Intravenous methotrexate at a dose of 1 g/m² incorporated into RCHOP prevented CNS relapse in high-risk DLBCL patients: A prospective, historic controlled study

To the Editor:

Central nervous system (CNS) relapse is a devastating event of diffuse large B cell lymphoma (DLBCL). It occurs in 4%-7% of DLBCL in general and the rate is considerably higher in high-risk patients, resulting in a poor outcome.1 Effective methods of CNS prophylaxis have not yet been developed. In the past, intrathecal (IT) therapy had been widely used but its role has been questioned nowadays.2 Systemic high-dose MTX (methotrexate, HD MTX) has been proven effective in several studies. With IV MTX at a dose of 3-3.5 g/m² is recommended and is often delivered on day 15 of RCHOP.3 This routine was efficacious but may delay or interrupt subsequent chemotherapy due to increased toxicity, especially for elderly patients. Although IV MTX could also be delivered every 2 to 3 weeks after the last R-CHOP course, it is better to adopt prophylactic measures early in the treatment course since CNS relapse mainly occurs within the first year (median: 6 months).2,4

We herein report the result of our prospective, historic controlled study demonstrating that intravenous MTX at 1 g/m² could significantly decrease the CNS relapse rate in high-risk DLBCL patients. As IV MTX was delivered during RCHOP, it was a convenient regimen which did not increase admission times.

From July 2015 to February 2017, 90 patients diagnosed of DLBCL with high CNS relapse risk were enrolled in our study and given IV MTX at a dose of 1 g/m² to prevent CNS recurrence. A historical group of 91 patients with comparable age, sex, stage, ECOG and IPI who only received IT prophylaxis were used as controls. All these patients were similar in the following aspects: (a) age ≥ 18 years; (b) with high CNS risk, which was defined as involvement of more than one extranodal site, or involvement of particular extranodal sites such as bone marrow, breasts, testes, paranasal sinuses, epidural space, adrenal glands, kidney and female genital system; (c) first-line treatment with RCHOP; (d) complete staging work-up; and (e) absence of CNS involvement at presentation, confirmed by cerebral spinal fluid (CSF) examinations and MRI. Patients with primary mediastinal lymphoma, primary CNS lymphoma, intravascular large B-cell lymphoma, plasmablastic lymphoma, DLBCL leg-type, Burkitt lymphoma, gray zone or high-grade lymphomas with or without double/triple hit were excluded. Informed consent was obtained at the time of clinical trial enrolment in accordance with the Declaration of Helsinki.

Note, CNS relapse was confirmed by positive CSF conventional cytology, CSF flow cytometry, or biopsy. For those who had clinical symptoms indicating a CNS involvement and typical lesions on MRI, we also considered a recurrence.

In the intravenous prophylaxis group (study group), at least 1 course of IV MTX 1 g/m² was given in concurrence with the RCHOP regimen. Rituximab was given on day 1, IV MTX on day 2 over 4 hours, and CHOP on day 3-7. Before IV MTX, rigorous hydration and urine alkylation was given. Methotrexate was rescued by leucovorin 24 hours after the infusion started. At the same time, the CSF concentration of MTX was tested in 33 patients. Patients in the study group also received IT cytarabine and dexamethasone, while patients in the control group (historic group) only received IT prophylaxis.

The characteristics of these patients were displayed in supplementary data (Table S1). More patients in study group were in advanced stage (91.1% vs 76.9%, P = .014), with more than one extranodal site involvement (62.2% vs 44.0%, P = .017), and with kidney involvement (12.2% vs 3.3%, P = .023). There were more patients older than 60 years in the historic group (46.2% vs 31.1%, P = .047). Other clinical variables were comparable between two groups.

All the patients were given RCHOP as initial therapy. Second line chemotherapy was chosen according to doctor’s preference if disease progressed. Those who were under 65 years old, had an age-adjusted IPI > 1, and received complete remission during interim evaluation, underwent autologous hematopoietic stem cell transplantation (Auto-HSCT) as consolidation. Details of treatment were shown in Table S2. 83 (91.2%) patients in the historic group had at least four courses of RCHOP while 86 (95.6%) patients in study group, with no significant difference found between them (P = .24). The proportion of patients underwent Auto-HSCT was also comparable, with 15 (16.5%) in the historic group and 20 (22.2%) in the study group (P = .352). There was no difference found in the course of IT prophylaxis between two groups (P = .961). In the study group, a median of four courses (range: 1-6) of intravenous MTX were given to the patients. We planned to give at least two courses of IV MTX in concurrence with two serial RCHOP from the second cycle, five(6%) patients only received one time of IV MTX because of mucositis (grade 3).

Since the observation time was longer in the historic group (median 37 months, range 5-84 months) than the study group (median 27 months, range 5-64 months), we compared the 2-year CNS relapse rate, 2-year PFS and OS between them. The 2-year CNS relapse rate was 12.1% in the historic group and 1.1% in the study group (P = .003, Figure 1A). The relapse patterns were summarized in
Table S3. The 2-year PFS and 2-year OS of the historic group was 58% and 82% separately, while it was 74% and 87% in the study group. There was a significant difference in the 2-year PFS (P = .028, Figure 1B) between the two groups, but wasn’t in the 2-year OS (P = .300, Figure 1C).

We also performed a multivariate logistic regression test for CNS relapse, which included prophylaxis strategies, CNS risk factors and age. The results indicated IV MTX was the only variable that affected CNS relapse (Table S4). The odds ratio (OR) was 14.675 (95% CI 1.804-119.359, P = .012). For 2-year PFS, multivariate COX regression was done and the results demonstrated that treatment (HR 2.148, 95% CI 1.257-3.670, P = .005), IPI≥4 (HR 0.398, 95% CI 0.173-0.919, P = .031) and stage III-IV (HR 0.329, 95% CI 0.112-0.962, P = .042) predicted disease progression (Table S5).

We examined the CSF concentration of MTX 24 hours after infusion started in 33 patients. The median was 0.34 μmol/L (range: 0.04-1.6).

After hydration and leucovorin rescue, the concentration of MTX could fall below 0.1 μmol/L in all cases 48 hours after infusion. A total of 332 courses of IV MTX were given to 90 patients. The most common side effects were mucositis, nephrotoxicity and hepatotoxicity. There were 38 (11.4%) cases of mucositis, with 32 (9.6%) in grade 1 and 6 (1.8%) in grade 3. Acute kidney injury happened in 16 (4.8%) cases, with only 1 in grade 2, others were all grade 1. All these adverse events were expected and resolved quickly.

The present study evaluated the effects of systemic treatment with intravenous MTX on CNS relapse and survival in high risk DLBCL patients in a real world setting. In the past, we used IT cytarabine and dexamethasone to prevent CNS relapse. But it turned out to be ineffective in IT prophylaxis and compared it to our historic population. As the immunomotherapy and IT treatment were comparable in two groups, we draw our conclusion that intravenous methotrexate at an intermediate dose of 1 g/m² incorporated to standard R-CHOP regimen is associated with decreased 2-year CNS recurrence risk (1.1% vs 12.1%, P = .003). As MTX was delivered on day 2 of R-MTX-CHOP therapy, this routine was convenient and did not increase the admission times, which was important for our patients because most of them had to fly from their hometown far away to our hospital for each cycle of treatment. Our 2-year PFS of 74% is also encouraging in a cohort of patients with a preponderance of high-risk features for systemic disease recurrence, including advanced stages (stage III-IV in 91.1%), high risk IPI scores (IPI ≥ 4 in 37.8% of patients) and poor clinical performance (ECOG ≥ 2 in 46.7%).

Our result was controversial to the main stream opinion that systemic methotrexate lower than 3 g/m² was unable to achieve adequate therapeutic levels in the CSF to yield significant benefit. Those conclusions, however, were mainly drawn from early studies in the 1990s, in which they did not find any significant difference in CNS relapse rate between chemotherapy with or without intermediate IV MTX, like the M-BACOS regimen for instance, which contained 1 g/m² IV MTX.8 But we should notice that in the 1990s, the risk factors predicting CNS recurrence were not well defined yet. Patients in those studies were DLBCL in general and the percentage of high risk patients was not clear, so it was hard to get a significant difference between prophylaxis therapies, given the CNS relapse rate in the general population was low.8 Another point against our result is that CSF MTX level should be more than 1 μmol/L, as the CSF concentration in our patients did not meet this threshold. But the therapeutic concentration of CSF MTX came from experiences of prophylaxis in acute lymphocytic leukemia.9 The relapse patterns of these two diseases are quite different. As in ALL, meningeal is the site where relapse mainly occurs, while in DLBCL CNS involvement mostly happens in parenchymal. The concentration of MTX in the CSF could not represent the efficiency of this medicine to prevent parenchymal involvement.

Prevention strategies of CNS relapse also learn a lot from the treatment of primary central nervous system (CNS) lymphoma, in which significant advances have been made nowadays. Novel insights into the pathophysiology of PCNSL led to introduction of targeted agents such as BTK inhibitor and immunomodulatory drugs into the salvage setting. In the recent study of Ayed et al., they analyzed records for patients with DLBCL treated with R2CHOP as frontline therapy in two clinical trials (NCT00670358, NCT00907348), and found the estimated 2-year CNS relapse rates by Kaplan-Meier method are 0.9% for the entire R2CHOP cohort and 5.0% for the high CNS-IPI risk group. This indicates incorporation of lenalidomide into

![Figure 1](image-url)  Kaplan-Meier curves for 2-year CNS relapse-free survival (A; P = .003), 2-year progression-free survival (B; F = 0.028) and 2-year overall survival (C; P = .30) for study (black line; n = 90) vs historic (red line; n = 91) group.
RCHOP is associated with a lower-than-expected rate of CNS relapse. Limited by the small number of patients (only 25 CNS-IPI high risk patients), this conclusion needs further confirmation. We do agree that novel agents such as lenolidomide, ibrutinib and venetoclax might be effective in CNS prophylaxis, especially in elderly patients who could not tolerate IV MTX. But considering the cost of these new agents, IV MTX would still be the standard treatment in China.

In conclusion, our prospective, historic controlled study in DLBCL patients with high CNS risk provided an effective, convenient, and safe regimen to prevent CNS relapse. The better 2-year PFS also indicated this is a promising strategy. Further study with more patients should be taken to validate our exploration.

CONFLICT OF INTEREST
The authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS
W.Z. and D.B.Z. designed experiments, W.W. analyzed all the data, and wrote the first draft of the manuscript, Y.Z., L.Z., C.Y., J.F., H.C.C., M.C., X.X.C., J.L., provided the data, W.Z., and D.B. Z., revised the final draft. All authors reviewed and edited the final manuscript before submission.

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REFERENCES

SUPPORTING INFORMATION
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