse large B cell lymphoma (DLBCL), often accompanies HCV infection. Direct Acting Antivirals (DAAs) treatment of HCV has dramatically resolved this problem, but there has been debate about its relationship to the accompanying lymphoma. Cell-free DNA (cfDNA) is a reliable non-invasive biomarker for multiple malignancies, primarily lymphoma. It can aid in prognosis and drug-tailored treatment plans, especially with HCV and DAAs.

Objective: This study aimed to evaluate cfDNA as a prognostic biomarker for DLBCL patients.

Method: The study was conducted on three groups of subjects: Group 1: DLBCL patients positive for HCV, this group was subdivided to: Group (1A): DLBCL patients positive for HCV [receiving DAAs].Group (1B): DLBCL patients positive HCV [did not receive any treatment for HCV]. Group 2: DLBCL patients negative HCV. Group 3: Control group. All participants underwent full history, clinical examination, routine laboratory investigations and quantitative measurement of cfDNA by real-time PCR.

Results: Our study reveals that cfDNA levels are significantly higher in DLBCL patients positive for HCV who did not receive any treatment for HCV (Group 1B), as well as in advanced lymphoma stages. Additionally, we found a high mortality rate among patients with elevated cfDNA. These findings highlight the complex relationship between DLBCL, HCV, and DAA treatment, emphasizing the need for tailored management strategies and increased surveillance in this patient population.

Conclusion: cfDNA has a valuable prognostic and monitoring role in DLBCL, particularly with HCV infection.

Keywords: Cell-free DNA, Diffuse Large B-Cell Lymphoma, Hepatitis C Virus, Direct Acting Antivirals
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1. Introduction:

DLBCL represents the most prevalent subtype of non-Hodgkin lymphoma, comprising approximately 30-40% of cases worldwide **[1].** The introduction of rituximab (R) to the cyclophosphamide, adriamycin, vincristine, and prednisone (CHOP) chemotherapy regimen a decade ago has significantly improved overall survival rates for DLBCL patients **[2].** Nonetheless, 30-50% of patients exhibit resistance to this standard treatment paradigm **[3].** Advances in molecular profiling have identified genetic and molecular markers that contribute to the prognostic landscape of DLBCL **[4].** The International Prognostic Index (IPI) is the standard tool for assessing risk in DLBCL patients, particularly those at high risk. It's often used to guide treatment decisions, like R-CHOP chemotherapy **[5].** However, IPI may not accurately predict how well

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R-CHOP will work for individual DLBCL patients **[6].**

The prevalence of HCV infection worldwide is approximately 3% **[7],** making it a leading cause of chronic liver disease. HCV infections can cause various extrahepatic complications, such as vasculitis, glomerulonephritis, and lymphoproliferative disorders, leading to increased morbidity and mortality **[8].** Identifying the specific HCV genotype is crucial for determining the appropriate type and duration of treatment, which can help predict the response to antiviral therapy and patient outcomes **[9].**

HCV treatment with DAAs has been suggested as a possible therapeutic intervention for patients with HCV related lymphoproliferative disorders, particularly in terms of improving patient outcomes **[10].**

Several cases of lymphoma regression following HCV eradication suggest a causal link between HCV infection and lymphoma development. While most patients with HCV-associated non-Hodgkin lymphoma (NHL) exhibit mild liver disease at diagnosis, early initiation of DAAs therapy is recommended regardless of liver status. This approach aims to eliminate HCV infection and potentially prevent extrahepatic complications like NHL **[11].** Studies have indicated that a high HCV-RNA viral load is associated with a poorer prognosis in patients with DLBCL **[12].** While R-CHOP therapy is a standard treatment for DLBCL, it can lead to significant hepatic toxicity in a subset of patients, with rates ranging from 14% to 28% for grade 3-4 adverse events **[13].** This is particularly concerning for patients with DLBCL and HCV infection. Although R-CHOP can exacerbate HCV, the efficacy of newer DAAs, such as glecaprevir and pibrentasvir, in preventing or managing HCV flares during R-CHOP therapy remains unstudied **[14].**

cfDNA is present in low concentrations in the plasma of healthy individuals. Elevated cfDNA levels have been documented in various clinical conditions, including malignancies, autoimmune diseases, myocardial infarction, trauma, and pregnancy-related complications **[15].**

Circulating tumor DNA (ctDNA) derived from the primary tumor can be detected in the blood of cancer patients. This non-invasive approach offers a valuable source of surrogate tumor material for analysis **[16].** Studies have demonstrated that methylation patterns within ctDNA, such as DAPK1 methylation in DLBCL patients, can be used to assess the efficacy of treatments like R-CHOP **[17].** Additionally, cfDNA provides a convenient and minimally invasive biomarker for real-time monitoring of tumor genetic and epigenetic characteristics **[18].** Recent research has highlighted the significance of 5-hydroxymethylcytosine (5hmC) in cfDNA as a valuable biomarker for cancer diagnosis, staging, prognosis, and recurrence in various cancer types, including gastric, lung, hepatocellular, and esophageal cancers **[19].**

A recent genome-wide analysis of 5-hydroxymethylcytosine (5hmC) in cfDNA from patients with newly diagnosed DLBCL and follicular lymphoma (FL) revealed distinct molecular signatures, differentiating these two subtypes **[20].**

2. Materials and Methods

2.1. Patients

This case control study was conducted on 97 participants , 71 DLBCL and 26 apparently healthy individuals.

They were divided into 3 groups:

Group (1A): 22 cases DLBCL patients positive HCV received DAAs.

Group (1B): 19 cases DLBCL patients positive HCV did not receive any treatment for HCV.

Group (2): 30 cases DLBCL patients negative HCV.

Group (3): 26 cases healthy subjects who were matched in age and sex with patients.

Patients were selected from outpatient clinics and inpatient department of Clinical Oncology, Menoufia University, Egypt.

Each subject provided informed consent, strictly adhering to the ethical guidelines of the Ethics Committee at Menoufia University.

2.2. Inclusion criteria:

- DLBCL by histopathology.
- Positive CD20.
- Performance Status (0–2) assessment by using ECOG **[21].**

2.3. Exclusion criteria:

- Other Malignancy.
- Hepatic viral infection e.g (HBV)
- Decompensated liver due to liver cirrhosis.
- Inadequate renal function.

2.4. All study participants were subjected to:

2.4.1. Comprehensive medical history and physical examination, including a thorough palpation of all lymph node regions, as well as the spleen and liver.

2.4.2 Radiology

Liver cirrhosis, lymph nodes, ascites and splenomegaly were diagnosed by ultrasonography & Computed Tomography & Bone Marrow Aspirate & Biopsy.

2.4.3 Histopathologyical Study

DLBCL is ideally diagnosed from an excisional biopsy of a suspicious lymph node, which shows sheets of large cells that disrupt the underlying structural integrity of the follicle center and stain positive for pan-B-cell antigens, such as CD20 and CD79a. COO is determined by immunohistochemical stains, while molecular features such as double-hit or triple-hit disease are determined by fluorescent in situ hybridization analysis. Commercial tests for frequently recurring mutations are currently not routinely used to inform treatment **[22].**

2.4.4 Lab work

Prior to initiating lymphoma treatment, blood samples were obtained and analyzed for blood chemistry, hematology, and HCV RNA. These analyses were conducted using automated laboratory equipment: the SYNCHRON CX9ALX (Randox, CA, USA), Sysmex K-21 (SysmexCorporation, Kobe, Japan), and COBAS® TaqMan® HCV assay (Roche Molecular Diagnostics, CA, USA). The COBAS® TaqMan® HCV assay had a lower limit of quantitation of less than 10 IU/mL.

2.4.5 Molecular study

Sampling

3ml Whole blood samples were collected on EDTA tube at diagnosis, before starting treatment of lymphoma.

Plasma samples were isolated by two consecutive centrifugations at 2500 xg for 10 minutes at 4°C, without time brake, to minimize cell contamination. The supernatants were carefully collected and stored at -80°C. Aliquots of 400-800 microliters were used for cfDNA extraction.

CfDNA extraction

CfDNA was extracted using the QIAamp® UltraSens® kit (Hilden, Germany). according to manufacturer's settings. Then stored at -20°C for subsequent analysis.

Primer preparation

To form a stock solution of primers the lyophilized primers were diluted by a volume of nuclease free water equal to multiplication of the number of nanomoles (nmol) of primer in the tube by 10.

Working solution was formed from stock solution by dilution (1 to 10) .

Real-time PCR (Real-time fast 7500 system PCR, Applied Biosystems, Life Technologies CA, USA) was used for absolute quantification of cfDNA.

Quantification of cfDNA in plasma

Cell-free DNA in plasma was quantified using a SYBR green-based real-time PCR assay using b-globin gene as reference gene, in a reaction volume of 20 µl, containing: 10 µl Maxima SYBR Green Master Mix (2X) , 2 µl of nuclease free water, 1µl of each forward and reverse primers of b-globin gene , 0.05 µl Rox dye solution (Thermo Fisher Scientific Baltics UAB, Lithuania) and 6 µl of the extracted cfDNA.

Sequence of b-globin gene primers

Forward
[5`-GTGCACCTGACTCCTGAGGAGA 3`and
Reverse 5`CCTTGATACCAACCTGCCCG-3`]
(GenBank Sequence accession number U01317).

A standard curve was prepared via a serial 10-fold dilutions of known concentration- genomic DNA, 100 ug/ml (100, 10,1, 0.1 respectively) . These dilutions were used in parallel with each PCR of samples (Genomic DNA promega USA).

The cycle parameters were as follows:

An initial denaturation step at 95°C for 10 minutes. This is followed by 40 cycles of denaturation (95°C for 15 seconds), annealing (60°C), and extension (1.5 minutes).

To ensure the accuracy of the amplified PCR products, a melting curve analysis was performed at the conclusion of each PCR reaction.

2.5 Statistical analysis

2.5.1 Calculation of sample size

Unmatched case control study, the sample size was calculated using the web site (https://www.stat.ubc.ca/~rollin/stats/ssize/n2.html).

Statistical analysis was conducted using SPSS version 28.0. Descriptive statistics, such as means and standard deviations, were calculated for continuous variables, while categorical variables were summarized using counts and percentages. To compare groups, we utilized appropriate statistical tests, including chi- square tests for categorical variables and independent t- tests for continuous variables. The significance level was set at $p < 0.05$. Moreover, survival analysis was conducted using Kaplan-Meier plots. All statistical tests were two-tailed, and p-values less than 0.05 were considered statistically significant.

3. Results:

3.1. Association between different groups regarded to clinical characteristics

Our study provided a comprehensive overview of the associations between different groups with baseline and clinical characteristics within the DLBCL cases. We observed significant variations in several key characteristics among these groups. Notably, the distribution of Body Mass Index (BMI), Diabetes Mellitus (DM), and Hypertension (HTN) showed significant differences among the subgroups ($p<0.05$). Furthermore, the presence of HCV antibodies (HCV Ab), HCV-RNA PCR results, liver condition (cirrhotic, enlarged, normal), spleen status (enlarged, normal), and drug treatment for HCV displayed statistically significant variations ($p<0.001$). Additionally, the death rate significantly differed among the groups (group 1A is the lowest and group II is the highest) with implications for patient outcomes. (Table 1)

3.2. Association between different patients group and laboratory investigation.

Associations between different patient groups and blood biomarkers, offering mean values and standard deviations for each group. Several biomarkers, including AST, ALT, T.B, ALB, Creatinine, Lactate Dehydrogenase (LDH), Hb, TLC, AFP and B2 Macroglobulin, display statistically significant differences among the groups (p-values < 0.001). These differences suggest distinct physiological profiles or disease states among the patient groups (Table 2)

3.3. Association between cfDNA expression and DLBC.

The mean cfDNA level in DLBCL cases was higher significantly than control group (p< 0.001). This suggests a potential link between elevated free DNA expression and the presence of DLBC in our study participants. (Table 3)

3.4. Association between cfDNA expression and Lymphoma stage

cfDNA demonstrated a significant increase as Lymphoma stage advanced. Mean cfDNA expression levels rose progressively from 4.12 ± 3.16 in Stage I to 44.03 ± 22.04 in Stage II, 156.26 ±113.02 in Stage III, and notably surged to 1243.02 ±604.06 in Stage IV (p<0.001, One-Way ANOVA). These results highlight a strong association between Lymphoma stage and elevated cfDNA expression, indicating its potential as a valuable staging and prognostic marker. (Table 4)

3.5. Death rate among DLBC.

Mortality rates within the lymphoma groups Notably, 67.6% of DLBCL cases who were alive at the time of assessment remained alive, while 32.4% had passed away.(Table 5)

3.6. Association between death rate and cfDNA expression in cases groups.

It showed higher mortality rate in patient who had high cfDNA level (407.15) compared to those who were alive (299.20) (p < 0.001). This suggests a potential correlation between elevated cfDNA expression and mortality in case group and its importance prognostic biomarker. (Table 6)

3.7. Association between different patients group and cfDNA expression

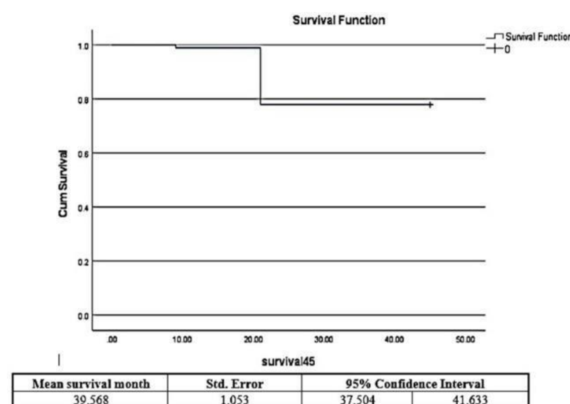
It provided mean values and standard deviations of cfDNA expression for each group. The highly significant p-value (<0.001) indicates substantial differences in cfDNA. Expression among the groups. Notably, it showed lower mean for control, negative HCV, positive HCV after treatment lastly positive HCV without treatment (respectively) the potential clinical relevance of cfDNA expression in this context. (Table 7)

3.8. Multivariate and Univariate Analysis of multiple predicting factors of lymphoma in participants (Logistic regression mode).

The logistic regression analysis revealed important findings. Age exhibited a significant association with the outcome in both univariate (OR = 0.913, p<.001) and multivariate (OR = 0.859, p=0.121) analyses. cfDNA Expression was also significantly associated in both univariate (OR = 0.792, p=0.002) and multivariate (OR = 0.793, p=0.020) analyses. Drug treatment for HCV was significant in univariate analysis (OR = 1.765, p=0.044) but not in multivariate analysis (OR = 3.121, p=0.059). These results highlight cfDNA as independent predictor of the outcome, emphasizing their importance in risk assessment. Other variables showed varying degrees of significance in either the univariate or multivariate analysis. (Table 8)

3.9. Kaplan Meier Plot for the overall survival

The Kaplan-Meier analysis for overall survival revealed a mean survival of 39.568 months (95% CI: 37.504-41.633) with a standard error of 1.053 months. This analysis provides a valuable insight into the overall survival pattern, offering a median time within which a certain percentage of individuals survived. The findings signify a substantial duration of survival within the studied population, indicating a noteworthy outcome measure for this study.(Figure 1).



Mean survival month	Std. Error	95% Confidence Interval	
39.568	1.053	37.504	41.633

Figure 1

4. Discussion

Our study investigated the demographic profiles of patients with DLBCL in the context of HCV infection, with a focus on cfDNA as a potential diagnostic and prognostic marker. Our findings confirm several associations between DLBCL and clinical parameters, including comorbidities such as DM, HTN, and HCV infection, all of which are known to be linked to an increased risk of lymphoma development.

Table 1: Association between different groups regarded to clinical characteristics

		Groups				
		Group IA Positive HCV Treated	**Group IB Positive HCV Not Treated**	**Group II Negative HCV**	**Control**	**P-value**
		N %	**N %**	**N %**	**N %**	
Age (Mean ± SD)		56.5 ± 9.2	57.5 ± 6.1	59 ± 11.12	56 ± 13	P1=0.233 P2=0.166 P3= 0.116
Sex	**Female**	23.6%	20.0%	30.9%	25.5%	P1=0.128 P2=0.421 P3= 0.116
	Male	21.4%	19.0%	31.0%	28.6%	
BMI	**Normal**	28.6%	31.0%	31.0%	9.5%	P1=0.611 P2=0.217 **P3=0.003**
	Overweight	18.2%	10.9%	30.9%	40.0%	
DM	**No**	23.5%	18.5%	25.9%	32.1%	P1=0.221 **P2=0.023** **P3 <0.001**
	Yes	18.8%	25.0%	56.3%	0.0%	
HTN	**No**	21.8%	15.4%	29.5%	33.3%	P1=0.252 **P2=0.01** **P3 <0.001**
	Yes	26.3%	36.8%	36.8%	0.0%	
HCV Ab	**Negative**	0.0%	0.0%	53.6%	46.4%	P1=0.257 P2=0.325 **P3<0.001**
	Positive	53.7%	46.3%	0.0%	0.0%	
HCV-RNA PCR	**Negative**	28.2%	0.0%	38.5%	33.3%	**P1 <0.001** P2=0.366 P3=0.411
	Positive	0.0%	100.0%	0.0%	0.0%	
Liver	**Cirrhotic**	21.4%	71.4%	7.1%	0.0%	
	Enlarged	37.9%	13.8%	48.3%	0.0%	**<0.001**
	Normal	14.8%	9.3%	27.8%	48.1%	
Spleen	**Enlarged**	40.0%	25.7%	34.3%	0.0%	P1=0.211 **P2=0.01** **P3<0.001**
	Normal	13.1%	14.8%	29.5%	42.6%	
Drug treatment for HCV	**No**	0.0%	38.8%	61.2%	0.0%	**P1<0.001** **P2<0.001** **P3<0.001**
	Yes	100.0%	0.0%	0.0%	0.0%	
Death rate	**Alive**	21.6%	16.2%	27.0%	35.1%	P1=0.361 **P2=0.006** **P3<0.001**
	Dead	26.1%	30.4%	43.5%	0.0%	

SD: Standard deviation, Chi-square test was used for categorical variables; One way ANOVA was used fornumerical test. *: Statistically significant at p≤ 0.001

Table 2: Association between different patients group and laboratory investigation.

	Groups				
	Group IA	**Group IB**	**Group II**	**Control**	**P-value**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	
AST(U/I)	33 ± 15	34 ± 14	27 ± 13	21 ± 7	P1=0.251 **P2 <0.001** **P3 <0.001**
ALT(U/I)	36 ± 17	30 ± 9	26 ± 11	23 ± 6	**P1<0.001** **P2 <0.001** **P3<0.001**
T.B(mg/dl)	1.40 ± 1.64	1.01 ± .53	1.13 ± 1.63	.55 ± .25	**P1 <0.001** P2=0.231 **P3 <0.001**
D.B(mg/dl)	.53 ± 1.14	.33 ± .22	.49 ± 1.26	.14± .05	P1=0.421 P2=0.221 P3=0.172
ALB. (g/dl)	3.7 ± .5	3.7 ± .2	3.8 ± .3	4.3 ± .2	P1=0.81 P2=0.611 **P3<0.001**
Urea(mg/dl)	30 ± 10	29 ± 8	33 ± 15	30 ± 4	P1=0.653 P2=0.231 P3=0.722

Creat. (mg/dl)	.98 ± .24	.87 ± .19	a. .28	.95 ± .13	**P1<0.001** **P2<0.001** **P3<0.001**
UA(mg/dl)	5.1 ± 1.4	4.9 ± 1.2	4.9 ± 1.3	5.5 ± 1.0	P1=0.721 P2=0.112 P3=0.114
LDH(U/I)	437 ± 287	470 ± 348	528 ± 279	158 ± 17	**P1<0.001** **P2<0.001** **P3<0.001**
Hb(g/dl)	11.9 ± 2.0	12.0 ± 1.7	11.1 ± 2.3	14.6 ± 1.5	**P1<0.001** **P2<0.001** **P3<0.001**
PLT *10^3	219 ± 105	256 ± 104	256 ± 132	254 ± 53	P1=0.096 P2=0.315 P3=0.114
TLC *10^3	7.7 ± 3.4	8.3 ± 2.6	7.2 ± 2.2	12.3 ± 17.3	P1=0.231 P2=0.112 **P3=0.043**
AFP(ng/ml)	2.52 ± 1.20	2.96 ± 1.10	2.02 ± .87	1.54 ± .24	P1=0.211 P2=0.321 **P3=0.049**
B2 Macroglobulin **(mg/dl)**	2.74 ± 1.11	2.99 ± 1.26	2.45 ± 1.30	1.32 ± .16	P1=0.411 P2=0.512 **P3<0.001**

SD: Standard deviation, *: Statistically significant at p≤ 0.001

Chi-square test was used for categorical variables;One way ANOVA was used for numerical test.

Table 3: Association between free DNA expressions

	Group		**P-value**
	Case	**Control**	
	Mean ± SD	**Mean ± SD**	
Free DNA expression	347.8 ± 261.3	3.1 ± 2	**<0.001**

SD: Standard deviation, Student T test was used. *: Statistically significant at p≤ 0.05

Table 4: Association between Free DNA expression and Lymphoma stage

Free DNA expression	**Lymphoma stage**				**P-value**
	I	**II**	**III**	**IV**	
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	
	4.12 ± 3.16	**44.03 ± 22.04**	**156.26 ± 113.02**	**1243.02 ± 604.06**	**<0.001**

SD: Standard deviation, One way ANOVA was usedfor numerical test.

*: Statistically significant at p≤ 0.001

Table 5: Death rate among Lymphoma (DLBC).

Group			
Case			
		Count	**N %**
Death rate	**Alive**	48	67.6%
	Dead	23	32.4%

Death Rate - Indicates mortality rates among DLBCLcases.

Table 6: Association between death rate and free DNA expression in cases groups.

Free DNA Expression				
		Mean	**StandardDeviation**	**P-value**
Death rate	**Alive**	299.20	190.9	**<0.001**
	Dead	**407.15**	210.3	

SD: Standard deviation, Student T test was used. *: Statistically significant at p≤ 0.05

Table 7: Association between different patients group and free DNA expression

Free DNA. Expression				**P-value**
		Mean	**Standard Deviation**	
Groups	**Group IA Positive HCV Treat**	363.0	300.4	**<0.001**
	Group IB Positive HCV N.T	384.5	292.5	
	Group II Negative HCV	313.4	209.9	
	Group III Control	3.1	2.0	

SD: Standard deviation, One way ANOVA was used for numerical test.

*: Statistically significant at p≤ 0.05

Table 8: Multivariate and Univariate Analysis of multiple predicting lymphoma in study participants (Logistic regression mode).

Univariate					P-value	Multivariate			P-value
		OR	95% CI.for OR			OR	95% CI.for OR		
			Lower	Upper			Lower	Upper	
Demographics	**Sex**	0.874	0.326	2.087	.731	0.031	0	7.97	0.997
	Age	**0.933**	0.848	0.939)	**<.001**	0.857	0.706	1.039	0.997
	BMI	0.178	0.029	0.485	**.002**	0.056	0.001	2.709	0.996
	Free DNA. Expression	0.812	0.662	0.901	**0.002**	0.791	0.65	0.962	**0.03**
Lab markers	**AST**	1.745	1.22	3.045	**0.004**	3.119	0.956		0.997
	ALT	0.911	0.867	0.957	**0.009**	0.642	0	.	0.998
	T.B	0.916	0.87	0.963	**0.001**	1.666	0	.	0.998
	D.B	0.018	-0.015	0.266	**0.015**	0	0	.	0.998
	ALB	-0.019	-0.02	-0.244	**<.001**	0	0	.	0.999
	Urea	12662.517	170.36	941072.946	0.605	0	0	.	0.998
	Creat.	0.967	0.92	1.017	0.711	1.788	0	.	0.998
	UA	0.659	0.068	5.241	0.082	0	0	.	0.999
	LDH	1.377	0.939	2.017	0.004	15.337	0	.	0.999
	ESR	0.909	0.863	0.957	**<.001**	0.892	0		0.999
	Hb	0.689	0.577	0.821	**<.001**	1.456	0	.	0.998
	PLT *10^3	2.448	1.674	3.575	0.709	1.76	0	.	0.998
	TLC *10^3	0.981	0.977	0.985	0.145	0.95	0		0.997
	AFP	1.038	0.961	1.121	**0.001**	2.927	0	.	0.997
	B2 Microglobiun	0.166	0.047	0.495	**<.001**	176.792	0	.	0.996

The strong association between cfDNA levels and DLBCL, with higher cfDNA levels observed in patients with advanced-stage lymphoma (particularly stage IV), supports the emerging role of cfDNA as a non-invasive biomarker. Elevated cfDNA levels were significantly higher in DLBCL patients compared to healthy controls, and this association was even more pronounced in patients with HCV infection. The potential for cfDNA to act as a real-time marker of tumor burden and disease progression has been highlighted by previous studies, including those by Arzuaga-Mendez et al. **[23]** and Ashley et al. **[24]**, who demonstrated that cfDNA levels rise with advancing or relapsed disease. The higher cfDNA levels observed in deceased patients in our cohort further support its value as a prognostic tool for assessing survival outcomes in DLBCL patients.

Our study also corroborates earlier research linking comorbidities like DM and HTN to lymphoma risk. Wang et al. **[25]** identified these conditions as significant risk factors for non-Hodgkin lymphoma, which aligns with our findings that DLBCL patients frequently presented with metabolic comorbidities. Similarly, the association between obesity and non-Hodgkin lymphoma, as noted by Ingham et al. **[26]**, may further explain the link between metabolic syndrome and lymphoma pathogenesis. The chronic inflammatory state induced by these metabolic disorders may promote an environment conducive to lymphomagenesis.

In the context of HCV infection, our study strengthens the well-documented relationship between chronic viral infection and lymphoproliferative disorders. Chronic HCV infection is known to trigger prolonged immune stimulation, which can lead to B-cell proliferation and genetic instability, ultimately contributing to the development of lymphomas, as supported by the findings of Tasleem et al. **[27]**. This relationship is of particular importance in regions with high HCV prevalence, where monitoring patients for early signs of lymphoproliferative disorders becomes a critical aspect of healthcare. Additionally, the impact of DAAs on lymphoma progression remains an area for future research. Understanding how DAAs treatment modulates the immune system in the context of DLBCL could offer new insights into personalized therapeutic approaches.

Our study also revealed important laboratory findings, including elevated liver enzymes (AST, ALT), bilirubin, and LDH, all of which were associated with poor outcomes in DLBCL patients. Elevated LDH levels, in particular, have long been recognized as a prognostic factor in DLBCL, often linked with aggressive disease behavior and a higher risk of poor survival outcomes **[28]**. These biochemical abnormalities, particularly in patients with comorbid HCV infection, suggest that liver dysfunction may be an additional complicating factor in the disease course of DLBCL, warranting close hepatic monitoring in affected patients

Our study's findings on the prognostic significance of cfDNA are particularly noteworthy. Patients with DLBCL who exhibited higher cfDNA levels had significantly shorter survival times, as illustrated by the Kaplan-Meier survival analysis. This is consistent with the work of Mondelo-Macía et al. **[29]**, who demonstrated that elevated cfDNA levels were associated with shorter overall survival in lymphoma patients. This suggests that cfDNA could serve as a valuable biomarker for identifying high-

risk patients who may benefit from more aggressive treatment strategies. Moreover, the logistic regression analysis in our study revealed that cfDNA was an independent predictor of DLBCL, with an odds ratio of 0.791 (95% CI: 0.65-0.962, p=0.03). This highlights the potential utility of cfDNA not only as a prognostic marker but also as a predictive marker for lymphoma development.

However, our study's findings regarding cfDNA as a predictive marker require further validation. The discrepancy between our results and those of Wu et al. **[30]**, who did not find a significant difference in cfDNA levels between lymphoma patients and controls, may be attributed to differences in study design, patient populations, and methods of cfDNA quantification. The variability in cfDNA results across studies highlights the need for standardized protocols for cfDNA analysis, including uniform sample handling and processing, to ensure that cfDNA can be reliably used as a clinical biomarker in the future. This is particularly important in the context of diseases with complex pathophysiology, such as DLBCL, where factors like tumor heterogeneity, treatment regimens, and viral co-infections (such as HCV) may all influence cfDNA levels.

Our study’s Kaplan-Meier survival analysis showed a mean survival of 40 months for DLBCL patients, which is in line with the expected survival range but slightly lower than the 52 months reported by Xu et al. **[31]** for DLBCL patients treated with rituximab-based immunochemotherapy. This discrepancy could be due to variations in patient demographics, treatment protocols, and disease stages. Our findings highlight the importance of stratifying DLBCL patients based on risk factors such as cfDNA levels and metabolic comorbidities to better tailor therapeutic approaches.

5. Conclusion

Our study provides great insights into the potential role of cfDNA as both a diagnostic and prognostic biomarker in DLBCL, particularly in the context of HCV infection. The significant associations observed between elevated cfDNA levels and advanced disease stages, poor survival outcomes, and comorbid conditions such as DM and HTN suggest that cfDNA could be a key factor in personalizing treatment for DLBCL patients. However, further research is needed to clarify the predictive role of cfDNA in lymphoma risk, as well as to standardize cfDNA measurement techniques to enhance its clinical utility.

6. References

1. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, Advani R, Ghielmini M, Salles GA, Zelenetz AD. (2016). The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood, The Journal of the American Society of Hematology. 127(20):2375-90.

2. Singh R, Dubey A, Rathore A, Kapoor R, Sharma D, Singh NK, Maggo S. (2018). Diffuse large B-cell lymphoma-review. Journal of Medical Sciences. 38(4):137-43.

3. Kim S-H, Go S-I, Seo J, Kang MH, Park SW, Kim H-G, Lee G-W. (2018). Prognostic impact of pretreatment albumin to globulin ratio in patients with diffuse large B-cell lymphoma treated with R-CHOP. Leukemia Research. 71:100-5.

4. Elad S, Cheng KKF, Lalla RV, Yarom N, Hong C, Logan RM, Bowen J, Gibson R, Saunders DP, Zadik Y, Ariyawardana A, Correa ME, Ranna V, Bossi P, Mucositis Guidelines Leadership Group of the Multinational Association of Supportive Care in C, International Society of Oral O. (2020). MASCC/ISOO clinical practice guidelines for the management of mucositis secondary to cancer therapy. Cancer. 126(19):4423-31.

5. Song C-X, Yin S, Ma L, Wheeler A, Chen Y, Zhang Y, Liu B, Xiong J, Zhang W, Hu J, Zhou Z, Dong B, Tian Z, Jeffrey SS, Chua M-S, So S, Li W, Wei Y, Diao J, Xie D, Quake SR. (2017). 5-Hydroxymethylcytosine signatures in cell-free DNA provide information about tumor types and stages. Cell Res. 27(10):1231-42.

6. Deng Y, Chen X, Huang C, Chen G, Chen F, Lu J, Shi X, He C, Zeng Z, Qiu Y, Chen J, Lin R, Chen Y, Chen J. (2019). EZH2/Bcl-2 Coexpression Predicts Worse Survival in Diffuse Large B-cell Lymphomas and Demonstrates Poor Efficacy to Rituximab in Localized Lesions. J Cancer. 10(9):2006-17.

7. Defrancesco I, Zerbi C, Rattotti S, Merli M, Bruno R, Paulli M, Arcaini L. (2020). HCV infection and non-Hodgkin lymphomas: an evolving story. Clinical and Experimental Medicine. 20(3):321-8.

8. Negro F, Forton D, Craxì A, Sulkowski MS, Feld JJ, Manns MP. (2015). Extrahepatic Morbidity and Mortality of Chronic Hepatitis C. Gastroenterology. 149(6):1345-60.

9. Lauer EM, Mutter J, Scherer F. (2022). Circulating tumor DNA in B-cell lymphoma: technical advances, clinical applications, and perspectives for translational research. Leukemia. 36(9):2151-64.

10. Soliman WG, Zaghloul AM, Ali AM, Gaber MS. Lymphoid Malignancies and Direct-Acting Antivirals. The Egyptian Journal of Hospital Medicine. 2022;89(1):4540-3.

11. Lawitz E, Sulkowski MS, Ghalib R, Rodriguez-Torres M, Younossi ZM, Corregidor A, et al. Simeprevir plus sofosbuvir, with or without ribavirin, to treat chronic infection with hepatitis C virus genotype 1 in non-responders to pegylated interferon and ribavirin and treatment-naive patients: the COSMOS randomised study. The Lancet. 2014;384(9956):1756-65.

12. Merli M, Defrancesco I, Visco C, Besson C, Di Rocco A, Arcari A, Sica A, Cencini E, Tisi MC, Frigeni M. (2020). Direct-acting antivirals in relapsed or refractory hepatitis C virus-associated diffuse large B-cell lymphoma. Leukemia & Lymphoma. 61(9):2122-8.

13. Visco C, Finotto S. Hepatitis C virus and diffuse large B-cell lymphoma: Pathogenesis, behavior and treatment. World Journal of Gastroenterology: WJG. 2014;20(32):11054.

14. Umemura M, Suda G, Tsukamoto S, Ebata K, Takahash S, Sasaki T, Nakajima S, Hirata K, Ozasa M, Takano M, Katagiri M, Sakamoto N. (2021). Successful treatment by on-demand glecaprevir and pibrentasvir for hepatitis C flare during R-CHOP in patients with diffuse large B-cell lymphoma: a case report. BMC Infect Dis. 21(1):389-.

15. Tsang JC, Lo YD. Circulating nucleic acids in plasma/serum. Pathology-Journal of the RCPA. 2007;39(2):197-207.\

16. Fleischhacker M, Schmidt B. Circulating nucleic acids (CNAs) and cancer—a survey. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer. 2007;1775(1):181-232.

17. Kristensen LS, Hansen JW, Kristensen SS, Tholstrup D, Harsløf LBS, Pedersen OB, De Nully Brown P, Grønbæk K. (2016). Aberrant methylation of cell-free circulating DNA in plasma predicts poor outcome in diffuse large B cell lymphoma. Clin Epigenetics. 8(1):95-.

18. Tan G, Chu C, Gui X, Li J, Chen Q. (2018). The prognostic value of circulating cell-free DNA in breast cancer: A meta-analysis. Medicine (Baltimore). 97(13):e0197-e.

19. Liu X, Liu L, Ji Y, Li C, Wei T, Yang X, Zhang Y, Cai X, Gao Y, Xu W, Rao S, Jin D, Lou W, Qiu Z, Wang X. (2019). Enrichment of short mutant cell-free DNA fragments enhanced detection of pancreatic cancer. EBioMedicine. 41:345-56.

20. Chiu BCH, Chen C, You Q, Chiu R, Venkataraman G, Zeng C, Zhang Z, Cui X, Smith SM, He C, Zhang W. (2021). Alterations of 5-hydroxymethylation in circulating cell-free DNA reflect molecular distinctions of subtypes of non-Hodgkin lymphoma. NPJ Genom Med. 6(1):11-.

21. Azam F, Latif MF, Farooq A, Tirmazy SH, AlShahrani S, Bashir S, Bukhari N. (2020). Performance status assessment by using ECOG (Eastern Cooperative Oncology Group) score for cancer patients by oncology healthcare professionals. Case reports in oncology. 12(3):728-36.

22. Liu Y, Barta SK. Diffuse large B-cell lymphoma: 2019 update on diagnosis, risk stratification, and treatment. American journal of hematology. 2019;94(5):604-16.

23. Arzuaga-Mendez J, Prieto-Fernández E, Lopez-Lopez E, Martin-Guerrero I, García-Ruiz JC, García-Orad A. (2019). Cell-free DNA as a biomarker in diffuse large B-cell lymphoma: A systematic review. Critical Reviews in Oncology/Hematology. 139:7-15.

24. Ashley CW, Selenica P, Patel J, Wu M, Nincevic J, Lakhman Y, Zhou Q, Shah RH, Berger MF, Da Cruz Paula A, Brown DN, Marra A, Iasonos A, Momeni-Boroujeni A, Alektiar KM, Long Roche K, Zivanovic O, Mueller JJ, Zamarin D, Broach VA, Sonoda Y, Leitao MM, Friedman CF, Jewell E, Reis-Filho JS, Ellenson LH, Aghajanian C, Abu-Rustum NR, Cadoo K, Weigelt B. (2023). High-Sensitivity Mutation Analysis of Cell-Free DNA for Disease Monitoring in Endometrial Cancer. Clin Cancer Res. 29(2):410-21.

25. Wang Y, Liu X, Yan P, Bi Y, Liu Y, Zhang ZJ. (2020). Association between type 1 and type 2 diabetes and risk of non-Hodgkin's lymphoma: A meta-analysis of cohort studies. Diabetes & Metabolism. 46(1):8-19.

26. Ingham RR, Reagan JL, Dalia S, Furman M, Merhi B, Nemr S, Zarrabi A, Mitri J, Castillo JJ. (2011). The Relationship Between Obesity and Lymphoma: A Meta-Analysis of Prospective Cohort Studies. Blood. 118(21):5198-.

27. Tasleem S, Sood GK. (2015). Hepatitis C Associated B-cell Non-Hodgkin Lymphoma: Clinical Features and the Role of Antiviral Therapy. J Clin Transl Hepatol. 3(2):134-9.

28. Wu W, Miao L, Zhao L, Zhu Y, Mao J, Cai Z, Ji Y, Wang L, Wang Y, Jia T. (2023). Prognostic value of lactate dehydrogenase, serum albumin and the lactate dehydrogenase/albumin ratio in patients with diffuse large B-cell lymphoma. Hematology. 29(1).

29. Mondelo-Macía P, García-González J, Abalo A, Mosquera-Presedo M, Aguín S, Mateos M, López-López R, León-Mateos L, Muinelo-Romay L, Díaz-Peña R. (2022). Plasma cell-free DNA and circulating tumor cells as prognostic biomarkers in small cell lung cancer patients. Transl Lung Cancer Res. 11(10):1995-2009.

30. Wu J, Tang W, Huang L, Hou N, Wu J, Cheng X, Ma D, Qian P, Shen Q, Guo W, Peng W, Liu Y, Jiang C, Feng J. (2019). The analysis of cell-free DNA concentrations and integrity in serum of initial and treated of lymphoma patients. Clinical Biochemistry. 63:59-65.

31. Xu J, Wang B-Y, Yu S-H, Chen S-J, Yang S-S, Liu R, Chen L-J, Hou J, Chen Z, Zhao W-H, He A-L, Mi J-Q, Chen S-J. (2024). Long-term remission and survival in patients with relapsed or refractory multiple myeloma after treatment with LCAR-B38M CAR T cells: 5-year follow-up of the LEGEND-2 trial. J Hematol Oncol. 17(1):23-.