Circulating tumor DNA mutation profile is associated with the prognosis and treatment response of Chinese patients with newly diagnosed diffuse large B-cell lymphoma

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Background: Characterization of gene mutation profiles can provide new treatment options for patients with diffuse large B-cell lymphoma (DLBCL). However, this method is challenged by the limited source of tissue specimens, especially those of DLBCL patients at advanced stages. Therefore, in the current study, we aimed to describe the gene mutation landscape of DLBCL using circulating tumor DNA (ctDNA) samples obtained from patients' blood samples, as well as to explore the relationship between ctDNA mutations and the prognosis and treatment response of patients with newly diagnosed DLBCL.

Methods: A total of 169 newly diagnosed Chinese DLBCL patients were included in this study, among which 85 patients were divided into a training set and 84 were assigned into a validation set. The mutation profile of a 59-gene panel was analyzed by targeted next generation sequencing (NGS) of the patients' ctDNA samples. Differences in clinical factors between patients with and without ctDNA mutations were analyzed. In addition, we also explored gene mutation frequencies between GCB and non-GCB subtypes, and the relationship between gene mutation status, clinical factors, mean VAF (variant allele frequencies) and the patients' overall survival (OS) and progression-free survival (PFS).

Results: ctDNA mutations were detected in 64 (75.3%) patients of the training set and 67 (79.8%) patients of the validation set. The most commonly mutated genes in both sets were *PCLO*, *PIM1*, *MYD88*, *TP53*, *KMT2D*, *CD79B*, *HIST1H1E* and *LRP1B*, with mutation frequencies of >10%. Patients with detectable ctDNA mutations trended to present advanced Ann Arbor stages (III-IV), elevated LDH (lactate dehydrogenase) levels, shorter OS and PFS, and a lower complete response (CR) rate to the R-CHOP regimen compared with DLBCL patients without ctDNA mutations. In addition, mean VAF ($\geq$4.94%) and *PCLO* mutations were associated with poor OS and PFS.

Conclusion: We investigated the ctDNA mutation landscape in Chinese patients with newly diagnosed DLBCL and found that ctDNA could reflect tumor burden and patients with detectable ctDNA mutations trended to have shorter OS and PFS and a lower CR rate.

KEYWORDS

diffuse large B cell lymphoma, circulating tumor DNA, targeted next-generation sequencing, mutation, prognosis

Background

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin's lymphoma (NHL) worldwide with high clinical and genetic heterogeneity and worse outcomes (1). Gene expression profiling (GEP) divides DLBCL into two main subtypes, namely the germinal center B-cell (GCB) and activated B-cell (ABC) subtypes, with different responses to chemotherapy and targeted agents (2, 3). Recently, Schmitz et al. (4) and Wright et al. (5) classified DLBCL into five and seven genetic subtypes based on gene mutation and translocation profiles. Although these current genotyping techniques are widely accepted, they are challenged by the limited source of tissue specimens, especially for the detection of minimal residual disease (MRD). Thus, it is vital to develop alternative genotyping methods based on patients' body fluids.

Liquid biopsy is a non-invasive method reflecting intra-tumor heterogeneity with no need for fresh tissues (6) and has potential values in diagnosis, MRD monitoring and treatment choice of lymphomas (7, 8). Circulating tumor DNA (ctDNA) is the DNA fragment derived from tumor cells, which accounts for about 0.1% of cell-free DNA (cfDNA) and emerges as one of the most powerful tools for the early diagnosis of cancers (9). Evidence has demonstrated that the allele frequencies (AFs) of individual mutations detected in tumor samples are highly correlated with those observed in paired plasma cfDNA samples (8, 10). Thus, an analysis of ctDNA in cancer patients can reveal both genetic alterations, including single nucleotide variants (SNVs), insertions/deletions (Indels), chromosomal rearrangements, and copy number variations (CNVs), which can be used for genotyping, and ctDNA content, which can reflect tumor burden

(11). Kurtz et al. (11) explored the prognostic value of ctDNA level before and during immunochemotherapy for patients with DLBCL from North America and Europe; they found that pretreatment ctDNA level was an independent prognostic factor in DLBCL. Liu et al. (10) explored the mutation profiles in Chinese patients with newly diagnosed and relapsed/refractory (R/R) DLBCL and observed highly consistent ctDNA and tissue mutation profiles in these patients (sensitivity: 87.50%).

Considering that different races can have varied gene mutation profiles and that the clinical value of ctDNA in Chinese patients remains largely unknown, in this study, we explored the clinical significance of ctDNA in 169 newly diagnosed Chinese DLBCL patients. These patients were first divided into a training set and a validation set. Then we assessed the relationship between ctDNA mutations and clinicopathological features, as well as the roles of ctDNA mutations, including the detected mutation site/gene number, the mean VAF (variant allele frequency) and the mutation status of genes, in the overall survival (OS) and progression-free survival (PFS) in these patients.

Patients and methods

Patients

A total of 169 newly diagnosed Chinese DLBCL patients were enrolled at Shanxi Cancer Hospital from June 2018 to December 2019. Patients were considered eligible for inclusion if they aged $\geq$ 18 years and had histologically confirmed DLBCL according to the 2016 World Health Organization (WHO) Classification of Tumors

of Haematopoietic and Lymphoid Tissues (12). The patients were classified into GCB and non-GCB subgroups according to the Hans algorithm (13). The disease was staged based on the 2014 Lugano Classification and the international prognositic index (IPI) was applied for risk stratification. Bone marrow involvement was assessed by flow cytometry, combined with immunoglobulin (Ig) gene rearrangement and positron emission tomography-computed tomography (PET-CT). A tumor lesion was judged as a bulky disease if the product of length and width of the tumor was ≥ 7.5 cm. All the patients were treated with the R-CHOP regimen (rituximab, cyclophosphamide, doxorubicin, vindesine, prednisone) and followed up until March, 2022. The treatment response, including CR (complete response), PR (partial response), SD (stable disease) and PD (progression disease), was assessed by CT/ magnetic resonance imaging (MRI) and PET/CT according to the 2022 Guidelines of Chinese Society of Clinical Oncology (CSCO) after two to four cycles of the R-CHOP regimen. The data described in this manuscript were approved by the Ethics Committee of Shanxi Cancer Hospital (Ethical approval No.2021013) and conducted in accordance with the Helsinki declaration.

All study activities were approved by the Ethics Committee of Shanxi Cancer Hospital (Ethical approval No.2021013), and informed consent was obtained in accordance with the Declaration of Helsinki.

DNA extraction and targeted sequencing

Ten milimeter of peripheral blood samples were collected using EDTA-containing tubes within 1 week of receiving anticancer treatment and centrifuged at 820 g for 10 min to obtain plasma samples, which were centrifuged at 20,000 g for 10 min. Next, cfDNA was extracted using the QIAamp Circulating Nucleic Acid Kit (QIAGEN, Gemany) according to the manufacturer's instructions. Subsequently, the mutation profile of a 59-gene panel based on literatures (8, 14) was analyzed by targeted next generation sequencing (NGS) of the cfDNA samples (Shanghai Rightongene Bio-tech Co. Ltd, Shanghai, China; Supplementary Table 1) with Illumina NovaSeq 5000 (2×150-bp paired-end sequencing). In this study, VAF was defined as the ratio of the number of mutated alleles to the total number of alleles detected by NGS at a specific genome locus. Mutations with a VAF value ranging from 45% to 55% and ≥ 95% were identified and considered as heterozygous and homozygous germline mutations, respectively. Mean VAF was calculated as follows: Mean VAF = The sum of VAF values of all mutations/the total number of mutations.

Statistical analysis

The maftools ("clinical Enrichment") package of R was used to analyze the differences in clinical factors and gene mutation frequencies between the GCB and non-GCB subgroups using Chi-square test or Fisher's exact test. The tableone package of R was applied to analyze the differences in mean VAF between the two groups. Survival probabilities were estimated using the Kaplan-Meier method. We considered two survival endpoints: PFS, the time intervals from diagnosis to progression, relapse, or death from any cause; and OS, the time intervals from diagnosis to death resulting from any cause. Factors with a P value <0.1 were included in the multivariate Cox regression models. P values < 0.05 were considered as statistically significant.

Results

Relationship between clinicopathological features and ctDNA mutation status in patients with newly diagnosed DLBCL

A total of 169 newly diagnosed DLBCL cases with valid targeted NGS data were included in this study, with 85 patients in the training set and 84 in the validation set. Detailed clinical information of the 169 patients is provided in Supplementary Table 2. Sixty-four (75.3%) patients of the training set carried ctDNA mutations. These patients were significantly enriched in Ann Arbor stages III-IV (69.8% vs. 38.1% in Ann Arbor stages I-II, P=0.002) and tended to have elevated LDH (lactic dehydrogenase) levels (53.1% vs. 14.3%, P=0.004) as compared with the patients without detectable ctDNA mutations (Table 1). Similar results were observed in the validation set, in which 67 (79.8%) patients having detectable ctDNA mutations were significantly enriched in advanced Ann Arbor stages (76.1% vs. 47.1%, P=0.041) and exhibited elevated LDH levels (56.7% vs. 18.8%, P=0.014) as compared with those without detectable ctDNA mutations (Table 2). In addition, the presence of ctDNA mutations was also associated with a higher incidence of bulky disease (41.8% vs. 0.0%, P=0.003) only in the validation group (Table 2). These results indicated that ctDNA mutation status was closely associated with the staging and LDH level of newly diagnosed DLBCL patients.

ctDNA mutation profiles of patients with newly diagnosed DLBCL

Next, we explored the ctDNA mutation profiles of DLBCL patients of the training and validation sets. Detailed information on mutation sites is provided in Supplementary Table 3 and Supplementary Table 4. On average, we detected 6.1 ± 7.1 genetic mutations in patients with confirmed ctDNA mutation. Mutations in PCLO (26%), PIM1 (25%), MYD88 (21%), TP53 (20%), KMT2D (16%), CD79B (12%), HIST1H1E (12%) and LRP1B (11%) genes were the most frequently detected variations, with mutations in each of the genes being found in no less than 9 patients (10%) of the training set (Figure 1A).

TABLE 1 The clinicopathologic features of DLBCL patients with mutation or without in training group.

Clinicopathologic features	Non-mutation group	Mutation group	P
Age, n (%)			0.663
≤60	10 (47.6)	25 (39.1)	
>60	11 (52.4)	39 (60.9)	
Gender			1.000
Female	8 (38.1)	24 (37.5)	
Male	13 (61.9)	40 (62.5)	
Bone marrow involvement, n (%)			0.192
No	21 (100.0)	54 (87.1)	
Yes	0 (0.0)	8 (12.9)	
Bulky disease, n (%)			0.131
No	19 (90.5)	44 (71.0)	
Yes	2 (9.5)	18 (29.0)	
Ann Arbor stage, n (%)			0.002
I-II	13 (61.9)	19 (30.2)	
II-IV	8 (38.1)	44 (69.8)	
Han's classification, n (%)			0.319
GCB	9 (45.0)	19 (29.7)	
Non-GCB	11 (55.0)	45 (70.3)	
IPI score, n (%)			0.103
0-3	20 (95.2)	47 (75.8)	
4-5	1 (4.8)	15 (24.2)	
LDH, n (%)			0.004
Normal	18 (85.7)	30 (46.9)	
Elevated	3 (14.3)	34 (53.1)	
Response to therapy, n (%)			0.048
CR	14 (20.3)	26 (37.7)	
PR+SD+PD	4 (5.8)	25 (36.2)	

LDH, lactic dehydrogenase; CR, complete response; PR, partial response; SD, stable disease; PD, progression disease.

Consistently, *PCLO* (26%), *PIM1* (24%), *MYD88* (20%), *TP53* (24%), *KMT2D* (17%), *CD79B* (17%), *HIST1H1E* (17%) and *LRP1B* (20%) genes also showed high mutation frequencies in the validation set (Figure 1B). In addition, we assessed the difference in ctDNA mutation profile between the GCB and non-GCB subtypes in the 169 DLBCL patients. The results demonstrated that the mutation frequencies of *PIM1* (30.4% vs. 12.7%) and *CD79B* (18.8% vs. 5.5%) were significantly higher in patients of the non-GCB subtype than in those of the GCB subtype (Figure 1C). These results depicted the ctDNA mutation landscape of patients with newly diagnosed DLBCL.

ctDNA mutation status was associated with the response to R-CHOP and clinical manifestation in newly diagnosed DLBCL patients

Next, we assessed the relationship between ctDNA mutation status and the response to R-CHOP regimen in newly diagnosed DLBCL patients. The CR rate in DLBCL patients without ctDNA mutations was obviously higher than that in those carrying ctDNA mutations in both the training (P=0.048) and validation sets (P=0.050) (Tables 1, 2). However, there were no valid differences in the rates of PR, SD and PD between DLBCL patients with different mutation numbers, which is mean VAF values and mutation profiles because the training and validation sets exhibited inconsistent findings. In addition, we compared the mean VAF value in patients with different ages (≤60 vs. >60 years), genders (male vs. female), bone marrow involvement statuses (positive vs. negative), Hans classifications (GCB vs. non-GCB), bulky disease statuses (positive vs. negative), IPIs (1-3 vs. 4-5), Ann Arbor stages (I-II vs. III-IV) and LDH levels (high vs. low). The results showed that the mean VAF value was significantly increased in patients with bone marrow involvement, higher IPI scores (4, 5) and elevated LDH levels in both of the training (Figures 2A–C) and validation sets (Figures 2D–F). Collectively, these results demonstrated that ctDNA mutations were associated with a lower CR rate and

TABLE 2 The clinicopathologic features of DLBCL patients with mutation or without in validation group.

Clinicopathologic features	Non-mutation group	Mutation group	*P*
Age, n (%)			0.029
≤60	14 (82.4)	33 (49.3)	
>60	3 (17.6)	34 (50.7)	
Gender, n (%)			1.000
Female	7 (41.2)	29 (43.3)	
Male	10 (58.8)	38 (56.7)	
Bone marrow involvement, n (%)			0.525
No	17 (100.0)	58 (92.1)	
Yes	0 (0.0)	5 (7.9)	
Bulky disease			0.003
No	17 (100.0)	39 (58.2)	
Yes	0 (0.0)	28 (41.8)	
Ann Arbor stage, n (%)			0.041
I-II	9 (52.9)	16 (23.9)	
III-IV	8 (47.1)	51 (76.1)	
Han's classification, n (%)			0.085
GCB	9 (52.9)	18 (27.3)	
Non-GCB	8 (47.1)	48 (72.7)	
IPI score, n (%)			0.058
0-3	17 (100.0)	51 (76.1)	
4-5	0 (0.0)	16 (23.9)	
LDH, n (%)			0.014
Normal	13 (81.2)	29 (43.3)	
Elevated	3 (18.8)	38 (56.7)	
Response to therapy, n (%)			
CR	12 (16.9)	26 (36.6)	
PR+SD+PD	4 (5.6)	29 (40.8)	

LDH, lactic dehydrogenase; CR, complete response; PR, partial response; SD, stable disease; PD, progression disease.

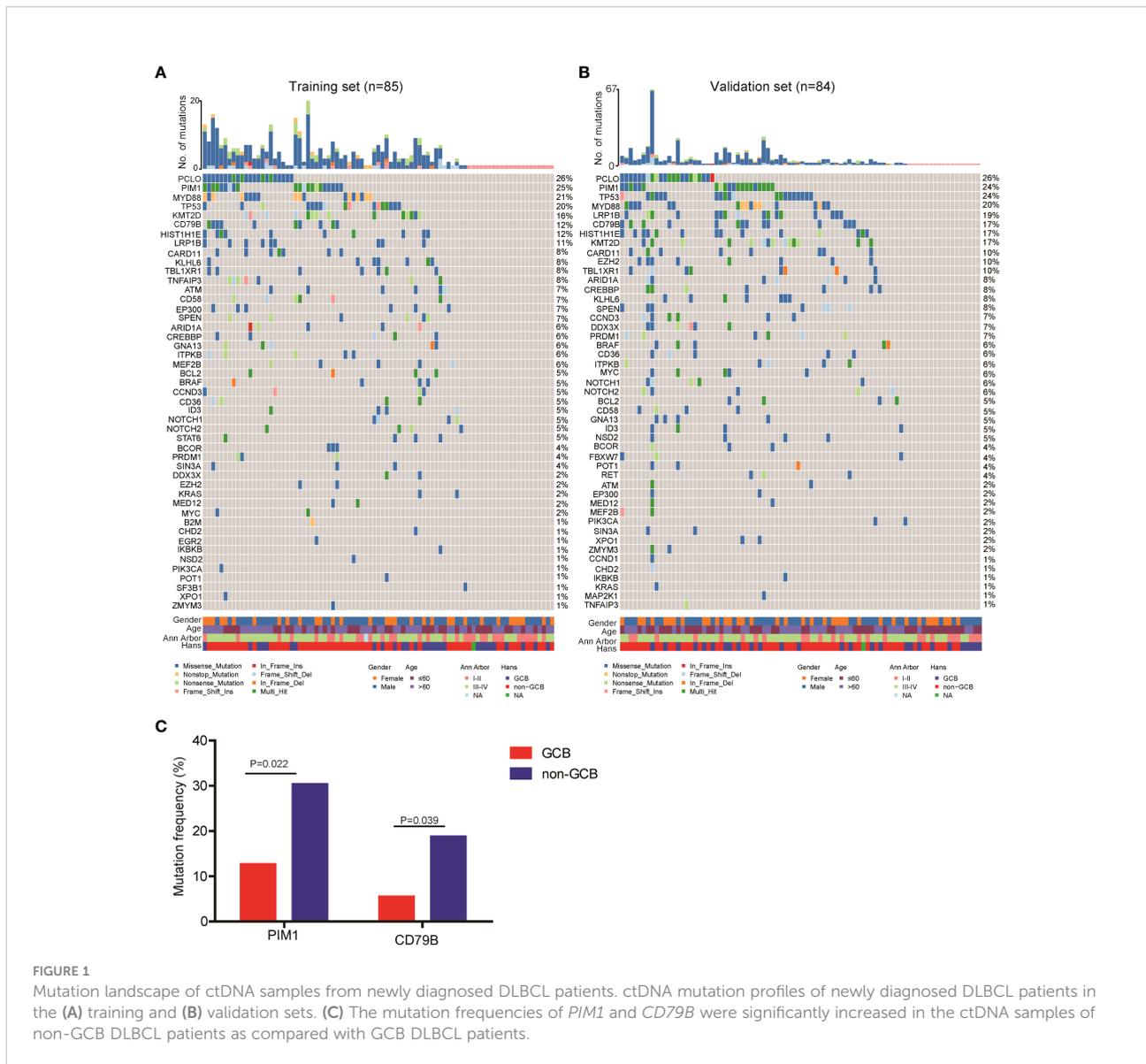aggressive clinical manifestation in patients with newly diagnosed DLBCL.

associated with poor prognosis in patients with newly diagnosed DLBCL.

DLBCL patients carrying ctDNA mutations demonstrated poor prognosis

We also compared survival outcomes between patients with and without ctDNA mutations. In the training set, the 64 patients with ctDNA mutations exhibited significantly shorter OS than the 19 patients without ctDNA mutations (P=0.03) (Figure 3A). PFS was also shorter in patients with ctDNA mutations, albeit the difference was not statistically significant (P=0.095) (Figure 3B). Next, we validated these results in the validation set, in which 67 patients had detectable ctDNA mutations and 17 patients did not. Compared with patients without ctDNA mutations, both OS (P=0.011) (Figure 3C) and PFS (P=0.0032) (Figure 3D) were significantly shorter in patients with ctDNA mutations. These results indicated that ctDNA mutations were

Mean VAF and *PCLO* mutations were associated with poor prognosis in patients with newly diagnosed DLBCL

To further explore the relationship between ctDNA mutation status and the prognosis of patients with newly diagnosed DLBCL, we assessed the effects of mutation number, mutated gene number and mean VAF on OS and PFS using Kaplan-Meier curves generated based the parameters' average/median values. The results demonstrated that only mean VAF (the median value of which was 4.94%) was closely associated with patients' prognosis in the training set. Specifically, the OS (P=0.024) and PFS (P=0.043) of patients with a mean VAF ≥ 4.94% were significantly shorter than those of patients with a mean VAF < 4.94% in the training set (Figures 4A, B). We next verified these findings in the

FIGURE 1

Mutation landscape of ctDNA samples from newly diagnosed DLBCL patients. ctDNA mutation profiles of newly diagnosed DLBCL patients in the **(A)** training and **(B)** validation sets. **(C)** The mutation frequencies of *PIM1* and *CD79B* were significantly increased in the ctDNA samples of non-GCB DLBCL patients as compared with GCB DLBCL patients.

validation set. Compared with those in patients with a mean VAF < 4.94%, the OS (P=0.093) and PFS (P=0.014) were shorter in patients with a mean VAF ≥ 4.94%, albeit the difference in OS was not statistically significant (Figures 4C, D).

In addition, we assessed the effects of gene mutation status on the OS and PFS of patients with newly diagnosed DLBCL. Due to the relatively small sample size, we only assessed genes with a mutation frequency ≥ 10%. In the training set, *LRP1B* (Supplementary Figures 1A, B) and *PCLO* mutations (Figures 5A, B) were significantly associated with shorter OS and PFS; whereas in the validation set, only *PCLO* mutations were significantly associated with shorter OS and PFS (Figures 5C, D). These results demonstrated that a high mean VAF value and *PCLO* mutations predicted poor prognosis in patients with newly diagnosed DLBCL.

Multivariate analysis of prognostic factors in patients with newly diagnosed DLBCL

Finally, multivariate Cox analysis was performed to further explore prognostic factors in patients with newly diagnosed DLBCL. The univariate Cox analysis showed that age > 60 years was an influencing factor on both OS (P=0.038) and PFS (P=0.083) in the training set; meanwhile, bulky disease status (P=0.099) was an influencing factor on PFS in the training set (Table 3). Afterwards, factors with a P value < 0.1, namely the clinical factors (age and/or bulky disease status), mean VAF, and *PCLO* mutation status, were included in the multivariate analysis. The results showed that age (> 60 years) and mean VAF (≥ 4.94%) were independent influencing factors on both OS
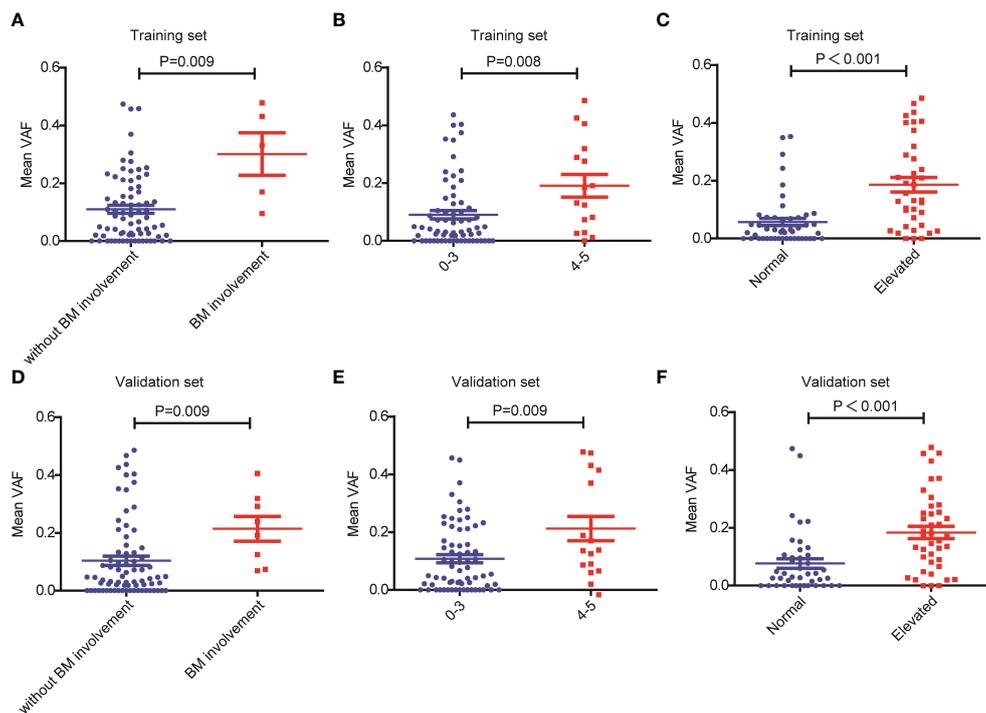
FIGURE 2

Relationship between mean VAF and clinical features in patients with newly diagnosed DLBCL. Mean VAF was increased in patients with **(A, D)** bone marrow involvement, **(B, E)** higher IPI scores and **(C, F)** elevated LDH levels in the training and validation sets.

and PFS in the training set (Table 4). In the validation set, age (> 60 years) and *PCLO* mutation status were influencing factors on OS, while age (> 60 years) and bulky disease status were influencing factors on PFS (Table 4) . These results further verified the close relationship between ctDNA mutation and the prognosis of patients with newly diagnosed DLBCL.

Discussion

Genetic heterogeneity is a major cause of increased risk and treatment failure in DLBCL. Several studies (8, 10, 14–16) have proved that the mutations detected in blood samples were similar to those identified in tumor tissue, with a concordance rate over 80%.In the present study, we performed targeted sequencing of 59 lymphoma-related genes, the same panel as Liu et al. (10) to analyze the clinical value of ctDNA mutation in 169 Chinese patients with newly diagnosed DLBCL. To increase the reliability of our findings, the 169 patients were randomly divided into a training set (n=85) and a validation set (n=84). Our results demonstrated that detectable ctDNA mutations, a mean VAF value ≥ 4.94%, and *PCLO* mutations were strongly associated with shorter OS and PFS in the newly diagnosed DLBCL patients.

We found that *PCLO* (piccolo presynaptic cytomatrix protein), *PIM1, CD79B* and *MYD88* (genes involved in the

NF-κB signaling pathway), *LRP1B* and *TP53* (tumor suppressive genes), as well as *KMT2D* and *HIST1H1E* (histone modifying genes) were the most commonly mutated genes in the 169 newly diagnosed DLBCL patients. According to the genetic landscape of DLBCL in western countries, the most frequently mutated genes are sequentially *KMT2D*, *MYD88*, *CREBBP*, *TP53* and *PIM1* (17, 18). In contract, the most frequently mutated genes in Chinese DLBCL patients are sequentially *PIM1*, *BTG2*, *TP53*, *HIST1H1E* and *KMT2D* (19). The higher proportion of non-GCB DLBCL cases in Chinese patients may be a reason for this difference. According to literature, genes related to histone methylation or acetylation (*EZH2*, *EP300*, *CREBBP* and *KMT2D*) and the PI3K/AKT and JAK/STAT pathways are commonly mutated in the GCB subtype of DLBCL patients, while genes related to the B-cell receptor and NF-κB signaling pathways, such as *MYD88*, *CD79A/B*, *CARD11*, *PIM1* and *TNFAIP3*, are commonly mutated in the ABC subtype (20). Consistently, we found that the mutation frequencies of *PIM1* and *CD79B* were significantly higher in DLBCL patients with the non-GCB subtype than in those with the GCB subtype.

In addition, we were able to detect ctDNA mutations in 64 (75.3%) out of 85 patients in the training set and 67 (79.8%) out of 84 patients in the validation set. Rivas-Delgado et al. (21) were able to detect at least one ctDNA mutation in 69 of 79 patients (87%) with DLBCL. This slight difference in ctDNA mutation
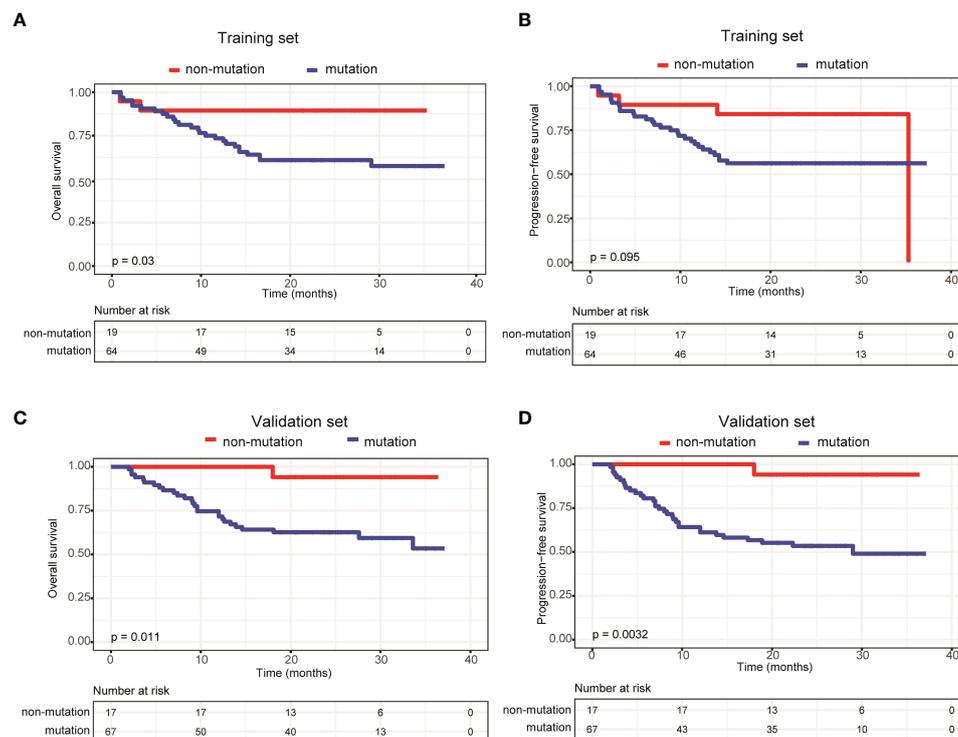
FIGURE 3

Patients carrying ctDNA mutations trended to have poor prognosis. Kaplan-Meier curves were applied to compare the **(A)** OS and **(B)** PFS between patients with and without ctDNA mutations in the training set. Kaplan-Meier curves were applied to compare the **(C)** OS and **(D)** PFS between patients with and without ctDNA mutations in the validation set.

detection rate may be caused by different panels of genes sequenced: Rivas-Delgado et al. (21) performed targeted sequencing on 112 genes, while we analyzed 59 genes. In addition, we found that patients with detectable ctDNA mutations had shorter OS and PFS in both the training and validation sets. Furthermore, patients carrying ctDNA mutations were significantly enriched in more advanced Ann Arbor stages (stages III-IV) and generally exhibited elevated LDH levels. These findings establish a link between ctDNA mutation status and the prognosis of patients with DLBCL. Recently, Kurtz et al. (22) indicated that 25% of ctDNA-negative patients demonstrated by cancer personalized profiling by deep sequencing (CAPP-Seq) were found to be ctDNA-positive, as revealed by phased variant enrichment and detection sequencing (PhasED-Seq), after two cycles of therapy and presented with poor outcomes.

ctDNA VAF has been closely associated with the clinical features and prognosis of various cancers, and is considered as a new biomarker for tumor burden (23, 24). For example, Fu et al. (25) found that the VAF values of *TP53* p.Y88C and *LATS2* p.F972L were decreased in B-cell lymphoma patients with CR. Desch et al. (26) reported that ctDNA VAF values were strongly associated with total metabolic tumor volume (TMTV) and the

incidence of bulky disease in pediatric Hodgkin's lymphoma. In addition. the median VAF of non-DNMT3A clones increased from 1% at the time of autologous stem cell transplantation (ASCT) to 37% at the diagnosis of therapy-related myeloid neoplasms (tMNs) (27). In the present study, we found that the mean VAF values were significantly increased in patients with bone marrow involvement, higher IPI scores and elevated LDH levels in both the training and validation sets. Additionally, we observed that in the training set, patients with a mean VAF $\geq$ 4.94% showed inferior OS and PFS as compared with patients with a mean VAF < 4.94%. This finding was verified in the validation set.

Moreover, we assessed the relationship between ctDNA mutation status and the prognosis of patients with newly diagnosed DLBCL. Notably, we found that patients with *PCLO* mutations had shorter OS and PFS. *PCLO* encodes a protein that functions as a part of the presynaptic cytoskeletal matrix, which is thought to be involved in neurotransmitter release regulation. It has been suggested that *PCLO* might play a role in calcium sensing. *PCLO* mutations have been detected by whole-exom sequencing in a variety of tumors, including DLBCL (28–31). In the mesenchymal subtype of glioblastomas, *PCLO* mutations have been shown to be associated with poor prognosis (31), but
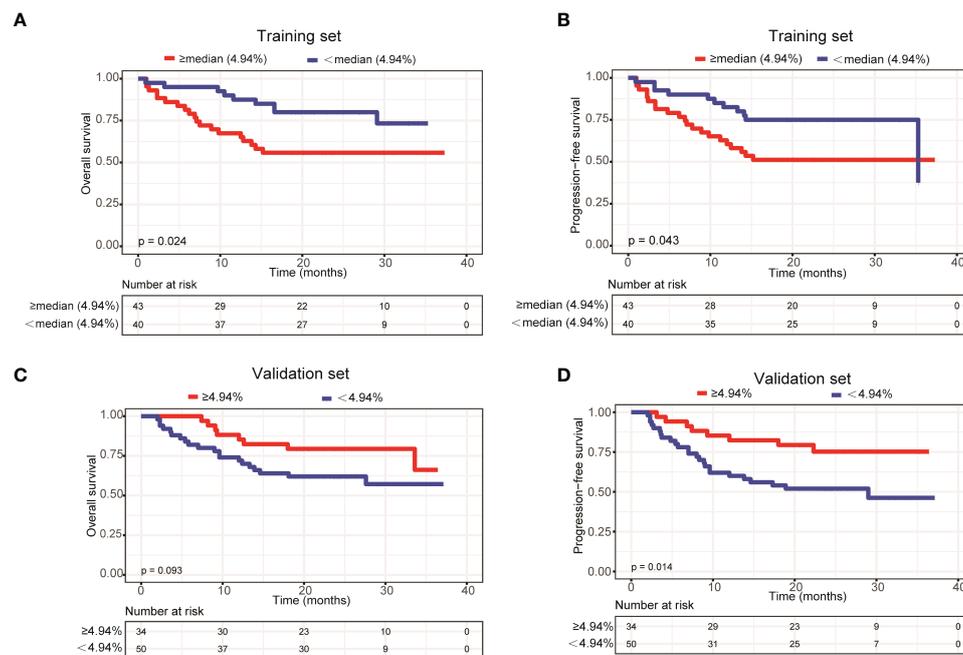
FIGURE 4
Patients with a mean VAF value ≥ 4.94% trended to have poor prognosis. Kaplan-Meier curves were applied to compare the **(A)** OS and **(B)** PFS between patients with mean VAF values ≥ 4.94% and < 4.94% in the training set. Kaplan-Meier curves were applied to compare the **(C)** OS and **(D)** PFS between patients with mean VAF values ≥ 4.94% and < 4.94% in the validation set.

its association with the prognosis of DLBCL has not been reported. Mutations in *PCLO* are usually considered as passenger mutations with no functional consequences in DLBCL (28). In this study, *PIM1* (34.1%), *MYD88* (31.8%) and *TP53* (20.5%) were the most common co-mutated genes with *PCLO* mutations detected in the ctDNA samples of DLBCL patients. Furthermore, we found that the mutation frequency of *TNFAIP3* in *PCLO* mutated DLBCL patients was significantly higher than that of DLBCL patients without *PCLO* mutations [1.6% (2/125) vs. 13.6% (6/44)]. These four genes (*PIM1*, *MYD88*, *TP53*, *TNFAIP3*) has been identified to be the mutational drivers in DLBCL, which might partly explain the poor prognosis of patients carrying *PCLO* mutations (32–35). Additional work is needed to resolve the mechanism of action and role of *PCLO* mutations in DLBCL.

Evidence has demonstrated that ctDNA mutations are correlated with treatment response in DLBCL patients (36). According to the current gold standard for evaluating treatment response in lymphoma, the sensitivity and specificity of ctDNA profiling were 94.7% and 83.3% in refractory or relapse (r/r) DLBCL patients after CAR-T treatment; the median numbers of baseline ctDNA mutations in patients who remained long-term CR and in patients who relapsed or became refractory to CAR-T therapy were 3.0 and 14.3, respectively (36). Herein, we explored the relationship between ctDNA mutation status, the number of ctDNA mutations and mean VAF and the

curative effect of R-CHOP regimen in DLBCL patients. Our results showed that patients without detectable ctDNA mutations had a higher CR rate to R-CHOP treatment as compared with patients with detectable ctDNA mutations, while the ctDNA mutation number and mean VAF showed no significant impacts on the CR rate.

Our study showed that age (> 60 years) and mean VAF (≥ 4.94%) were independent influencing factors on prognosis in the training set, while age (> 60 years), *PCLO* mutations and bulky disease status were independent influencing factors on prognosis in the validation set. The high heterogeneity of DLBCL may have caused these differences between the training and validation sets. Of course, the small sample size of our study may be another reason for the differences. In fact, the relatively small sample size is the main limitation of the present study, although we have recruited the largest cohort of Chinese DLBCL patients to date. To this end, we intend to include more Chinese DLBCL patients for analysis in the future.

Taken together, we herein have described the ctDNA mutation landscape of a largest cohort of Chinese patients with newly diagnosed DLBCL to date. Our results suggested that patients with detectable ctDNA mutations, a higher mean VAF value or *PCLO* mutations trended to have shorter OS and PFS and a lower CR rate. Our study provides evidence to support the feasibility of using ctDNA samples obtained from patients' blood in prognosis prediction of newly diagnosed DLBCL.
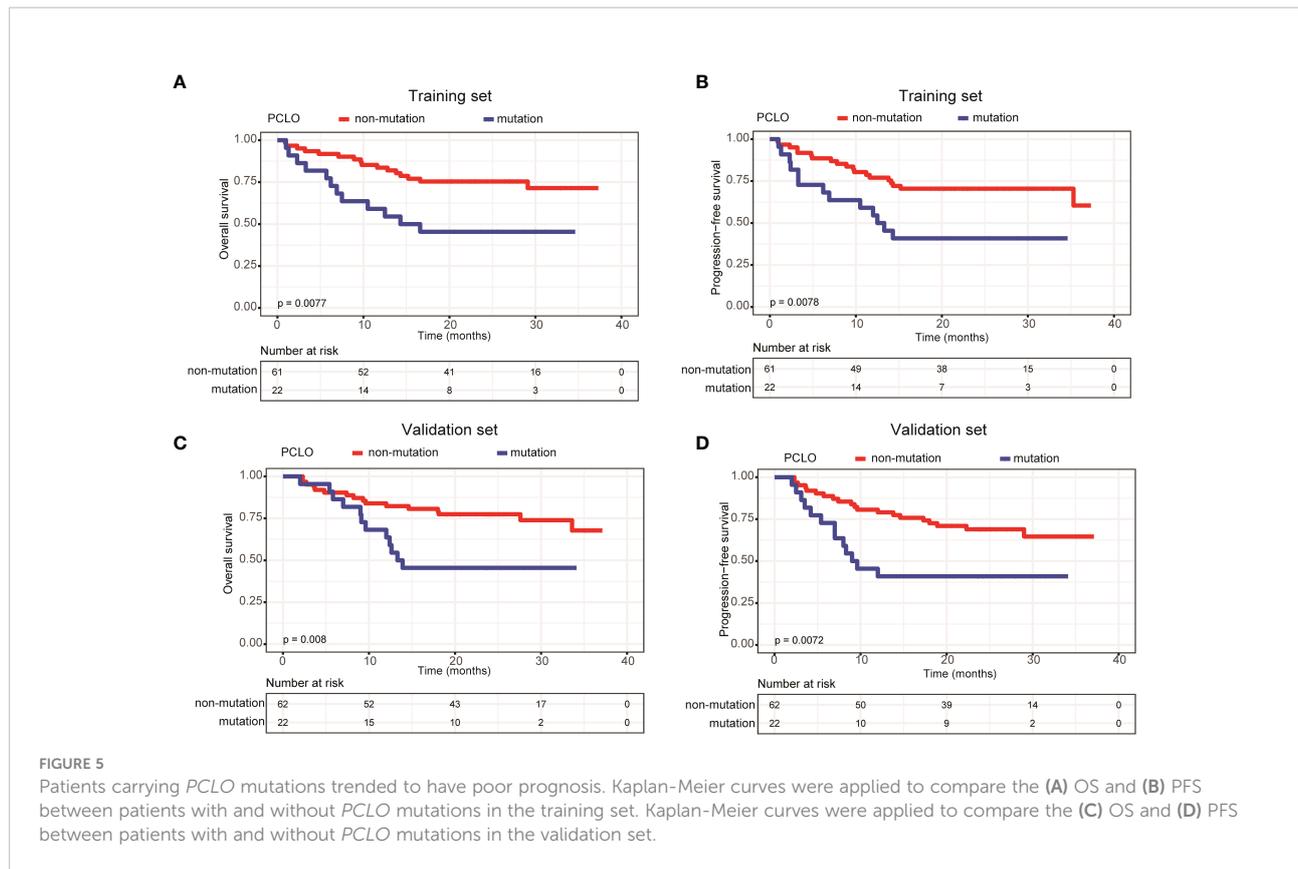
FIGURE 5
Patients carrying *PCLO* mutations trended to have poor prognosis. Kaplan-Meier curves were applied to compare the **(A)** OS and **(B)** PFS between patients with and without *PCLO* mutations in the training set. Kaplan-Meier curves were applied to compare the **(C)** OS and **(D)** PFS between patients with and without *PCLO* mutations in the validation set.

TABLE 3 Univariate Cox analysis of the influencing factors of PFS and OS in the training set.

Clinical factors	OS		PFS	
	HR (95% CI)	P	HR (95% CI)	P
Age (>60)	2.651 (1.053-6.671)	0.038	2.066 (0.909-4.694)	0.083
Ann Arbor stage (III-IV)	1.673 (0.697-4.013)	0.249	1.583 (0.697, 3.594)	0.273
Han's classification (non-GCB)	0.710 (0.314-1.607)	0.411	0.870 (0.394-1.924)	0.731
Bulky disease	1.920 (0.846-4.360)	1.920	1.918 (0.885-4.159)	0.099
LDH level (elevated)	1.760 (0.799-3.881)	0.161	1.699 (0.808-3.572)	0.162

HR, Hazard ratio; CI, confidence interval.

TABLE 4 Multivariate Cox analysis of the influencing factors of PFS and OS in the training set and validation set.

Clinical factors	Training set				Validation set			
	OS		PFS		OS		PFS	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Age (>60)	3.6 (1.4-9.2)	0.01	2.6 (1.1-6.4)	0.03	2.2 (1.0-4.9)	0.05	2.1 (1.0-4.4)	0.04
Bulky disease	—	—	1.2 (0.5-2.7)	0.69	—	—	2.4 (1.1-5.0)	0.03
VAF (4.94%)	2.6 (1.1-6.4)	0.04	2.5 (1.1-5.8)	0.04	1.4 (0.6-3.5)	0.51	1.4 (0.6-3.6)	0.48
PCLO mutation	1.9 (0.8-4.3)	0.15	1.8 (0.8-4.0)	0.17	2.7 (1.2-4.3)	0.02	2.0 (1.0-4.3)	0.06

HR, Hazard ratio; CI, confidence interval.

Data availability statement

Ethics statement

The studies involving human participants were reviewed and approved by The Ethics Committee of Shanxi Cancer Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

Concept and design: LS, WQ, JD. Acquisition, analysis, or interpretation of data: TG, MeZ, JL, XL, YR, YY, HW, XG, YH. Patients' follow-up: MiZ, YY, MeZ. Statistical analysis: BX, TG, MJZ. Drafting of the manuscript: TG, MiZ, BX. Supervision: LS. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Author BX and YR are employed by Shanghai Yuanqi Biomedical Technology Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.1003957/full#supplementary-material

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