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Jing Zhan

Peking Union Medical College Hospital

Wei Zhang (✉ vv1223@vip.sina.com)

Peking Union Medical College Hospital <https://orcid.org/0000-0001-9489-5965>

Daobin Zhou

Peking Union Medical College Hospital

Yan Zhang

Peking Union Medical College Hospital

Wei Wang

Peking Union Medical College Hospital

Chong Wei

Peking Union Medical College Hospital

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Intensive immunochemotherapy improves prognosis of young patients with double-expressor lymphoma comparison to non-double-expressor lymphoma: a single-center retrospective cohort study

Jing Zhan[†], Wei Zhang^{*†}, Daobin Zhou[†], Yan Zhang[†], Wei Wang[†], Chong Wei[†]

[†] Department of Hematology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China

* Correspondence: vv1223@vip.sina.com

Email addresses for all authors: Jing Zhan: zj_thupumc@126.com; Daobin Zhou: zhoudb@pumch.cn; Yan Zhang: zhangyan10659@pumch.cn; Wei Wang: wangweipumc@163.com; Chong Wei: QH5035@163.com

Abstract

Background Double-expressor lymphoma (DEL), defined as cases with concurrent MYC and BCL2 proteins overexpression, is associated with poor prognosis when treated with R-CHOP alone. Prognostic data on DEL treated with intensive treatment are limited and controversial.

Methods We retrospectively report our experiences about 221 consecutive de-novo diffuse large B cell lymphoma patients and analyze the role of intensive therapies for 65 patients with DEL.

Results No significant difference in clinical characteristics and survival outcomes between

DEL and non-DEL was observed. Overall, 32 (76.2%) and 20 (87.0%) patients with DEL received R-CHOP and intensive treatment achieved partial response or complete response, respectively. Intensive therapies may improve the prognosis over R-CHOP in patients with DEL (P = 0.029 for PFS, P = 0.026 for OS). Subgroup analysis according to clinical characteristics showed intensive therapies resulted in better prognosis than R-CHOP regimen in DEL patients aged under 60 years (P = 0.004 for PFS; P = 0.090 for OS), whereas no significant superiority was found in DEL patients aged over 60 years (P = 0.646 for PFS; P = 0.361 for OS).

Conclusions This second Chinese cohort study suggests that intensive immunochemotherapy may have the ability to improve the prognosis of young patients with DEL.

Keywords: Double-expressor lymphoma, Intensive treatment, R-CHOP, Therapy, Prognosis

Background

Diffuse large B cell lymphoma (DLBCL) is a leading cause of cancer morbidity in non-Hodgkin lymphoma, accounting for over 30% of all subtypes(1). RCHOP immunochemotherapy (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone)(2, 3) contribute to high average cure rates (approximately 70%) and great prognostic improvement in progression-free survival (PFS) and overall survival (OS) in the majority of patients with DLBCL(4) and has been regarded as a standard treatment in recent years.

However, underlying the molecular and morphologic heterogeneity of different pathologic

subtypes, there has been increasingly more attention in several subgroups of B-cell lymphoma with poor outcomes after RCHOP treatment, especially Double-hit lymphoma (DHL) and Double expressor lymphoma (DEL). DHL is defined as cases with genetic rearrangement for MYC along with BCL2 or BCL6 or both genes, which newly categorized into an individual subtype named “High-grade B-cell lymphoma” with a median OS is only about 13-16.8 months for patients treated with RCHOP(5-8). DEL is defined as cases with concurrent MYC and BCL2 proteins overexpression, representing 18–34% of patients with DLBCL(9, 10). Several research studies have demonstrated an abysmal prognosis for patients with DEL treated with R-CHOP alone, with 5-year PFS and OS rates less than 40%(8-10). There is an urgent need to dissect clinical characteristics and determine more beneficial therapeutic strategies than RCHOP for patients with DHL and DEL. Though intensive induction treatment proves significant prognostic improvement for patients with DHL in prior retrospective studies(11-13), prognostic data on patients with DEL treated with intensive treatment strategies are limited and controversial.

With large unmet medical needs, we conducted this retrospective cohort study, aiming to analyze clinical characteristics and survival outcomes of Chinese patients with DEL comparison to non-DEL and further determine the safety and feasibility of the first-line intensive induction regimens compared to R-CHOP regimen in patients with DEL in subset analyses, which will provide more clinical evidence for better risk-stratified treatment decisions.

Methods

Patient selection

This is a retrospective cohort study, including 221 consecutive patients who were diagnosed

with de-novo DLBCL and had available pathological specimens at Peking Union Medical College Hospital between January 2015 and December 2018. All cases were diagnosed with clinical symptoms and results of biopsy according to the World Health Organization (WHO) classification criteria. Data on laboratory clinical characteristics were retrieved from medical records, including gender, age, stage based on Ann Arbor staging system(14), IPI score(15), serum lactate dehydrogenase (LDH), serum β 2-microglobulin (β 2-MG), initial induction regimen, number of extranodal disease sites and CNS involvement evaluated with enhancement computed tomography (CT) scan or ^{18}F -fluorodeoxyglucose positron emission tomography (PET) or lumbar puncture with examination of cerebrospinal fluid. $\text{LDH} \geq 250 \text{ IU/L}$, β 2-MG $\geq 1.8 \text{ mg/L}$, and IPI score ≥ 3 were considered high, respectively. The date of the last follow-up is May 2019. Our study was with ethic board approval. The Ethics Committee of the Peking Union Medical College Hospital, Chinese Academy of Medical Sciences has approved this retrospective study, in which informed consent was waived, but patient confidentiality was protected.

Immunohistochemistry and FISH

Two experienced morphological and pathological specialists re-stained all formalin-fixed, paraffin-embedded samples and independently reanalyzed the immunohistochemical results, employing the most recent biopsy specimens and a panel of antibodies including MYC (clone Y69; Abcam), BCL2 (clone 124; Dako), BCL6(clone LN22, Dako), CD10 (clone 56C6, Dako) and MUM1(clone MUM1p, Dako). Any discrepancy was resolved by discussion. As previously described(6, 16), the positive cut-off of MYC, BCL2, BCL6 protein is 40%, 50%, and 30% expressing cells, respectively. Cases with concurrent positive MYC and BCL2 protein were

considered as DEL. Additionally, the GCB and non-GCB subtype of all cases were determined with cell-of-origin (COO) classification according to Hans algorithm based on the histologic results of CD10, BCL6, and MUM1 proteins(17). Furthermore, DHL refers to B-cell lymphoma with genetic rearrangement for MYC and BCL2 and/or BCL6 genes, which newly categorized into a separate category named “High-grade B-cell lymphoma”. FISH was conducted to detect the genetic rearrangement in the majority of our samples, applying two different probes to evaluate MYC on 8q24 or BCL6 on 3q27.

Treatment

Initial induction treatment was categorized into two groups: (i)Standard regimen: R-CHOP (ii)Intensive induction regimen, including: DA-EPOCH-R (rituximab, etoposide, prednisone, vincristine, cyclophosphamide and doxorubicin)(18, 19), R-hyperC-VAD/MA (rituximab, hyperfractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone, alternating with cytarabine plus methotrexate)(20), R-DHAP(rituximab, dexamethasone, cytarabine, and cisplatin)(21), (iii)Other weak regimen such as CHOP without rituximab or rituximab only. Notably, patients that initially treated with R-CHOP less than four courses and later converted into intensive regimen because of DHL or DEL rather than disease progression were considered as intensive regimen group.

Supportive treatment

During treatment, long-lasting granulocyte colony-stimulating factor (G-CSF) and prophylactic antibiotics were applied regularly when peripheral blood leukocyte counts $<2.0 \times 10^9/L$.

Concentrated red blood cell infusion was conducted when hemoglobin <60 g/L or be complicated by severe cardiopulmonary decompensation. Thrombopoietin was employed when platelet <30 × 10⁹/L, and platelets concentrate were infused when platelet <20 × 10⁹/L.

Treatment evaluation

Treatment evaluation and progression of disease were evaluated with a lumbar puncture, 18F-fluorodeoxyglucose positron emission tomography (PET), or enhancement computed tomography (CT) scan, complied with revised International Workshop Group response criteria for malignant lymphoma(22). Regular imaging evaluations were conducted at the end of regular treatment courses and every six months after diagnosis. The extra evaluation was performed when physicians suspected disease progressed. PFS duration is defined as the duration between diagnostic date and the date of disease progression, recurrence date, or date of death from any cause. OS duration is described as the interval between the diagnostic date and the date of death or the last follow-up.

Statistical analysis

Data on clinical characteristics were analyzed using Statistical Package for the Social Sciences (SPSS) software for windows (version 25.0). Dichotomous statistical analyses were performed with the chi-square test or Fisher's exact test. PFS and OS were estimated and calculated with the Kaplan-Meier method and log-rank test in subgroups comparison and univariate analyses(23). Characteristics with P value <0.15 in univariate analyses were estimated as potential correlative factors in the multivariate analyses, using the Data on clinical characteristics were analyzed using Statistical Package for the Social Sciences (SPSS) software for windows (version 25.0).

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Results

Patient characteristics

We reviewed a total of 221 patients in our present study, including 77 (34.8%) patients with DEL and 144 (65.2%) patients with non-DEL. The clinical, immunohistochemical and FISH characteristics were summarized in **Table 1**. Overall, the characteristics of patients with DEL were very similar to non-DEL. The median age for all cases was 55 years old (range 13-87). 173 (78.3%) cases were observed at advanced stages (III-IV) at diagnose, and 114 (51.6%) case was found with high IPI score (3-5). Though the condition of serum β 2-MG and rearrangement were not determined in 94 and 78 cases respectively, the majority of cases had elevated serum LDH (122, 55.2%) and serum β 2-MG (111, 87.4% in 127, 94 was undetected), respectively. 96 (43.4%) of cases had more than two different extranodal disease sites, and only 3 (1.4%) patients were found with and central nervous system involvement (CNS involvement) at diagnosis. Germinal center B-cell-like (GCB) subtype (134, 60.6%) was relatively more common than the non-GCB subtype, and the proportion of non-GCB in the DEL group was higher than the non-DEL group without significant difference (66.2% vs. 57.6%, P=0.213). Only 143 cases had explicit FISH results, and 8 cases (5.6%) were positive for MYC and BCL2 genetic rearrangement, which is mostly consistent with prior literature.

Generally, there was no significant difference in each baseline clinical characteristic between the DEL group and the non-DEL group.

Table 1 Clinical characteristics of 221 patients diagnosed with DLBCL

Parameter	Total	DEL(n=77) n(%)	Non-DEL(n=144) n (%)	P value
Gender				
Male	106	35(45.5)	71(49.3)	0.585
Female	115	42(54.5)	73(50.7)	
Age groups				
≤60	136	45(58.4)	91(63.2)	0.489
>60	85	32(41.6)	53(36.8)	
Staging				
I-II	46	15(19.5)	33(22.9)	0.555
III-IV	143	62(80.5)	111(77.1)	
IPI score				
Low (0-2)	107	34(44.2)	73(50.7)	0.354
High (3-5)	114	43(55.8)	71(49.3)	
Serum LDH				
Normal	99	31(40.3)	68(47.2)	0.321
High	122	46(59.7)	76(52.8)	
Serum β2-MG				
Normal	16	5(13.5)	11(12.2)	1.000
High	111	32(86.5)	79(87.8)	
Not determined	94	40	54	
Extranodal disease sites				
<2	125	38(49.4)	87(60.4)	0.114
≥2	96	39(50.6)	57(39.6)	
CNS involvement				
No	218	76(98.7)	142(98.6)	1.000
Yes	3	1(1.3)	2(1.4)	
Cell of origin				
GCB	87	26(33.8)	61(42.4)	0.213
Non-GCB	134	51(66.2)	83(57.6)	
DHL				
No	135	42(93.3)	93(94.9)	1.000
Yes	8	3(6.7)	5(5.1)	
Not determined	78	32	46	

DLBCL diffuse large B cell lymphoma, DEL double-expressor lymphoma, IPI International Prognostic Index, LDH lactate dehydrogenase, β2-MG β2-microglobulin, CNS central nerve system, GCB germinal center B-cell-like, DHL double-hit lymphoma

Treatment choice

Notably, considering that patients with DHL should receive more intensive immunochemotherapeutic regimens according to the updated National Comprehensive Cancer Network guidelines and the preference for aggressive therapy for patients with known DHL in our practical clinical work, all known 8 cases with DHL were excluded in following statistical analysis in order to avoid potential selection bias caused by treatment choice. Another 31 cases were excluded from the comparison of treatment choice and survival analysis because they received weaker treatment than R-CHOP or no treatment. Finally, 182 cases were included in the comparison of treatment choices, as shown in **Table 2**. The most common regimen performed in our study was R-CHOP (131, 72.0%), followed by intensive therapies (51, 28.0%). The proportion of patients with intensive treatment in the DEL group was higher than the non-DEL group without significant difference (35.4% vs. 23.9%, P=0.099). A total of 28 (15.4%) patients received frontline stem cell transplantation (SCT) regardless of autologous or allogeneic. Consolidative radiation therapy and CNS prophylaxis, defined as more than three times of intrathecal injection with cytosine arabinoside and dexamethasone or combination methotrexate with induction treatment, was applied on 16(8.8%) and 149 (74.2%) patients respectively. Additionally, intensive therapies were applied in patients with a maximum age of 74 and 76 for the DEL group and the non-DEL group, respectively. In summary, treatment choices were comparable, with no significant difference between the two groups.

Table 2 Treatment choices of 182 patients diagnosed with DLBCL

Parameter	Total	DEL(n=65) n(%)	Non-DEL(n=117) n (%)	P value
Treatment				
R-CHOP	131	42(64.6)	89(76.1)	0.099
Intensive (vs R-CHOP)	51	23(35.4)	28(23.9)	
DA-EPOCH-R		19(29.2)	21(17.9)	

R-hyperC-VAD/MA		1(1.5)	4(3.4)	
R-DHAP		3(4.6)	3(2.6)	
CNS prophylaxis				
No		39(60.0)	69(59.0)	0.893
Yes		26(40.0)	48(41.0)	
Radiotherapy				
No	166	60(92.3)	106(90.6)	0.696
Yes	16	5(7.7)	11(9.4)	
SCT				
No	154	58(89.2)	96(82.1)	0.198
Yes	28	7(10.8)	21(17.9)	

DLBCL diffuse large B cell lymphoma, DEL double-expressor lymphoma, R-CHOP rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone, DA-EPOCH-R rituximab, etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, R-HyperC-VAD/MA rituximab, hyperfractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone, alternating with cytarabine plus methotrexate, R-DHAP rituximab, dexamethasone, cytarabine, and cisplatin, CNS central nerve system, SCT stem cell transplantation

Response and toxicity

After 23.3 months' median follow-up time, 42 cases with DEL received 1-8 cycles of (median, 6) R-CHOP treatment. 22 (52.4%) and 10 (23.8%) patients achieved a complete response (CR) and partial response (PR) respectively. Progressive disease (PD) and stable disease (SD) both occurred in 5 (11.9%) patients. 2-8 cycles of intensive regimen (median, 6) were conducted in 23 patients with DEL, resulting in 16 (69.6%) patients had CR, 4 (17.5%) had PR, and 3 (13.0%) PD. As for 89 patients with non-DEL treated with 1-8 cycles of (median, 6) R-CHOP, 58 (65.2%) cases achieved CR, 17 (19.3%) had PR, and 14 (15.7%) SD or PD. 1-8 cycles (median, 6) of intensive therapy resulted in 14 (50%) CR, 7 (25%) PR, and 7 (25%) PD or SD of patients with non-DEL.

Furthermore, toxicity was evaluated in 131 patients treated with R-CHOP and 51 with intensive treatment. Cases treated with R-CHOP were less likely to be associated with grade 3/4 neutropenia (48,36.6% vs. 35,68.6%), anemia (15,11.5% vs. 11,21.6%) and thrombocytopenia (19,14.5% vs. 16,31.4%) comparison to intensive regimens. However, the incidence of neutropenic fever seemed similar (27,20.6% vs 14,27.5%) between the R-CHOP group and the intensive regimen group.

Treatment-related mortality, defined as deaths due to infection or massive hemorrhage rather than disease progression, was observed in 3 (2.3%) patients received R-CHOP and 1 (2.0%) patients received intensive treatment, respectively.

Survival outcomes of PFS and OS

Generally, there was no statistical difference in survival outcomes between the DEL group and the non-DEL group ($P = 0.991$ for PFS, $P = 0.632$ for OS) (Fig. 1A &1B). Median PFS and OS for the DEL group or non-DEL group were undefined because of the limited follow-up time and sample size. Considering the consensus that DEL indicated poor prognosis when treated with R-CHOP than non-DEL, we compare the PFS and OS outcomes between subgroups, including DEL with R-CHOP, DEL with intensive therapy, and non-DEL with RCHOP for further exploration (Fig.2A &2B). These results suggest that better PFS and OS were found in DEL treated with intensive treatment compared to DEL treated with R-CHOP ($P = 0.029$ for PFS, $P = 0.026$ for OS), which means intensive immunochemotherapy may have the ability to overcome the poor prognosis of double-expressor DLBCL. There was no statistical difference for PFS and OS between DEL patients treated with R-CHOP compared to non-DEL with R-CHOP ($P = 0.332$ for PFS, $P = 0.112$ for OS) and DEL patients treated with intensive therapy compared to non-DEL with R-CHOP ($P = 0.116$ for PFS, $P = 0.109$ for OS), though there seems a tendency for survival curves to separate. 2-year PFS for DEL treated with R-CHOP, intensive regimens, and non-DEL treated with R-CHOP were 54.8%, 87.0%, and 67.4%. Correspondingly, 2-year OS were 69.0%, 91.3% and 79.8%. 3-year PFS were 47.6%,

87.0%, 62.9% and 3-year OS were 52.4% ,91.3% and 77.5%, respectively.

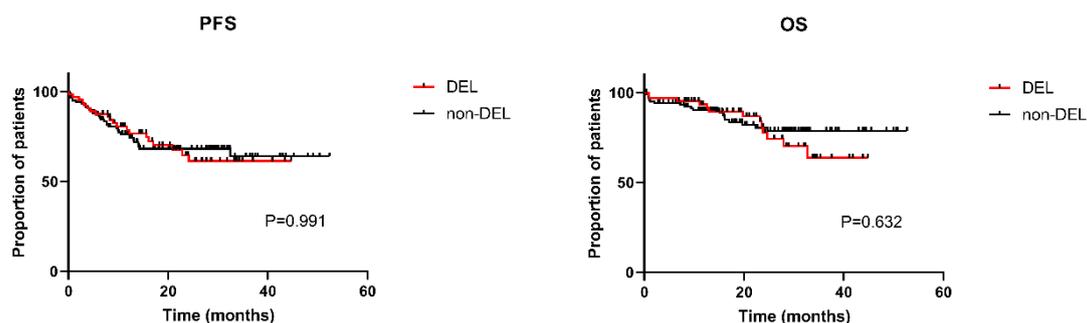


Fig. 1A

Fig. 1B

Fig. 1 Survival outcomes by protein expression. (a) PFS of patients with DEL and non-DEL. (b) OS of patients with DEL and non-DEL.

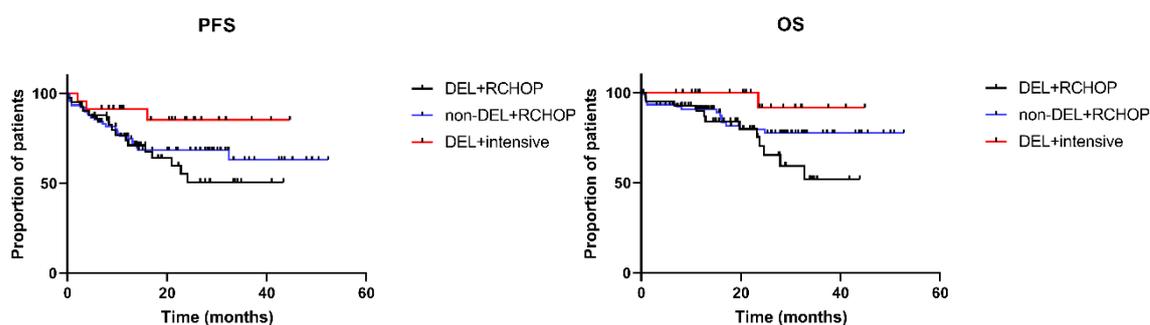


Fig.2A

Fig. 2B

Fig. 2 Subgroup survival outcomes by protein expression and therapies. (a) PFS of patients with DEL who treated with R-CHOP, with DEL who treated with intensive therapy and with non-DEL who treated with RCHOP. (b) OS of patients with DEL who treated with R-CHOP, with DEL who treated with intensive therapy and with non-DEL who treated with RCHOP

Identification of characteristics associated with outcomes for DEL subset

To evaluate the impacts of all clinical characteristics and therapeutic factors on clinical outcomes and eliminate interferences between different factors as much as possible, we carried out univariate and multivariate analyses on several factors of total 65 patients with DEL, including age, stage, IPI, LDH, β 2-MG, extranodal site involvement, central nerve system involvement (CNS involvement), cell of origin, BCL6 protein expression, immunochemotherapeutic regimens, CNS prophylaxis, radiotherapy and stem cell transplant (SCT), as shown in **Table 3** and **4**. Nevertheless, we excluded IPI and SCT factors due to their non-independence.

Table 3 Correlation with outcomes of 65 patients with DEL by univariate analyses

Parameter	n	PFS	OS
		P	P
Age>60 vs ≤60	30/35	0.055	0.029
Staging (III-IV vs I-II)	52/13	0.399	0.516
High IPI vs Low IPI	33/32	0.075	0.257
High LDH vs normal	37/28	0.062	0.142
High β2-MG vs normal	28/5	0.454	0.287
Extranodal disease sites≥2 vs <2	32/33	0.726	0.275
CNS involvement	1/64	0.482	0.582
Non-GCB vs GCB	43/22	0.245	0.416
MYC+BCL2+BCL6+ vs MYC+BCL2+BCL6-	58/7	0.128	0.646
Treatment			
Standard (R-CHOP)	42		
Intensive (vs R-CHOP)	23/42	0.029	0.026
DA-EPOCH-R	19/42	0.005	0.039
R-hyperC-VAD/MA	1/42	0.012	0.744
R-DHAP	3/42	0.520	0.418
CNS prophylaxis	26/39	0.708	0.221
Radiotherapy	5/60	0.793	0.241
SCT	7/58	0.088	0.135

P < 0.15 is in bold except IPI and SCT

DEL double-expressor lymphoma, PFS progression-free survival, OS overall survival, IPI International Prognostic Index, LDH lactate dehydrogenase, β2-MG β2-microglobulin, CNS central nerve system, GCB germinal center B-cell-like, R-CHOP rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone, DA-EPOCH-R rituximab, etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, R-HyperC-VAD/MA rituximab, hyperfractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone, alternating with cytarabine plus methotrexate, R-DHAP rituximab, dexamethasone, cytarabine, and cisplatin, CNS central nerve system, SCT stem cell transplantation

The univariate analysis suggested that intensive regimen and normal LDH indicated significant superior PFS, and age under 60 and CNS prophylaxis were associated with significant superior OS correspondingly. Furthermore, all factors with P value <0.15 in univariate analysis were contained in multivariate analysis. As for the model analyzing PFS outcomes, in which therapeutic regimens and LDH were included, intensive treatment was an independent prognostic factor for superior PFS (HR 0.266, 95%CI 0.078-0.910, P=0.035). Similar survival superiority of intensive treatment was observed in OS outcomes (HR 0.118, 95%CI 0.015-0.915, P =0.041). Further subgroup analysis

with the treatment subset was not possible due to the limited number of patients treated with R-hyperC-VAD/MA and R-DHAP. We found that clinical characteristics and treatment choices of patients with DEL in this study were comparable without significant difference ($P > 0.05$) between the R-CHOP group and intensive treatment group except that patients treated with intensive therapies were younger than patients treated with R-CHOP ($P = 0.016$). The median age of the R-CHOP group was 60 years and 48 years in the intensive treatment group. Despite the small number of patients in each subgroup, we further conducted the subgroup analysis according to age, which demonstrated that intensive regimen resulted in significantly better PFS and OS outcomes than R-CHOP regimen in 35 DEL patients aged under 60 years ($P = 0.004$ for PFS; $P = 0.090$ for OS), whereas no significant survival superiority of intensive therapies in 30 DEL patients aged over 60 years was observed ($P = 0.646$ for PFS; $P = 0.361$ for OS). These findings can reveal that intensive immunochemotherapeutic regimens, especially DA-EPOCH-R regimen, may have the ability to overcome the poor prognosis of patients with double-expressor DLBCL aged under 60 years.

Table 4 Correlation with outcomes of 65 patients with DEL by multivariate analyses

Parameter	PFS			Parameter	OS		
	HR	95%CI	P		HR	95%CI	P
LDH	2.673	0.969-7.375	0.058	LDH	3.117	0.853-11.387	0.085
Intensive vs R-CHOP	0.266	0.078-0.910	0.035	Intensive vs R-CHOP	0.118	0.015-0.915	0.041

$P < 0.05$ is in bold

DEL double-expressor lymphoma, PFS progression-free survival, OS overall survival, LDH lactate dehydrogenase

Discussion

Double-expressor lymphoma (DEL) is a relatively common subgroup of DLBCL with poor prognosis compared to non-DEL when treated with R-CHOP regimen. Patients with DEL were more likely to be associated with higher tumor-proliferation property and inferior response rates to R-

CHOP therapy(24). Intensive treatment, especially DA-EPOCH-based regimen, demonstrated promising results in DHL, relapsed or refractory DLBCL, and subgroups of DLBCL with high proliferation(25, 26). The optimal therapeutic strategies for patients with DEL remain a significant challenge because previous results have shown great controversies on intensive immunochemotherapeutic regimens recently. This study demonstrated that intensive treatment, especially DA-EPOCH-R, was associated with higher response rates and better survival outcomes than the R-CHOP regimen in DEL patients and might have the ability to eliminate the inferior prognosis of DEL over non-DEL to some extent.

To our knowledge, this is the second report analyzing the survival of a consecutive cohort of Chinese patients with double-expressor DLBCL treated with intensive therapies or R-CHOP, but our findings and conclusions differ from the first Chinese cohort study conducted by Xinyu Zhang(27). We found the majority of patients with DLBCL in our study had some characteristics including advanced stage, aged under 60, elevated LDH and β 2-MG, extranodal disease sites \leq 2 at diagnosis and there was no significant difference between two groups, which means patients with DEL was not more severe than non-DEL at baseline. In our cohort, 77 (34.8%) and 5.6% DLBCL cases were associated with the DEL subgroup and DHL subgroup, respectively. 66.2% DEL cases had a non-GCB subtype, which is consistent with the past findings (63%) (8), while in the first Chinese cohort, only 189 of 398 patients identified the data on MYC and BCL2 protein expression. 53 (28.0% of 189) cases were characterized as DEL, and 40 (75.5% of 53) DEL patients were non-GCB subtype.

Importantly, our findings suggest that PFS and OS were similar between DEL and non-DEL groups, and there was no significant difference between DEL patients treated with intensive

regimens and non-DEL patients treated with R-CHOP. Considering the unsatisfactory prognosis of DEL when treated with the R-CHOP regimen, these results might be associated with differences in treatment choice and overall response rates. A higher proportion (35.4%) of DEL patients were treated with intensive regimens, but only 23.9% of non-DEL patients were treated with intensive regimens. Moreover, the intensive treatment resulted in higher overall response rates (87.1%) than R-CHOP (76.2%) in patients with DEL. These might be the possible reasons for encouraging prognostic outcomes of intensive treatment that it achieved significant superiority in PFS and OS compared to R-CHOP for patients with DEL, especially those aged under 60, which is in line with a recent European retrospective study of 114 cases by Dodero et al. (2019) that DA-EPOCH-R can improve the PFS and OS of young patients with DEL(28).

Though we tried to address the bias by excluding all known DHL cases, considering the retrospective property and inevitable selection imbalance of this study, we further conducted the univariate and analysis for the DEL subset and identified the PFS and OS superiority of intensive regimen and normal LDH. The prognostic benefit of intensive therapies, especially DA-EPOCH-R, was mainly found in DEL patients aged under 60 years. Our study indeed has other disadvantages of limited follow-up time, small sample size, and unequal treatment assignment that intensive therapies were conducted more in patients aged under 60. We would continue our follow-up for long-time survival outcomes and expand the number of patients in future studies. Additionally, according to previous findings that 2-year PFS is highly consistent with long-time OS in DLBCL patients(29), the PFS superiority of intensive treatment in our study might analogize long-time OS superiority to some extent.

Interestingly significant prognostic superiority of intensive treatment was not observed in the

first Chinese cohort and some other unplanned subset analyses from trials, potentially due to their limited sample sizes and lower proportions of GCB subtype, which proved superior survival than non-GCB for patients with DLBCL(30). Additionally, given that their patients were mainly diagnosed before 2016, more application of advanced supportive treatment in recent years might significantly improve the safety and prognosis of intensive regimens in our study. Other possible reasons for the discrepancy in outcomes include selection bias caused by DHL, potentially different proportions of patients received radiotherapy or SCT. More importantly, different genetic subtype distribution between races, such as MCD, in Hesperian and Chinese(31, 32).

As for frontline SCT, it seemingly resulted in less prognostic benefits than intensive therapies for patients with DEL due to no significant comparisons, which may prove the opinion that DLBCL may be a “one-shot” disease. Kawashima (2018) retrospectively demonstrated the inferior prognosis of DEL compared to non-DEL, even after allogeneic hematopoietic cell transplantation. The definite benefits of SCT and intensive therapies for patients with DEL should be determined by larger randomized trials. Furthermore, due to the increased toxicity and treatment-related complications of intensive treatment, overall consideration of age, comorbidities, tolerance of the initial chemotherapy, performance status, and marrow function are necessary to make individualized therapy before conducting SCT or induction therapy. Adequate supportive treatment is a prerequisite for the safety of the intensive treatment. Although hematological toxicities were more common in patients with intensive treatment, it does not necessarily increase the incidence of neutropenic fever and treatment-related mortality, possibly due to more active application of prophylactic antibiotics and long-lasting G-CSF in patients with intensive regimens, which significantly reduced the duration of neutropenia.

Innovative strategies are urgently needed to improve DEL patients' outcomes. Especially, innovative targeted drugs lay the groundwork for future therapeutic strategies, such as ABT-199(33, 34), (a BCL2 inhibitor, showed high CR rate in patients with DEL after combination with CHOP-based regimens in phase I trial), lenalidomide (a targeted drug for drivers of MYC expression, demonstrated safety and feasibility of lenalidomide combined with DA-EPOCH-R in patients with DHL and DEL in phase I study)(35), KPT-330 (an XPO1 inhibitor, was confirmed the ability to decrease MYC protein expression for DHL tumor cells in vitro when combined with BCL2 inhibitor)(36) and several advanced immunotherapies, such as checkpoint inhibitors and cellular immunotherapies(37). The combination of new targeted drugs with R-CHOP regimen or intensive regimens may show survival superiority than conventional intensive treatment in the future and bring more treatment opportunities for patients with DEL. Though this is a promising therapeutic strategy, before there is sufficient medical evidence, we still recommend intensive chemotherapy for patients with DEL, especially for young patients.

Conclusions

In conclusion, clinical characteristics were comparable between DEL and non-del. The results of this study suggest that intensive immunochemotherapy may improve the prognosis of patients with double-expressor lymphoma aged under 60 years, which should be considered for future prospective clinical trials.

List of abbreviations

DEL: Double-expressor lymphoma; DLBCL: diffuse large B cell lymphoma; R-CHOP: rituximab,

cyclophosphamide, doxorubicin, vincristine, prednisone; PFS: progression-free survival; OS: overall survival; DHL: double-hit lymphoma; CNS: Central nervous system; IPI: International Prognostic Index; LDH: lactate dehydrogenase; β 2-MG: β 2-microglobulin; GCB: germinal center B-cell-like; PET: 18 F-fluorodeoxyglucose positron emission tomography; CT: computed tomography; DA-EPOCH-R: rituximab, etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin; R-HyperC-VAD/MA: rituximab, hyperfractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone, alternating with cytarabine plus methotrexate, R-DHAP rituximab, dexamethasone, cytarabine, and cisplatin; SCT stem cell transplantation; G-CSF: granulocyte colony-stimulating factor; CR: complete response; PR: partial response; PD: progressive disease; SD: stable disease; HR: hazard ratio; CI: confidence interval

Declarations

Ethics approval and consent to participate

All procedures conducted in studies involving human participants comply with the ethical standards of the institutional and national research committee, the 1964 Helsinki Declaration and subsequent amendments or similar ethical standards. The Ethics Committee of the Peking Union Medical College Hospital, Chinese Academy of Medical Sciences has approved this retrospective study, in which informed consent was waived, but patient confidentiality was protected.

Consent for publication

Consent was obtained from all cases included in the study.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

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Authors' contributions

JZ performed the analysis and wrote the manuscript. WZ conceived the study and edited the manuscript.

JZ, DZ, YZ, WW, and CW retrieved data and followed up the patients. All authors read and approved the final manuscript.

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Figures

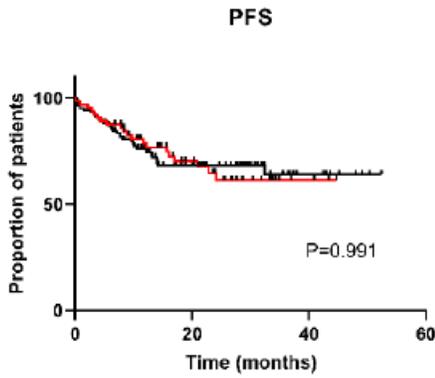


Fig. 1A

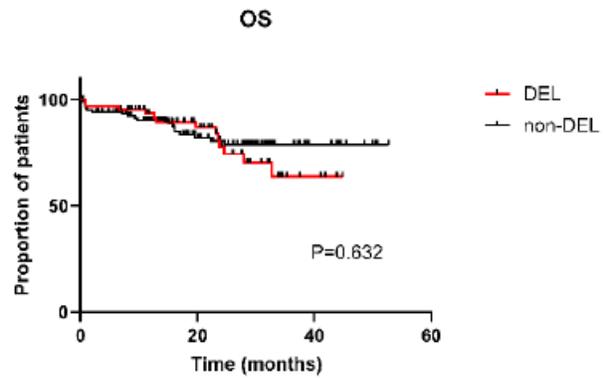


Fig. 1B

Figure 1

Survival outcomes by protein expression. (a) PFS of patients with DEL and non-DEL. (b) OS of patients with DEL and non-DEL.

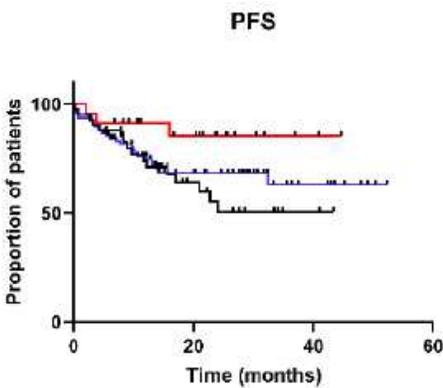


Fig. 2A

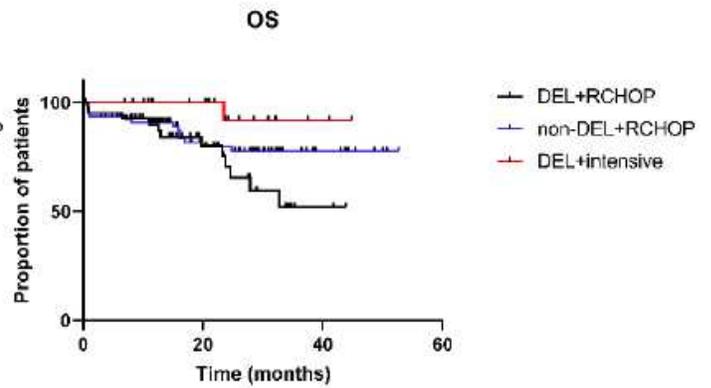


Fig. 2B

Figure 2

Subgroup survival outcomes by protein expression and therapies. (a) PFS of patients with DEL who treated with R-CHOP, with DEL who treated with intensive therapy and with non-DEL who treated with RCHOP. (b) OS of patients with DEL who treated with R-CHOP, with DEL who treated with intensive therapy and with non-DEL who treated with RCHOP