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Biweekly CHOP therapy improves therapeutic effect in the non-GCB subtype of diffuse large B-cell lymphoma

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Abstract: CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) is accepted as the best available standard treatment for diffuse large B-cell lymphoma (DLBCL) patients; however, the therapeutic efficacy seems unsatisfactory. Additional rituximab will improve the cure rate, but it is not popular in China because of its increased medical cost. Germinal center B-cell (GCB) and non-GCB subtypes distinction have been described as independent prognostic factors, and provides likelihood for cure with chemotherapy. The aim of the study is to explore the association between Immunophenotype and treatment regimen. Between August 2003 and May 2006, 66 patients with DLBCL were enrolled, according to immunohistochemistry results (GCB and non-GCB phenotype), randomly assigned to receive either six to eight cycles of CHOP every 2 weeks or standard CHOP every 3 weeks. After a median follow-up duration of 32 months (range of 4 to 42 months), an estimated 3-year overall survival (OS) rate for the GCB patients were 68.2% and 55.6% for the biweekly CHOP regimen and standard CHOP regimen respectively, while the data were 62.8% and 37.9% respectively for the non-GCB cases. The biweekly CHOP therapy showed higher efficacy than standard treatment, and its superiority was more obvious with the non-GCB subgroup.

Keywords: Diffuse large B-cell lymphoma (DLBCL); GCB; non-GCB; CHOP

1 Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common type of lymphoma in adults, accounting for about 30 − 40% of all cases. DLBCL can occur at any time between adoles-

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cence and old age. Early studies with the cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) regimen have showed that long-term remission could be achieved, but only 35 − 40% of patients are cured with this standard therapy. Today, the addition of the monoclonal antibody, rituximab, to CHOP has been shown to improve the outcome in both the elderly [1, 2] and younger patients [3], but its high price restricts its applications in China.

DLBCL can be divided into prognostically important subgroups with germinal center B-cell-like (GCB), activated B-cell-like (ABC), and type 3 gene expression profiles [4]. Bcl-6 and CD10 are markers of germinal center B-cells [7, 8]; MUM1 is expressed in plasma cells and the later stages of B-cell development [9], and it is associated with the ABC group in gene expression profiling studies. Hans *et al.* [4] using CD10, bcl-6, and MUM1 expression classify cases of DLBCL into GCB and non-GCB groups. The prediction for the GCB subgroup is better than that for the non-GCB group [5, 6]. It is thus important to select a proper curative regimen for these non-GCB group patients who are unlikely to be cured.

Except for rituximab, increasing the therapeutic intensity of CHOP is effective too; since therapeutic effect is associated with histotype, we speculate that the non-GCB subtype of DLBCL would preferentially benefit from dose escalation and interval shortening, while other subtypes have little if any benefit.

Here we report the results of a phase II "dose-densified" biweekly CHOP trial in newly diagnosed non-GCB subtype DLBCL patients, and G-CSF is added if necessary.

2 Statistical methods and Experimental Procedures

2.1 Eligibility criteria and staging

Between August 2003 and May 2006, 66 patients with DLBCL (from the Department of Medical Oncology, Tumor Hospital of Harbin Medical University, Harbin, China) were enrolled in this study.

The following criteria had to be fulfilled for inclusion into the study: 1) newly diagnosed patients with diffuse large B-cell lymphoma; 2) age 15 − 70 years; 3) performance status (PS) 0 − 3 according to the ECOG grades [10]; 4) no serious cardiac, renal, pulmonary and hepatic co-morbidity; 5) negativity of the serologic test for human immunodeficiency virus.

Pre-treatment staging procedures included physical examination, complete blood count with differential and platelet counts, biochemical analyses, electrocardiogram (ECG), computed tomography (CT) scan of the chest and abdomen, bilateral bone marrow aspiration and biopsy. The clinical stages accorded with the Ann Arbor staging classification [11], and the prognostic evaluation allowed by the score of the International Prognostic Index (IPI) [12] was considered for each patient. Written informed consent was obtained from all patients before randomization.

After randomization, 2 patients were excluded for combined leukemia. Thus, 64 pa-

tients were eligible for the study and all were evaluable for the end-points of the study. The details were seen in table 1.

Table 1 Clinical characteristics of the 64 eligible patients of the study, subdivided by treatment arm.

Characteristics	Bi-CHOP [n(%)]	S-CHOP [n(%)]	Total [n(%)]
Age (years)			
Median	46	44	45
Range	16–70	18-68	16-70
Gender			
Male	15(48)	16(48)	31(48)
Female	16(52)	17(52)	33(52)
Clinical Stage			
I	4(12)	6(18)	10(17)
II	7(23)	6(18)	13(20)
II	16(52)	17(52)	33(52)
IV	4(12)	4(12)	8(12)
Performance status			
ECOG			
0	19(61)	22(66)	41(64)
1	6(19)	5(15)	11(17)
2	4(12)	3(9)	7(11)
3	2(6)	3(9)	5(7)
IPI score			
0–1	16(52)	16(48)	32(50)
>1	15(48)	17(52)	32(50)
B symptoms	11(35)	13(39)	24(37)
Bone marrow involvement	2(6)	3(9)	5(7)
Immunohistochemistry			
GCB	11(35)	12(36)	23(36)
non-GCB	20(65)	21(64)	41(64)

2.2 Immunohistochemistry

The paraffin blocks of these 64 cases were from the Department of Pathology, Tumor Hospital of Harbin Medical University, Harbin, China.

Immunophenotyping was performed using a universal secondary antibody kit that used a peroxidase-conjugated labeled-dextran polymer (Zhongsan Biotechnology Limited Company, Beijing, China; Peroxidase DAB), with nonimmune serum added. The following commercially purchased primary antibodies were used: anti-Bcl-6, anti-CD10, anti-MUM1 (Zhongsan Biotechnology Limited Company, Beijing, China). They were washed three times with Phosphate Buffered Solution (PBS), and stained with standard H&E or hematoxylin counterstain for immunohistochemistry.

The cell of origin distinction was determined through the method used by Hans [4]. Immunohistochemistry results for CD10, bcl-6, and MUM1 were used to subclassify the

cases (Figure 1). Cases were assigned to the GCB group if CD10 alone was positive or if both bcl-6 and CD10 were positive. If both bcl-6 and CD10 were negative, the case was assigned to the non-GCB subgroup. The expression of MUM1 determined the group in the patients who were positive in bcl-6 and negative in CD10: the case was assigned to the GCB group if MUM1 was negative, or assigned to the non-GCB group if MUM1 was positive.

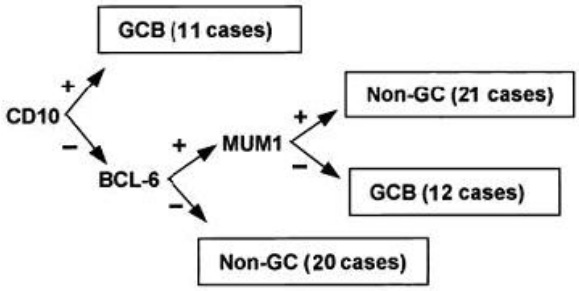

Fig. 1 Decision tree for immunohistochemistry classification of DLBCL (Hans *et al.* [1]).

The immunoperoxidase stained sections were examined by light microscopy. Additional controls omitted the primary antibody. A positive result was rendered if more than 15% of the large atypical cells were unequivocally stained in an expected staining pattern.

2.3 Treatments

Arm A, biweekly CHOP therapy: 31 patients with DLBCL were assigned to receive six to eight cycles of biweekly CHOP (CPA 750 mg/m^2, DOX 50 mg/m^2, VCR1.4 mg/m^2 and PSL 100 mg for 5 days) every 2 weeks.

Arm B, standard-dose CHOP therapy: 33 patients with DLBCL were assigned to receive six to eight cycles of standard-dose CHOP (CPA 750 mg/ m^2, DOX 50 mg/ m^2, VCR1.4 mg/ m^2 and PSL 100 mg for 5 days) every 3 weeks.

Patients received the CHOP regimen for a minimum of six cycles for the documentation of a complete response (CR); if it had not been achieved, another two cycles of therapy would be added for a total of eight cycles.

A combined therapy including ramosetron, dexamethasone and rhG-CSF was used, with ramosetron (5-hydroxytryptamine receptor antagonist, 0.3 mg) and dexamethasone (10 mg) prophylactically administered (one hour before chemotherapy). Among leukopenia patients, rhG-CSF (Jilifen, China) was hypodermically injected at a fixed dosage of 5 μg/kg everyday until the WBC (white blood count) recovered to $> 4.0 \times 10^9$/L (3 to 5 days).

2.4 Response and toxicity

Clinical response, if any, was assessed one month after the end of chemotherapy. Complete response (CR), complete response unconfirmed (CRu), partial response (PR), stable disease (SD) and progressive disease (PD) were evaluated according to the International Workshop criteria [13].

Physical examination, complete blood count, biochemical analyses and electrocardiogram (ECG) should have been evaluated every cycle. Toxicity was evaluated according to the standard ECOG grades [10].

2.5 Statistical considerations

Overall survival (OS) and progression-free survival (PFS) were estimated using the Kaplan-Meier product-limit method. OS was measured from the date of diagnosis to the date of death or the last follow-up visit, and PFS was calculated from the date of randomization until the date of progression. The response rate and toxicity incidence were compared for statistical differences by using Fisher's exact test, with $p < 0.05$ considered statistically significant.

3 Results

3.1 Clinical Findings

Of the 64 eligible patients, 33 were randomized for treatment with standard-dose CHOP and 31 for the biweekly CHOP therapy .Table 1 reports the main clinical and prognostic characteristics of the two groups of patients and demonstrates the good comparability of the two treated groups. The patients were comprised of 31 males and 33 females, with an age range of 16 to 70 years and a median age of 45 years. Twenty-four patients (34%) were in stage I or II, and stage III or IV was seen in forty patients (66%). Simultaneous nodal and extra-nodal involvement were found in eight patients (16%), which showed marrow and/or liver involvement.

3.2 Immunohistochemistry

The results of the immunohistochemical studies are summarized in Table 1 and Table 2. Of the total 64 cases, 23 (36%) were considered GCB and 41 (64%) were considered non-GCB. 11 (50%) of the 22 cases in clinical stage I or II were GCB, while only 12 (29%) of the 42 cases in clinical stage III or IV were GCB; this was statistically significant ($p < 0.05$).

Table 2 Patient characteristics and immunohistochemistry classification.

Characteristics	Total	GCB [n (%)]	non-GCB [n (%)]	p
Clinical stage				
I+ II	22	11 (50)	11 (50)	< 0.05
III+ IV	42	12 (29)	30 (71)	
B symptoms	24	6 (25)	18 (75)	< 0.05

3.3 Response and toxicity

A total of 60 cases completed the planned treatment, with 30 receiving the biweekly CHOP therapy and 30 receiving the standard-dose CHOP therapy. One patient in the biweekly CHOP arm and three patients in the standard-dose CHOP arm withdrew because of progressive disease. In the standard-dose CHOP arm, the CR rate including CRu was 50% among all randomized patients in the GCB group and 14% in the non-GCB group; in the biweekly CHOP arm, the rate was 55% and 50% for the GCB and non-GCB respectively. The clinical responses of all randomized and eligible patients are shown in Table 3 below.

Table 3 Clinical response to and evolution after biweekly CHOP or standard-dose CHOP.

Response	S-CHOP		Bi-CHOP	
	GCB	non-GCB	GCB	non-GCB
CR + CRu (n)	6	3	6	10
PR (n)	2	3	2	4
SD (n)	3	13	2	5
PD (n)	1*	2*	0	1*
NE (n)	0	0	1	0
CR + CRu rate (%)	50	14	55	50

CR: complete response. CRu: complete response unconfirmed. PR: partial response. SD: stable disease. PD: progressive disease. NE: not evaluable.
*One patient in the biweekly CHOP arm and three patients in the standard-dose CHOP arm were withdrawn because of progressive disease.

Toxicities were evaluated in 60 patients. Hematological toxicities are shown in Table 4. Grade 3 or 4 leukopenia was the major toxicity and occurred more frequently in the biweekly CHOP arm (19/30) than in the standard-dose CHOP arm (12/30). It occurred in 51.7% of all patients and 15 of them had infectious events; luckily, they were not serious and the duration was short, generally less than a week with the use of antibiotics and C-GSF. Similarly, grade 3 or 4 thrombocytopenia was observed more frequently in the biweekly CHOP arm than in the standard-dose CHOP arm. Anemia grades were similar between the biweekly CHOP arm and the standard-dose CHOP arm, and no grade 4 was found.

Non-hematological toxicities, including nausea, vomiting and diarrhea, were mild and similar between both arms.

Table 4 Patient characteristics and immunohistochemistry classification.

Hematological toxicities	Bi-CHOP Grade					S-CHOP Grade				
	0	1	2	3	4	0	1	2	3	4
Leukopenia	0	3	3	6	18	4	3	4	5	14
Thrombocytopenia	8	9	5	6	2#	15	6	7	1	1
Anemia	1	1	15	13	0	2	2	16	10	0

One patient was intravenously injected with thrombocytes.

3.4 Survival

After a median follow-up duration of 32 months (range of 4−42 months), 12 patients were dead (10 of them died within one year). The 1-year OS of the GCB patients were 90.9% and 90% for the biweekly CHOP regimen and standard CHOP regimen respectively, while the data were 83.3% and 76.2% respectively for the non-GCB cases. The estimated 3-year OS for the GCB patients were 68.2% and 55.6% for the biweekly CHOP regimen and standard CHOP regimen respectively, and 62.8% and 37.9% respectively for non-GCB cases (Figure 2).

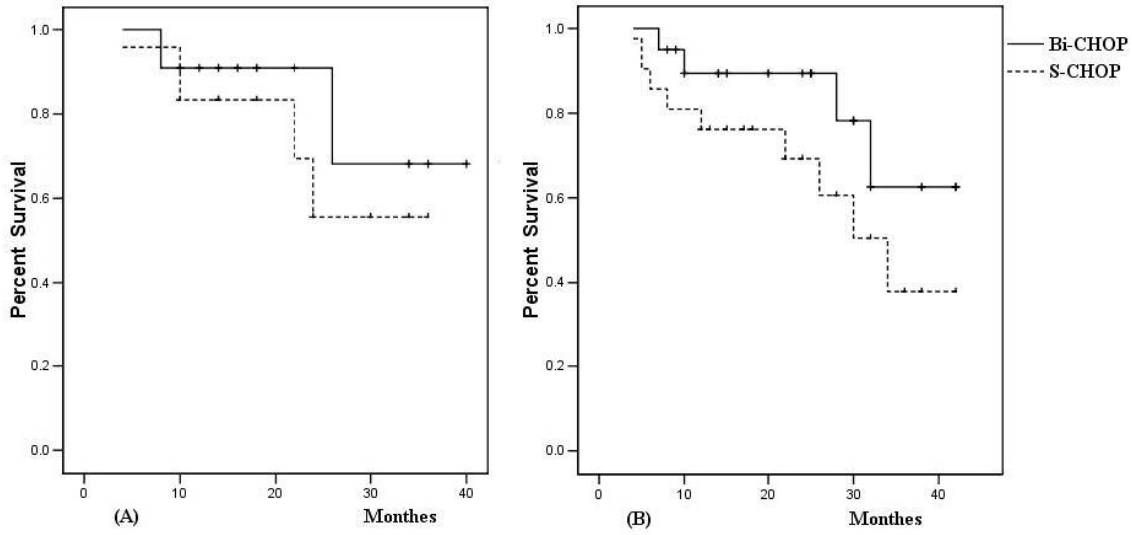

Fig. 2 Overall survival for 64 DLBCL based on cell origin.

4 Discussion

Diffuse large B-cell lymphoma (DLBCL) displays striking heterogeneity at the clinical, genetic, and molecular levels [14]. It is the type of aggressive intermediate- to high-grade non-Hidgkin's lymphoma. The CHOP regimen is still considered a very effective conventional treatment for aggressive lymphomas [15]. A cure is the overriding goal in the

treatment of DLBCL, because it is possible in a proportion of patients and because survival is typically short otherwise. Conventional therapy showed that long-term remission could be achieved, but the conditions are still unsatisfactory for more than half of the patients with DLBCL [16]. Today, the addition of the monoclonal antibody, rituximab, to CHOP (RCHOP) has been shown to improve the cure rate, but it was not popular in China due to its high price. For these people, it is more important to select an immediately applicable and proper approach to improve the cure rate and lower the unnecessary toxicity.

Increasing the doses of drug administration or shortening the schedules can both pursue a higher dose density. The favorable results of high-dose chemotherapy are supported by many [17, 18] but not all; some improved only partially [19–21] and produced multidrug resistance and severe toxicity. There was also some research about shortening the schedules of chemotherapy. The Japan Clinical Oncology Group [21] conducted an interesting randomized trial in aggressive lymphoma patients. Patients who were assigned standard CHOP every two weeks showed more benefits than those assigned dose-escalated CHOP every three weeks, with more hematological toxicity but lower response rate and progression-free survival. The Southwest Oncology Group (SWOG) [22] reported on a cohort of 88 intermediate- or high-grade lymphoma patients treated with a 2 week CHOP schedule; although the 2-year progression-free survival was lower than expected (51% versus 60%), the estimated 5-year overall survival was 14% better than that of patients treated with standard CHOP in an earlier SWOG study. In this trial a clear-cut intensification was reached by both escalating doses and shortening the interval between cycles. Another very instructive study was performed by the German High-Grade Non-Hodgkin's Lymphoma Study Group separately on patients younger than 60 [23] and on those older than 61 years of age [24]. In both trials, a biweekly regimen (CHOP 14) benefited more than a standard CHOP (CHOP 21) (53% versus 24%). In this study, the estimated 3-year OS of the biweekly CHOP arm was compared to the standard-dose CHOP arm in the GCB group (68.2% versus 55.6%, $P < 0.05$) and in the non-GCB group (62.8% versus 36.3%, $P < 0.05$). Compared with traditional standard chemotherapy, the biweekly CHOP regimen improved the curative effect, especially in the non-GCB group. Possible explanations of higher effectiveness coupled with shortening the schedules might be: (a) prolonged exposure time past a cytotoxic threshold; (b) penetration of sanctuary sites; (c) producing cytotoxicity through alternative mechanism, or d) inducement of drug-fast cells into the generation cycle. Though the hematological toxicity of leucocytopenia could be mitigated by G-CSF, proper selection of patients was important in order to gain effectiveness and avoid unnecessary adverse reactions.

Biological parameters, including expression of Bcl-6, Bcl-2, CD10, major histocompatibility complex class II, and categorization as germinal center (GC) type have been described as IPI-independent prognostic factors. In this study, we analyzed the expression of Bcl-6, CD10 and MUM1of 64 DLBCL patients. The immuno-phenotypic results of our study are suggestive of a non-germinal center stage of B-cell differentiation for most cases of DLBCL, and the non-GCB group is implied to have a more unfavorable prognosis than

the GCB group. This is in accordance with Daliu M et al [25]. Hence, poor prognostic factors can define a population at high risk for relapse following empirical chemotherapy. For these patients (the non-GCB group), we administrated the biweekly CHOP therapy instead of standard CHOP therapy because confers more advantages, including improved life quality, increased response rate and decreased medical costs. Based on the results of our clinical study, we would select more DLBCL patients for the biweekly CHOP therapy and conduct a long-term follow-up.

In summary, the biweekly CHOP therapy is a high performance, safe and economic regimen for DLBCL patients in China, especially for the non-GCB group that has the more unfavorable prognosis. Biologically directed therapy may be the future of DLBCL treatment.

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