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The analysis of cell-free DNA concentrations and integrity in serum of initial and treated of lymphoma patients

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ABSTRACT

Objective: To evaluate cell-free DNA (cfDNA) in plasma as a promising biomarker for lymphoma, altered levels of cfDNA and its association with clinical parameters are investigated in patients suffered from lymphomas.
Methods: Peripheral blood specimens were collected from 60 patients with lymphoma during initial diagnosis and those of another 107 patients with lymphoma during treated stage were also collected, 93 healthy volunteers were selected as control group. Quantitative PCR was used to detect cfDNA level in each group, cfDNA level in different groups was analyzed to understand its relationship with lymphoma patients' clinical features. After correlation analysis between cfDNA and clinical characteristics, Receiver operator characteristic curve was performed to analyze sensitivity and specificity of cfDNA and LDH.
Results: cfDNA concentration and integrity in initial stage of lymphoma patients were significantly higher than those in treated stage, and cfDNA concentration in treated phase was significantly higher than cfDNA concentration in control group. There was no significant difference in cfDNA integrity at treated stage compared with control group. There was no significant correlation between patient's age, gender, extranodal invasion and lymphoma pathological type and cfDNA concentration and integrity; In contrast, there was a significant correlation between ECOG score, LDH content, Ann Arbor stage, IPI, B-symptoms, Ki-67 expression and radiotherapy and cfDNA concentration and integrity, both at the time of initial diagnosis and treated stage. cfDNA concentration detection is an optimal diagnostic indicator, followed by cfDNA integrity detection, the sensitivity and specificity of both are superior to the traditional LDH detection.
Conclusion: cfDNA level is significantly increased in lymphomas patient plasma and may help lymphoma screening. cfDNA level may serve as a potential indicator of lymphomas treatment efficacy.

1. Introduction

Lymphomas is malignant tumors that originate in lymph nodes and extranodal lymphoid tissues. According to the different histopathological features, they can be divided into two categories: Hodgkin's lymphoma and non-Hodgkin's lymphoma [1]. In recent years, new cases of malignant lymphoma in Europe and the United States have been increasing year by year, and the annual incidence rate is as high as 11/100000–18/100000. In China, lymphoma accounts for about 4% of new malignant tumors each year; lymphoma accounts for about half of the newly diagnosed blood system tumors each year. The morbidity of lymphoma is only about 6.4/100000, but it has the high malignancy

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and poor prognosis [2]. The clinical manifestations of lymphoma are varied including painless mass or fever night sweats, vomiting, weight loss and other systemic symptoms [3]. The risk factors of lymphoma are known to be infection, immunodeficiency and so on, but the exact cause is still unknown [4]. In the past decades, lymphoma was characterized and classified into different subgroups according to the morphology. Recent discoveries in genetics and molecular biology have revolutionized our understanding of the initiation and progression of lymphoma. Various new and potentially powerful molecular markers for lymphoma have been identified by researchers. However, there is a need for a universal biomarker available for all lymphoma patients of different subgroups.

Cell-free DNA is a newly discovered biomarker in cancer research. It refers to a nucleotide fragment in plasma that has a DNA double-helical structure. In recent years, with the rapid progress of molecular genetics, basic research and clinical research of cfDNA has been gradually deepened, including non-tumor-specific plasma DNA content and tumor-specific gene level abnormalities [5]. According to reports in the literature, cfDNA plays an important role in the diagnosis, treatment, and prognosis of so many tumors, and it replaces tissue biopsy with a simple blood test [6]. The occurrence of this liquid biopsy overcomes the inconvenience in acquisition of tumor tissue, and there is little research on quantitative monitoring of cfDNA in lymphoma in China. To further understand the value of cfDNA in lymphomas, we used real-time quantitative PCR to detect the content of cfDNA and explored the important role of cfDNA in lymphoma screening.

2. Materials and methods

2.1. Case selection

60 patients which newly diagnosed by pathological examination after lymph node resection from June 2017 to March 2018 were selected (24cases of diffuse large B-cell lymphoma, 11 cases of Hodgkin's lymphoma, 9 cases of follicular lymphoma, 8 cases of NK-T cell lymphoma, 2 cases of mucosal-associated lymphoid tissue lymphoma, 3 cases of mantle cell lymphoma and 3 cases of peripheral T cell lymphoma). Among lymphoma patients, 32 were males and 28 were females, aged 12 to 80 years, with a median age of 46 years. At the same time, another 107 lymphoma patients which experienced treated stage during August 2017 to March 2018 were selected (45 cases of diffuse large B-cell lymphoma, 20 cases of Hodgkin's lymphoma, 14 cases of follicular lymphoma, 15 cases of NK-T cell lymphoma, and 4 cases of mucosal-associated lymphoid tissue lymphoma, 4 cases of mantle cell lymphoma and 5 cases of peripheral T cell lymphoma). Among lymphoma patients, 59 were males and 48 were females, aged 15 to 82 years, with a median age of 47 years. Lymphoma stage was assessed according to Ann. Arbor criteria, standard radiotherapy treatment based on histopatho -logical classification was given. The prognostic factors proposed by the International Prognostic Index (IPI) include: age $>$ 60 years, abnormal serum LDH, physical status 2–4, stage III or IV, and extranodal lesions $>$ 1. 93 healthy adults were selected as healthy controls, including 39 males and 54 females. The age range was from 4 to 78 years and the median age was 45 years. Exclusion criteria: Pregnancy, inflammation, cardiovascular and cerebrovascular disorders, heart and liver and kidney insufficiency, and autoimmune diseases. Pathological characteristics were shown in Table 1 (initial diagnosis) and 2 (treated).

Informed written consent for the study was obtained from all patients, and the study was approved by the Jiangsu Cancer Hospital Clinical Research Ethics Committee.

2.2. Separation of plasma and extraction of cfDNA

Venous blood samples were collected in EDTA-coated tubes and plasma was separated by centrifuging at 1600*g* for 10 min. Supernatant was transferred to a new tube and centrifuged at 16000*g* for 10 min. Purified plasma was carefully removed without disturbing lower residual layer. Minimum aliquot 200 μL plasma were used for DNA extraction immediately or stored at −80 °C freezer. Plasma samples were thawed on ice and spun at 10000 *g* for 3 min before DNA purification. DNA was purified from 200 μL of plasma and eluted by 50 μL elution buffer using QIAamp DNA Blood Mini Kits (Qiagen, Valencia, CA) according to the manufacturer's instructions. DNA samples were ready to use for quantification or stored at −20 °C freezer.

2.3. Quantitative Polymerase Chain Reaction (QPCR)

QPCR was performed on a LightCycler LC480 PCR machine (Roche Molecular Systems, Inc. Pleasanton, CA, USA). To measure the concentration of plasma cfDNA, the repetitive LINE 1(Long interspersed nuclear element 1) 97 bp (both for short and long) and LINE1 300 bp (only for long) DNA fragments were amplified as described respectively by [7]. The LINE1 97 bp primer amplified apoptotic and non-apoptotic DNA fragments while the LINE1 300 bp primer amplified non- apoptotic DNA fragments only. The total amount of plasma DNA was represented by the QPCR result with LINE1 97 bp primer. DNA integrity index was calculated as the ratio of LINE1 300 and LINE1 97 QPCR result. A serial diluted standardized solution of human genomic DNA (Thermo Fisher Scientific, Waltham, MA, USA) was used as a standard curve reference. The concentration of cfDNA in each sample was calculated according to the standard curve. QPCR reaction was performed in triplicate and mean values across triplicates were used for further analysis. The mixture of QPCR reaction was in 20 μL volume contained 1 μL DNA template, 0.5 μL of the each forward and reverse primer (LINE1 97 or LINE1 300), 10 μL UltraSYBR Mixture (Cwbiotech, Beijing, China) and 8 μL double-distilled water. Cycling conditions were 1 min at 95 °C, and 35 cycles of 95 °C for 8 s, and 60 °C for 15 s. Each plate consisted of a plasma DNA sample and a negative control (water template) and 7 serial diluted standard DNA solutions.

2.4. Immunohistochemistry staining

Immunohistochemistry (SP) method was used for Ki-67 detection. The PBS was used instead of the primary antibody as a negative control. The antigen was repaired using microwave method and the kit instruction was strictly followed. The positive expression of Ki-67 was located in the nucleus and stained with clear brown-yellow particles. Positive staining was observed in 5 high-magnification (×400) fields with 100 cells in each field and a total of 500 tumor cells. The positive expression of Ki-67 protein in each slice was calculated. Ki-67 high expression was defined as positive staining percentage ≥ 20%.

2.5. Statistical analysis

cfDNA quantification results were expressed as mean ± standard deviation (x ± SD). Kruskal-Wallis rank sum test was used for comparison between groups. The count data were compared using r-test, and the measurement data were compared using the *t*-test. The ROC curve assesses cfDNA quantification as a screening tool for patients with lymphoma, and the area under the ROC curve is used to calculate the accuracy for differentiating between two different diseases for different critical values. SPSS 21.0 software was used for statistical analysis, $P < .05$ was considered as statistical significance.

3. Results

3.1. Patients' clinical characters and demographic information

A total of 60 patients were enrolled in the initial diagnosis stage, including 8 NK/T type lymphomas, 11 HL type lymphomas, 9 FL type lymphomas, 24 DLBCL type lymphomas, and 8 other types of

Table 1
Demographic and clinical features of patients at initial diagnostic stage.

Variable	Total (n = 60)	NK/T (n = 8)	HL (n = 11)	FL (n = 9)	DLBCL (n = 24)	MALT (n = 2)	MCL (n = 3)	PTCL (n = 3)
Age (years)								
mean (range)	46 (2–80)	46 (5–65)	46 (7–74)	50 (17–82)	50 (19–78)	45 (8–73)	44 (10–71)	45 (9–72)
< 60	43	7	7	6	17	2	2	2
≥60	17	1	4	3	7	0	1	1
Gender								
Male	32	4	7	3	12	2	2	2
Female	28	4	4	6	12	0	1	1
ECOG score								
0–2	33	6	8	1	12	2	2	2
≥3	27	2	3	8	12	0	1	1
LDH								
Normal	40	5	9	7	12	2	3	2
Elevated	20	3	2	2	12	0	0	1
Ann Arbor stage								
I/II	27	6	1	6	8	2	2	2
III/IV	33	2	10	3	16	0	1	1
IPI								
0–2	35	6	6	5	14	1	2	1
≥3	25	2	5	4	10	1	1	2
IPS								
0–2			8					
≥3			3					
B-symptoms								
Yes	19	3	5	3	6	0	1	1
No	41	5	6	6	18	2	2	2
Lump size ≥5 cm								
Yes	13	1	2	3	5	0	1	1
No	47	7	9	6	19	2	2	2
Extranodal invasion								
Yes	24	3	3	4	11	0	2	1
No	36	5	8	5	13	2	2	2

Table 2
Demographic and clinical features of patients at treated stage.

Variable	Total (n = 107)	NK/T (n = 15)	HL (n = 20)	FL (n = 14)	DLBCL (n = 45)	MALT (n = 4)	MCL (n = 4)	PTCL (n = 5)
Age (years)								
mean (range)	47 (5–82)	46 (5–65)	46 (7–74)	50 (17–82)	50 (19–78)	45 (8–73)	44 (9–71)	46 (11–69)
< 60	79	14	14	9	31	4	3	4
≥60	28	1	6	5	14	0	1	1
Gender								
Male	59	7	14	5	24	3	3	3
Female	48	8	6	9	21	1	1	2
ECOG score								
0–2	64	11	18	1	24	3	3	4
≥3	43	4	2	13	21	1	1	1
LDH								
Normal	74	10	18	12	23	4	4	3
Elevated	33	5	2	2	22	1	1	0
Ann Arbor stage								
I/II	47	11	3	9	14	3	3	4
III/IV	60	4	17	5	31	1	1	1
IPI								
0–2	64	13	11	8	26	2	2	2
≥3	43	2	9	6	19	2	2	3
IPS								
0–2			16					
≥3			4					
B-symptoms								
Yes	32	5	7	4	12	1	2	1
No	75	10	13	10	33	3	3	3
Lump size ≥5 cm								
Yes	21	2	3	4	8	1	2	1
No	86	13	17	10	37	3	3	3
Extranodal invasion								
Yes	41	6	5	5	20	1	2	2
No	66	9	15	9	25	2	3	3

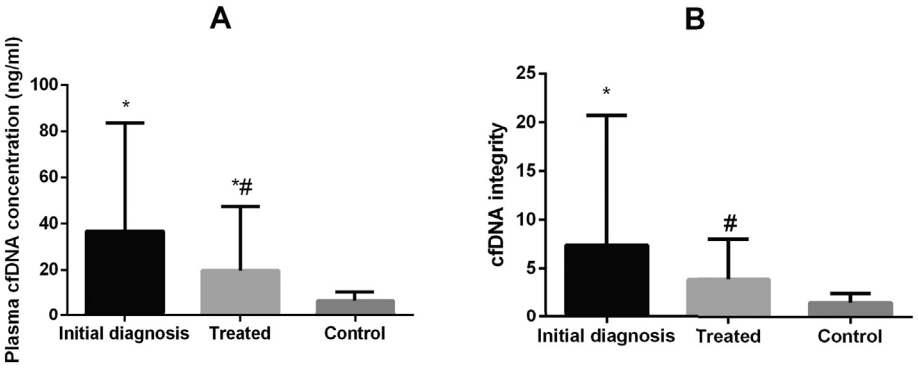

Fig. 1. QPCR results for quantification of cfDNA concentration and integrity (* indicated that $P < .05$ compared to control group, # indicated that $P < .05$ compared to Treated group).

lymphomas. Another 107 patients were collected during the treated stage. At the initial stage of diagnosis, there were 26 patients with an ECOG score of 3 or higher, 34 with 0–2; 40 with normal LDH and 20 with high LDH; The Ann Arbor stage was 27 in I/II and 33 in II/IV; For IPI scores, 25 were greater than or equal to 3, while 0–2 were 35; 19 patients had B-symptoms; 14 patients had tumors larger than 5 cm in diameter; 24 patients had extranodal invasion. At the treated stage, there were 43 people with an ECOG score of 3 or higher, 64 with 0–2, 74 had normal LDH and 33 with high LDH. The Ann Arbor stage was 47 in I/II, compared with 60 in II/IV; For the IPI score, 64 were greater than or equal to 3, compared with 43 for 0–2, 32 patients had B-symptoms; 21 patients had tumors larger than 5 cm in diameter, and 41 had extranodal invasion.

3.2. cfDNA concentration in plasma of healthy individuals and lymphoma patients

The results of QPCR for quantification of cfDNA concentration and integrity are shown in Fig. 1. The cfDNA concentration of the control group was 6.67 ± 3.75 ng/mL and the cfDNA integrity was 1.46 ± 0.93. The cfDNA concentration in lymphoma patients at the initial diagnosis stage was 36.85 ± 46.73 ng/mL, and the cfDNA integrity was 7.40 ± 13.31. The cfDNA concentration in lymphoma patients at the treated stage was 19.67 ± 27.91 ng/mL, and the cfDNA integrity was 3.87 ± 4.11. The cfDNA concentration and integrity in the initial stage of lymphoma patients were significantly higher than the cfDNA concentration and integrity in the treated stage, and the cfDNA concentration in the treated phase was significantly higher than the cfDNA concentration in the control group, both $P < .05$. However, there was no significant difference in cfDNA integrity at the treated stage compared with the control group, $P > .05$.

3.3. Correlation of cfDNA concentrations to patient characteristics

After determining the cfDNA concentration and integrity, an analysis of correlation between the clinical pathological features of patients and cfDNA was conducted. The results are shown in Table 3 (total cfDNA) and Table 4 (cfDNA integrity): regardless of the initial diagnosis or treated stage, there was no significant correlation between the patient's age, gender, extranodal invasion and lymphoma pathological type, degree of malignancy and cfDNA concentration and integrity, $P > .05$; In contrast, there was a significant correlation between ECOG score, LDH content, Ann Arbor stage, IPI, B-symptoms, Ki-67 expression and radiotherapy with cfDNA concentration and integrity, both at the time of initial diagnosis and treated stage, $P < .05$. Interestingly, after the analysis of results, it was found that this indicator of large lump showed no significant correlation with cfDNA concentration and integrity at the initial diagnosis stage, $P > .05$, but there was a significant correlation with cfDNA concentration and integrity at the treated stage. $P < .05$.

3.4. Receiver operator characteristic curve analysis of cfDNA levels in lymphoma

According to the content of cfDNA and LDH in each group, the sensitivity and specificity of cfDNA and LDH were calculated and compared for discrimination of lymphoma patients and healthy individuals, and ROC curve analysis was performed. The results are shown in Fig. 2. At the initial diagnosis stage, the area under curve (AUC) of LDH was 0.7686 (95% CI: 0.6876–0.8497), the area under curve (AUC) of cfDNA concentration was 0.8683 (95% CI: 0.8077–0.9290), and the area under curve (AUC) of cfDNA integrity was 0.8785. (95% CI: 0.8203–0.9368); at the treated stage, the the area under curve (AUC) for LDH was 0.7649 (95% CI: 0.6948–0.8350), and the area under curve (AUC) for cfDNA concentration was 0.8811 (95% CI: 0.8359–0.9263). The area under curve (AUC) for cfDNA integrity was 0.8375 (95% CI: 0.7835–0.8914). From the results, it can be seen that cfDNA concentration detection is the optimal screening standard, followed by cfDNA integrity detection, the sensitivity and specificity of both are superior to the traditional detection of LDH.

4. Discussion

As early as the 1970s, Leon et al. [8] proposed that the cell-free DNA levels of patients with malignant tumors were significantly higher than those of healthy people; molecular studies confirmed that plasma DNA of patients with lymphoma had tumor-associated genetic changes, such as microsatellite changes, IgH gene rearrangement, etc., supporting that cfDNA originated from tumor tissue. When cfDNA levels are significantly higher, it is more likely to indicate a possible tumor state [9].

Recently, emerging evidences elucidated that absolute concentration and DNA integrity of circulating cfDNA might be candidate biomarkers for diagnosis and prognosis of malignant tumors [10–15]. In malignant solid tumors, DNA integrity index was associated with tumor loads and expected to be one molecular diagnosis biomarker in clinic [16–19]. Debatably, DNA integrity index was higher in breast cancer patients than benign and health controls [16] while higher DNA integrity predicted to have lower risk of recurrence [19]. In colorectal cancer and hepatocellular cancer patients, DNA integrity was much higher than that in benign and health volunteers [17,18]. Thus, higher fragmentation of cfDNA was one hint of genetic aberration for malignant solid tumors.

This study was the first to quantitatively analyze cfDNA in healthy individuals and lymphoma patients at initial diagnostic stage and treated stage. The results showed that the cfDNA levels in lymphoma patients were significantly different from those in healthy volunteers. It is interesting to found that cfDNA levels are not significantly associated with disease stage, pathological classification and extranodal invasion,

Table 3
Correlation between total plasma cfDNA level investigation and clinical characters.

Variable	Initial diagnosis	Treated
Age (years)		
< 60	39.10 ± 45.79	19.49 ± 21.34
≥60	31.39 ± 27.44	25.63 ± 40.37
P	0.4041	0.3127
Gender		
Male	35.79 ± 36.92	21.33 ± 28.50
Female	38.73 ± 61.38	17.62 ± 27.33
P	0.76	0.4967
ECOG score		
0–2	22.58 ± 19.17	12.26 ± 15.20
≥3	119.33 ± 71.11	30.28 ± 37.31
P	< 0.0001	0.0008
LDH		
Normal	28.36 ± 33.02	12.94 ± 17.20
Elevated	75.44 ± 75.89	45.65 ± 43.12
P	< 0.0001	< 0.0001
Ann Arbor stage		
I/II	31.07 ± 47.82	13.08 ± 17.80
III/IV	51.82 ± 41.38	24.83 ± 33.03
P	0.0121	0.03
IPI		
0–2	26.16 ± 21.41	10.02 ± 8.43
≥3	55.75 ± 42.66	34.03 ± 38.82
P	< 0.0001	< 0.0001
B-symptoms		
Yes	51.87 ± 49.23	32.42 ± 37.13
No	30.63 ± 27.33	14.23 ± 20.93
P	0.003	0.0044
Lump size ≥5 cm		
Yes	49.1 ± 70.55	36.33 ± 43.10
No	32.12 ± 33.36	15.60 ± 21.19
P	0.229	0.0111
Extranodal invasion		
Yes	41.12 ± 39.71	21.78 ± 30.47
No	31.28 ± 27.65	18.36 ± 26.36
P	0.1121	0.5403
Pathological classification		
Hodgkin's lymphoma	35.12 ± 34.66	19.35 ± 15.98
non-Hodgkin's lymphoma*	32.34 ± 31.84	17.25 ± 16.62
P	0.7931	0.6861
Degree of malignancy		
Indolent lymphoma*	24.69 ± 27.29	12.61 ± 18.68
Aggressive lymphoma*	33.53 ± 35.14	18.14 ± 16.77
P	0.3798	0.3308
Ki-67		
≥20%	81.42 ± 56.71	41.63 ± 38.26
< 20%	24.63 ± 17.66	19.68 ± 15.22
P	< 0.0001	0.0008
Radiotherapy		
Yes		30.02 ± 36.34
No		15.05 ± 21.96
P		0.0091

* Aggressive Lymphoma include DLBCL and NK/T; indolent lymphoma: FL, MALT; non-Hodgkin's lymphoma: DLBCL, NK/T and FL.

Table 4
Correlation between integrity of cfDNA investigation and clinical characters.

Variable	Initial diagnosis	Treated
Age (years)		
< 60	8.57 ± 14.78	3.77 ± 4.22
≥60	3.10 ± 2.30	4.14 ± 3.85
P	0.0545	0.6844
Gender		
Male	6.46 ± 7.14	4.52 ± 4.84
Female	9.08 ± 20.24	3.07 ± 2.84
P	0.3562	0.0695
ECOG score		
0–2	4.25 ± 3.36	3.17 ± 3.61
≥3	25.61 ± 28.63	4.88 ± 4.60
P	< 0.0001	0.0337
LDH		
Normal	5.50 ± 5.97	3.03 ± 2.85
Elevated	16.05 ± 28.09	7.12 ± 6.23
P	0.0016	< 0.0001
Ann Arbor stage		
I/II	6.49 ± 14.64	3.05 ± 3.78
III/IV	9.75 ± 8.97	4.51 ± 4.27
P	0.1377	0.0679
IPI		
0–2	5.47 ± 4.88	2.78 ± 2.51
≥3	9.29 ± 8.71	5.49 ± 5.35
P	0.0027	0.0006
B-symptoms		
Yes	9.56 ± 8.35	5.50 ± 5.09
No	5.33 ± 4.64	3.17 ± 3.42
P	0.0005	0.0099
Lump size ≥5 cm		
Yes	5.11 ± 4.04	3.28 ± 3.10
No	13.33 ± 23.86	6.27 ± 6.42
P	0.078	0.0137
Extranodal invasion		
Yes	7.92 ± 6.22	4.19 ± 3.87
No	6.75 ± 5.17	3.67 ± 4.27
P	0.2655	0.5272
Pathological classification		
Hodgkin's lymphoma	5.58 ± 4.71	3.19 ± 2.73
non-Hodgkin's lymphoma*	5.97 ± 4.88	3.86 ± 3.14
P	0.7984	0.4759
Degree of malignancy		
Indolent lymphoma*	4.38 ± 4.27	2.42 ± 2.67
Aggressive lymphoma*	6.53 ± 5.87	3.85 ± 3.72
P	0.1932	0.1706
Ki-67		
≥20%	15.97 ± 10.24	7.06 ± 5.54
< 20%	7.84 ± 5.69	3.16 ± 2.97
P	< 0.0001	0.0004
Radiotherapy		
Yes		5.34 ± 5.20
No		3.21 ± 3.36
P		0.0114

* Aggressive Lymphoma include DLBCL and NK/T; indolent lymphoma: FL, MALT; non-Hodgkin's lymphoma: DLBCL, NK/T and FL.

while it is significantly correlated with lymphoma lactate dehydrogenase expression, ECOG score, Ann Arbor stage, B symptoms Ki-67 expression and radiotherapy, suggesting that cfDNA levels reflect active disease proliferation. We found also that cfDNA level and integrity were significantly different between initial diagnosis stage and treated stage, showing a correlation between them, indicating that cfDNA could serve as a potential *Indicator indicator* of treatment efficacy for lymphoma. Apropos of pathological classification, we found that the indolent lymphoma and the aggressive lymphoma showed no significant difference in cfDNA concentration and integrity, which was controversial with our expectation, it may be due to invasive behavior of partially indolent lymphomas. For example, the pathological classification of FL is class IIIB, and some invasive lymphomas such as MCL also have indolent behavior. This point needs further investigation until we have more samples. However, the results of this study were controversial as to whether there is a correlation between the presence of large lumps and the cfDNA level. The results suggested that there was no significant correlation between the expression level of cfDNA and the appearance of large lumps at initial diagnosis stage, but there was a significant correlation between the two at subsequent visit. This controversy may have something to do with the number of samples at the time of the initial diagnosis. We will increase the selection of sample size in future studies to further clarify the relevance of this indicator to cfDNA. In addition, previous studies have confirmed that cfDNA was related to tumor burden through rat epithelial tumor cell transplantation model [20,21].

In assessing the role of cfDNA levels in clinical lymphoma screening, this study calculated the sensitivity and specificity of cfDNA as a means of discrimination between lymphoma patients and controls, and then

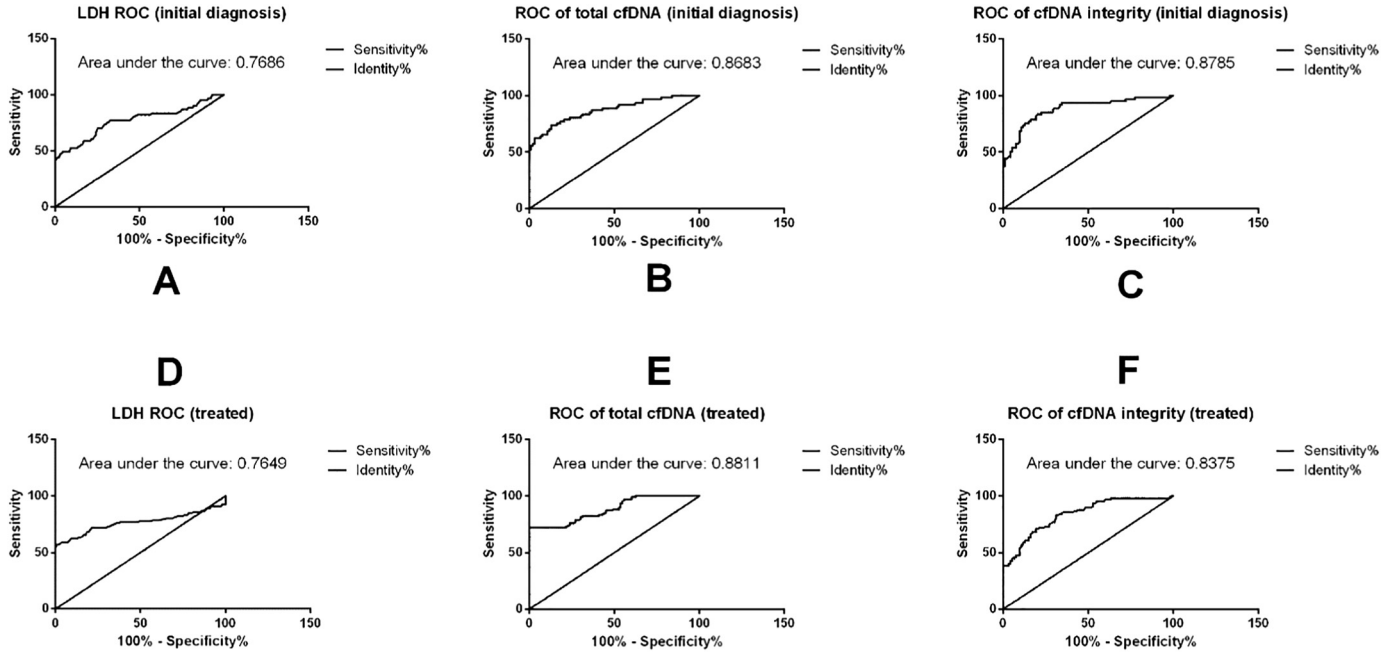

Fig. 2. ROC for discrimination between lymphomas patients and healthy individuals. A: the area under curve (AUC) of LDH was 0.7686 (95% CI: 0.6876–0.8497), B: the area under curve (AUC) of cfDNA concentration was 0.8683 (95% CI: 0.8077–0.9290), C: the area under curve (AUC) of cfDNA integrity was 0.8785. (95% CI: 0.8203–0.9368); D: the area under curve (AUC) for LDH was 0.7649 (95% CI: 0.6948–0.8350), E: the area under curve (AUC) for cfDNA concentration was 0.8811 (95% CI: 0.8359–0.9263). F: the area under curve (AUC) for cfDNA integrity was 0.8375 (95% CI: 0.7835–0.8914).

ROC curve analysis was performed. In order to make accurate cfDNA quantification in identifying lymphomas and healthy individuals with high sensitivity and specificity, the critical value of cfDNA concentration is set to 24.67 ng/mL. Because the number of cases in patient group is not too large, there are still more cases to be added in later studies, a series of prospective studies can be conducted to fully evaluate the value of cfDNA for the early screening of lymphoma. In order to improve the sensitivity of lymphoma detection, it can be combined with other traditional or newer diagnostic methods, such as PET-cT, which can greatly improve the sensitivity and specificity of tumor diagnosis and better use in the diagnosis of tumors.

Pathological and immunohistochemical examination is the gold standard for diagnosis of lymphoma and LDH detection is an important auxiliary means of diagnosis and prognosis [22,23].Therefore, this study analyzed the sensitivity and specificity of LDH in the diagnosis of lymphoma at the same time as analyzing the sensitivity and specificity of cfDNA as a means of differentiating between lymphoma patients and controls, in order to more intuitively evaluate the accuracy of cfDNA as a new detection method for lymphoma. The ROC curve analysis confirmed that the AUC of cfDNA was greater than that of LDH, suggesting that the detective effect of cfDNA as a supplementary diagnose for lymphoma, is better than that of LDH, and may be a reliable auxiliary method for the diagnosis of lymphoma in the future.

The clinical application of cfDNA is a newly proposed and rapidly developing research field. If this is used as a routine test item in clinical practice, there are still great challenges. For example, there is no standardized operating procedure for the analysis of cfDNA, such as the use of anticoagulants, and storage time and conditions, etc., these may affect the qualitative and quantitative analysis of circulating blood DNA. Therefore, if large-scale clinical trials are to be conducted, uniform standards need to be established for all aspects of cfDNA testing, and strict positive and negative controls also need to be established to reduce false positives and false negatives. cfDNA has become a good biomarker for cancer screening due to its advantages of being non-invasive, simple and stable. By detecting its changes, early recurrences of cancer can be discovered, providing basis for judging drug efficacy, formulating treatment plans, and guiding prognosis, ultimately helping to achieve personalized medicine and bringing a new revolution to cancer research field.

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Conflicts of interest

The authors declare no conflicts of interest.

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