Efficacy of intravenous high-dose methotrexate in preventing relapse to the central nervous system in R-CHOP(-like)-treated, high-risk, diffuse large B-cell lymphoma patients and its effect on mortality: a systematic review and meta-analysis

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Abstract

Central nervous system (CNS) relapse in patients with diffuse large B-cell lymphoma (DLBCL) carries a dismal prognosis and most clinical guidelines recommend CNS prophylaxis to patients deemed at high risk of CNS relapse. However, results from observational studies investigating the effect of CNS prophylaxis have yielded conflicting results. The aims of this study were to evaluate: (i) whether addition of prophylactic intravenous high-dose methotrexate (HD-MTX) reduces the risk of CNS relapse in high-risk DLBCL patients treated with R-CHOP or similar, and (ii) whether HD-MTX prophylaxis confers an overall survival benefit, irrespective of CNS relapse. We performed a systematic search of MEDLINE/PubMed and EMBASE for data on DLBCL patients at high risk of CNS relapse treated with R-CHOP or similar who received HD-MTX as an intervention and a comparator arm of patients who did not receive prophylaxis and/or intrathecal prophylaxis. A risk of bias was estimated using the ROBINS-I tool and the quality of the evidence was assessed by the GRADE approach. Finally, a meta-analysis based on the systematic review was conducted. A total of 1,812 studies were screened. No randomized controlled trials were identified. Seven observational studies comprising 1,661 patients met the inclusion criteria. We found a statistically non-significant relative risk of 0.54 (95% confidence interval: 0.27-1.07) of CNS relapse for patients receiving HD-MTX *versus* controls. The meta-analysis investigating mortality demonstrated a relative risk of death of 0.70 (95% confidence interval: 0.44-1.11) for patients treated with HD-MTX *versus* controls. The overall risk of bias was adjudged as “serious” and the quality of the evidence was rated as “low”. In conclusion, our data indicate that HD-MTX does not prevent or, at best, only slightly reduces the risk of CNS relapse and confers no survival benefit.

Introduction

Relapse in the central nervous system (CNS) is a rare, but serious, event in patients with diffuse large B-cell lymphoma (DLBCL). In the post-rituximab era, CNS relapse has been reported in 3-5% of DLBCL patients following first-line treatment.[1,2] In the majority, the relapse is diagnosed within the first year, suggesting that some patients harbor subclinical CNS disease at diagnosis.[1,3,4] The prognosis is extremely poor with a median overall survival after CNS relapse of only a few months.[3,4] Thus, improvement in prediction of CNS relapse and subsequent administration of effective CNS prophylaxis are critical.

In an effort to reduce the risk of CNS relapse, clinical guidelines have recommended CNS prophylaxis to high-risk patients.[5-7] Historically, intrathecal (IT) chemotherapy has been employed. However, increasing evidence has challenged the benefit of IT prophylaxis.[8] In recent years, intravenous high-dose methotrexate (HD-MTX) has been the most commonly recommended prophylactic strategy, both in clinical studies and treatment guidelines.[5-7,9,10] The toxicity of HD-MTX is considerable and may be a limiting

factor for administration to patients of advanced age or with comorbidities, especially impaired renal function.[10-12] Furthermore, administration of prophylaxis may derail primary treatment, thereby risking a worse outcome.[13]

No randomized controlled trial investigating the efficacy of CNS prophylaxis in addition to standard treatment has ever been performed. Thus, current recommendations are based on retrospective studies reporting a potential benefit of HD-MTX in the prevention of CNS relapse. However, several studies have found diverging results and in recent years, large retrospective studies have failed to demonstrate a significantly lower rate of CNS relapse after HD-MTX prophylaxis.[10,14,15] Retrospective studies are hampered by numerous limitations. The definitions of patients at high risk of CNS relapse differ and the delivery of HD-MTX (timing, dose, and number of cycles) and combination with IT prophylaxis also vary between studies. The chemo-immunotherapy regimens employed as the antilymphoma treatment backbone also differ; some of these regimens may inherently reduce the risk of CNS relapse[16] and thereby obscure the efficacy of HD-MTX alone. Finally, there is likely treatment selection bias, since younger patients with good performance status are more likely to receive CNS prophylaxis than are older or unfit patients.

Two meta-analyses[17,18] and a network meta-analysis[19] concerning CNS prophylaxis have recently been published with diverging conclusions. They are affected by the innate limitations of the retrospective studies included, cohort overlap (effectively counting some patients more than once), and patients receiving multiple types of both CNS prophylaxis and chemo-immunotherapy regimens, making interpretation of the results difficult.

The primary objective of the present study was to elucidate whether addition of prophylactic intravenous HD-MTX reduces the risk of CNS relapse in DLBCL patients treated with R-CHOP (rituximab plus cyclophosphamide, doxorubicin, vincristine, prednisone) or similar and considered at high risk of subsequent CNS relapse. The secondary objective was to investigate whether HD-MTX prophylaxis confers a reduced mortality risk irrespective of CNS relapse. Our approach differs from that of other meta-analyses in the field in important ways. Only patients treated with rituximab in combination with CHOP or similar, and only those considered at high risk of CNS relapse by the respective authors, were included (for a listing of high-risk criteria for each study, see Table 1 and the Methods section). Attempts were made to eliminate cohort overlap by including only one publication per cohort. Patients in the interventional arm were required to have received HD-MTX while those who received only IT prophylaxis were

Table 1. High-risk classification and criteria for central nervous system prophylaxis.

Study	High-risk sites	High-risk molecular subtypes	Criteria for prophylaxis
Cheah *et al.*[29]	Bone marrow, breast, testis, kidney, adrenal gland, paranasal sinus, nasopharynx, liver or paravertebral site	Not included	Two or more of the following: multiple extranodal sites, elevated LDH, or B-symptoms. In addition, involvement of high-risk sites
Ferreri *et al.*[33]	Testis, spine, skull, paranasal sinus, orbit, nasopharynx, kidney, adrenal gland, and/or breast	Not included	Involvement of high-risk sites or presence of both advanced stage and elevated LDH
Eyre *et al.*[32]	Not included	Not included	Physician preference
Bobillo *et al.*[12]	Testis, breast, kidney, adrenal gland, and/or bone marrow	Concurrent *MYC* and *BCL2* rearrangement	CNS-IPI 4-6 or involvement of high-risk sites or presence of high-risk molecular subtypes
Jeong *et al.*[30]	Kidney, adrenal gland, testis, breast, epidural space, or paranasal sinus	Co-expression of MYC and BCL2 (immunohistochemical analysis), or concurrent *MYC* and *BCL2* and/or *BCL6* rearrangements (fluorescence *in situ* hybridization)	CNS-IPI 4-6 or involvement of high-risk sites or >1 extranodal site and elevated LDH level or HIV$^+$ lymphoma or presence of high-risk molecular subtypes (MYC and BCL-2 double expressor only if IPI score ≥2)
Ong *et al.*[11]	Breast, testis, kidney or adrenal gland	Not included	CNS-IPI 4-6 or involvement of high-risk sites
Puckrin *et al.*[31]	Testicular involvement	From 2015: double-hit lymphoma	2012-2014: elevated LDH, ECOG >1, and >1 extranodal site or testicular involvement. 2015-2019: CNS-IPI score 4-6, double- hit lymphoma or testicular involvement

LDH: lactate dehydrogenase; CNS: central nervous system; IPI: International Prognostic Index; HIV: human immunodeficiency virus; ECOG: Eastern Cooperative Oncology Group performance status.

counted as controls. In an effort to maximize the number of patients available for analysis, authors of publications describing studies potentially able to meet eligibility criteria were contacted for supplementary data (see *Online Supplementary Table S5* and the Methods section for details on these requests).

Methods

A PROSPERO (International Prospective Register of Systematic Reviews) protocol (*CRD42022313841*) was submitted prior to commencing the review. The systematic review is reported in accordance with PRISMA (Preferred Reporting Items for Systemic Reviews and Meta-Analyses) guidelines.[20]

Eligibility criteria

Studies conducted on patients with DLBCL ≥18 years of age, treated with first-line R-CHOP or R-CHOP-like regimens, and considered at high risk of CNS relapse were included. Intervention groups included patients who received intravenous HD-MTX with or without IT prophylaxis while control groups consisted of patients receiving either no CNS prophylaxis or only IT prophylaxis. High-risk criteria of the studies included in this meta-analysis are listed in Table 1. If high-risk criteria were not explicitly listed in the study, administration of IT prophylaxis in the control group served as a proxy for high-risk estimation.

Studies of primary CNS lymphoma, CNS involvement at primary diagnosis, unknown primary chemotherapeutic treatment or without administration of rituximab, IT prophylaxis only, no comparator arm and with fewer than ten patients in the intervention group were excluded.

For studies fulfilling all but one eligibility criterion, corresponding authors were contacted for supplementary data. If they could not provide data, the study was not included. Requests for supplementary information are summarized in *Online Supplementary Table S5*.

Search strategy

MEDLINE/PubMed and EMBASE were searched until March 1, 2023. The search strategy and PICO (Population, Intervention, Comparison, Outcome) terms of the study are depicted in *Online Supplementary Tables S1* and *S2*, respectively. An additional manual search of references from included publications was conducted.

Selection process and data collection

Study selection and data extraction were conducted independently by two authors (ERT and THN). Search results were uploaded to the Covidence platform[21] and duplicates were removed. The title and abstract were screened, followed by full text screening. In cases of cohort overlap, studies published in peer-reviewed journals were preferred over abstracts and larger studies over smaller studies. In three of 13 cases, corresponding authors were able to provide relevant supplementary data (*Online Supplementary Table S5*). Details of data collection are provided in *Online Supplementary Table S1*.

Synthesis methods

The baseline characteristics of the studies were summarized using descriptive statistics. Time-to-event analyses were conducted using risk ratios (RR) as measures of effect, with a RR below 1 indicating a beneficial effect of HD-MTX. The Mantel-Haenszel inverse method was applied calculating pooled RR for all-cause mortality. We used a random-effects model due to an anticipated significant degree of statistical heterogeneity. Results are reported with 95% confidence intervals (95% CI) and double-sided *P* values and presented in Forest plots. I^2 statistics was used to differentiate to what extent the effect measured was due to chance *versus* heterogeneity. Supplementary estimates of heterogeneity were done by evaluating confidence interval overlaps visualized in the Forest plots. Two sensitivity analyses were conducted on the primary outcome: one including only studies using the CNS International Prognostic Index (IPI) score and one excluding studies that had patients treated with IT prophylaxis as controls. The Meta program in R statistics was applied for the data calculations.[22] Survival data were converted to mortality data using the formula: mortality = (1-survival).

Risk of bias assessment and certainty of evidence

Risk of bias was assessed by ROBINS-I (Risk Of Bias In Non-Randomized Studies - of Interventions) (2016).[23] All seven domains were assessed independently by the reviewers THN and ERT and disagreements were resolved by consensus. The quality of the body of evidence was estimated using the GRADE (Grading of Recommendations Assessment, Development and Evaluation) approach.[24]

Results

Study selection

The data search identified 1,812 studies, of which 326 titles were identified as duplicates by the Covidence software. Screening at title and abstract levels was performed on 1,486 studies and a secondary, full-text screening was performed on 101 studies. Ultimately, seven studies met the inclusion criteria (Figure 1). Among the 94 excluded studies, the main reasons for exclusion were “outcome of interest not being reported” (n=27), “cohort overlap” (n=23), or “wrong route of administration” (n=11). No automation tool was used in the exclusion process. Several studies were excluded due to prophylaxis not being HD-MTX alone,[1,10,25] the cohort overlapping with included studies,[14,26,27] or the patients not being risk stratified.[28]

Study characteristics

The baseline characteristics of the studies included are summarized in Table 2. For the studies by Cheah,[29] Jeong,[30] and Bobillo,[12] the authors provided supplementary data not published in the original report (see *Online Supplementary Table S5*). In the studies by Cheah[29] and Eyre,[32] data from the control groups are from patients who all received IT prophylaxis and in the study by Bobillo,[12] data from patients receiving IT prophylaxis and "no prophylaxis" were pooled as a joint control group. The total study cohort consisted of 1,661 patients across seven studies. All included patients received R-CHOP or similar regimens as first-line treatment. A CNS diagnostic work-up was listed and conducted to some extent in five studies: Cheah[29] and Ferreri[33] performed magnetic resonance imaging of the CNS and assessment of cerebrospinal fluid on all, or almost all, high-risk patients. In the studies by Ong[11] and Eyre,[32] only patients with clinically suspected CNS involvement were examined and in the study by Puckrin,[31] CNS examination of high-risk patients was recommended but not specified.

Criteria for adding CNS prophylaxis to first-line treatment varied between the included studies as outlined in Table 1. Risk stratification according to the CNS-IPI was employed in the studies by Ong,[11] Bobillo,[12] and Jeong[30] and in a subgroup of the patients in the study by Puckrin.[31] Studies by Cheah[29] and Ferreri,[33] conducted before the publication of the CNS-IPI in 2016, utilized adjusted combinations of CNS-IPI risk factors (e.g., advanced stage and lactate dehydrogenase, or lactate dehydrogenase and >1 extranodal site). Bobillo,[12] Jeong,[30] and Puckrin[31] included molecular data on co-expression of MYC and BCL-2 (identified by the use of immunohistochemistry) while Jeong[30] also included double-hit/triple-hit status (identified by fluorescence *in situ* hybridization) (Table 1).

All studies assessed the risk of CNS relapse and the indication for CNS prophylaxis based on the location of extranodal manifestations. High-risk characteristics were not described by Eyre,[32] but the control group consisted of patients all receiving IT prophylaxis.

The HD-MTX dose varied between 1-3.5 g/m^2 with the majority receiving 3-3.5 g/m^2. All patients received at least one cycle of HD-MTX. The number of HD-MTX cycles, dose of HD-MTX, and dose adjustments are shown in Table 3.

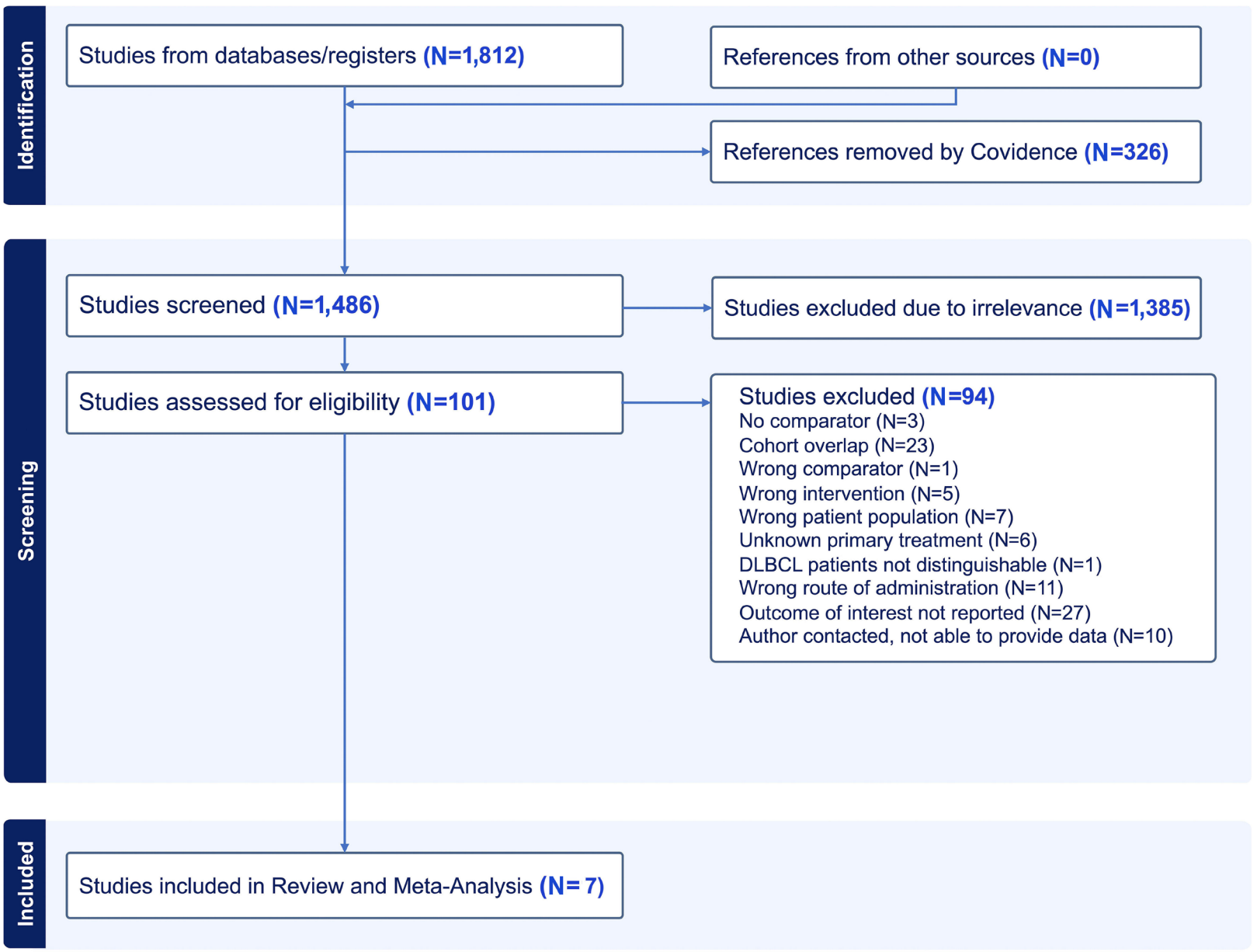


Figure 1. PRISMA flowchart of the study selection. PRISMA: Preferred Reporting Items for Systemic Reviews and Meta-Analyses; DLBCL: diffuse large B-cell lymphoma.

Risk of bias

Risk of bias was assessed using the ROBINS-I tool and is summarized in *Online Supplementary Table S3*. All studies were assessed to harbor a "serious" risk of bias due to confounding as none of the studies included information on comorbidity as a factor in the decision to offer CNS prophylaxis. Furthermore, all studies carried a "serious" risk of bias in their classification of intervention. As the prophylaxis ultimately was given on the basis of each physician's preference, the "criteria for considering individuals to have received each intervention" were not "clear and explicit".[23] All other categories were estimated to be associated with a "low" or "moderate" risk of bias.

The overall quality of the body of evidence was evaluated using the GRADE approach. As the risk of bias was assessed by the ROBINS-I tool, the body of evidence from the studies was initially categorized as "high".[34] However, we had to downgrade due to low ratings in "risk of bias", "inconsistency" and "imprecision". Thus the "Overall certainty of evidence" is categorized as "low" (*Online Supplementary Table S4*).

Results of individual studies

Cheah[29] and Ferreri[33] reported a statistically significant effect of HD-MTX in terms of reduction of the risk of CNS relapse (Table 4). Cheah[29] provided supplementary data on patients receiving rituximab. The study by Ong[11] showed a significantly reduced risk of CNS relapse when HD-MTX prophylaxis was added. However, when performing a multivariate analysis, the benefit was only maintained in patients with isolated CNS relapse, and not in patients with concomitant CNS and

Table 2. Baseline characteristics.

Study Country	Year	Journal	Design	N of DLBCL patients	Median age in years	Male sex %	First-line therapy	CNS-specific diagnostic work-up (pre-therapy)
Cheah *et al.*[29] Australia	2014	British Journal of Cancer	Retrospective cohort, multicenter	132**, ***	IT: 54.5 MTX: 63	66	R-CHOP	CSF analysis (cytology or flow cytometry) performed in 84%. MRI of the brain performed if CNS involvement was clinically suspected
Ferreri *et al.*[33] Italy	2014	British Journal of Haematology	Retrospective cohort, mono-institutional	107	66	50	R-CHOP or R-CHOP-like	Examination of CSF (biochemistry, cytology and flow cytometry) and whole-brain MRI in patients with increased risk of CNS involvement
Eyre *et al.*[32] UK	2019	British Journal of Haematology	Retrospective cohort, multicenter	130***	77.2	51	R-CHOP	Performed in patients with clinically suspected CNS involvement
Bobillo *et al.*[12] USA	2021	Blood	Retrospective cohort, mono-institutional	585	68	51	R-CHOP or R-CHOP-like	NP
Jeong *et al.*[30] South Korea	2021	Blood Advances	Retrospective cohort, ITT design, mono-institutional	244*	62	57	R-CHOP	NP
Ong *et al.*[11] Singapore	2021	Blood Cancer Journal	Retrospective cohort, multicenter	226	65 (mean)	53	R-CHOP	Performed in patients with neurological symptoms
Puckrin *et al.*[31] Canada	2021	American Journal of Hematology	Retrospective cohort, multicenter	237**	63	NP	R-CHOP or R-CHOP like regimens	Examination of CSF and MRI recommended in patients with neurological symptoms, involvement of high-risk sites, or combined elevated LDH, ECOG >1, and >1 extranodal site

*Supplementary data on patients receiving high-dose methotrexate as intended in the intervention group. Remaining data are from the entire cohort. **Supplementary data on patients only receiving R-CHOP therapy or similar. Remaining data are from the entire cohort. *** Data extracted on patients receiving high-dose methotrexate *versus* intrathecal prophylaxis. DLBCL: diffuse large B-cell lymphoma; CNS: central nervous system; IT: intrathecal; MTX: methotrexate; R-CHOP: rituximab plus cyclophosphamide, doxorubicin, vincristine, prednisone; CSF: cerebrospinal fluid; MRI: magnetic resonance imaging; NP: not provided; ITT: intention to treat; LDH: lactate dehydrogenase; ECOG: Eastern Cooperative Oncology Group performance status.

systemic relapse.

The four remaining studies by Bobillo,[12] Jeong,[30] Puckrin,[31] and Eyre[32] did not find that addition of HD-MTX reduced the risk of CNS relapse. In the study by Eyre,[32] the comparator included patients receiving IT prophylaxis only, in the study by Bobillo,[12] 253 of 543 patients received IT prophylaxis, and in the remaining studies by Jeong[30] and Puckrin,[31] the distribution of additional prophylaxis was not described. Supplementary data were received from Jeong[30] on a subgroup in which intention to treat and actual treatment were aligned and from Puckrin[31] where the subgroup treated with autologous stem cell transplantation was removed.

Survival was reported in five out of the seven studies (Table 4). Data from Cheah[29] and Ferreri[33] concluded that addition of HD-MTX was associated with a significant improvement in overall survival. The studies documented 5-year survival rates of 78% and 87%, respectively, among the HD-MTX-treated patients *versus* 50% and 54% among patients receiving no prophylaxis or IT prophylaxis. In contrast, Ong,[11] Jeong,[30] and Puckrin[31] did not find a survival benefit. Ong[11] reported a 3-year survival rate of 69.1% for patients receiving HD-MTX and 63.2% for controls (P=0.07) and Jeong[30] and Puckrin[31] provided supplementary data demonstrating similar 5-year survival rates in the HD-MTX-treated patients and controls (69.2% *vs*. 61.9% and 50% *vs*. 60%, respectively).

Results of synthesis

Central nervous system relapse

HD-MTX (± IT prophylaxis) was administered to a total of 452 patients. The control group consisted of 1,209 patients either given no prophylaxis or given IT prophylaxis alone (Figure 2). A total of 38 (8.4%) relapses occurred in the

Table 3. Administration of high-dose methotrexate (and intrathecal) prophylaxis.

Study	HD-MTX dose	Number of HD-MTX cycles	Dosage adjustments of HD-MTX	Timing of HD-MTX N (%)	Additional IT prophylaxis
Cheah *et al.*[29]	Median: NP Range: NP According to the methods section, each dose administered was 1-3 g/m^2	Median: NP Range: NP 2 cycles in 80% of patients, 1 cycle in 20% of patients.	Second cycle dose reduction/exclusion in 26.6% due to delayed clearance or toxicity, mainly renal	Intercalated: 0 (0) EOT: 122 (100)	HD-MTX: 99/122 Controls: 10/10
Ferreri *et al.*[33]	Median: NP Range: NP Dose: 3 g/m^2	Median: NP Range: NP 3-4 cycles	No cases of dose reduction	Intercalated: 0 (0) EOT: 33 (100)	HD-MTX: 10/23 Controls: 7/74
Eyre *et al.*[32]	Median: 3 g/m^2 Range: 1-3.5 g/m^2	Median: NP Range: NP Number of cycles: 63 in 31 patients (calculated mean: 2.0)	NP	NP	HD-MTX: 17/31 Controls: 99/99
Bobillo *et al.*[12]	Median: 3.5 g/m^2 Range: 2–3.5 g/m^2	Median: 2 cycles Range: 1-6	6 patients (14%) did not receive intended number of cycles due to renal toxicity	Intercalated: 19 (45) EOT: 23 (55)	HD-MTX: 11/42 Controls: 253/543
Jeong *et al.*[30]	Median cumulative dose: 7 g/m^2 Range: 1.5-17.5 g/m^2 According to the methods section, each dose administered was 3-3.5 g/m^2	Median: NP Range: NP 2-3 cycles	NP	Intercalated: 69 (61) EOT: 45 (39)	NP
Ong *et al.*[11]	Median: NP Range: NP Minimum dose: 1 g/m^2 81% received ≥3 g/m^2	Median: 2 cycles Range: 1-6	NP	Intercalated: 52 (79) EOT: 14 (21)	Yes, but not otherwise specified
Puckrin *et al.*[31]	Median: NP Range: NP Minimum dose ≥3 g/m^2 in 98.6%	Median: 2 cycles Range: 1-3	12 patients (10%) received only one dose of HD-MTX due to slow clearance or toxicity	Intercalated: 109 (94.8) EOT: 6 (5.2)	NP

HD-MTX: high-dose methotrexate; IT: intrathecal; NP: not provided; EOT: end of (R-CHOP) treatment.

HD-MTX group *versus* 125 (10.3%) in the control group. The meta-analysis found a non-significant RR of 0.54 (95% CI: 0.27-1.07; *P*=0.08) for CNS relapse for patients receiving HD-MTX compared to controls.

Mortality

Survival data were available for 379 patients in the intervention group and 567 patients in the control group (Figure 3). Among the patients in the HD-MTX group, 107 (28.2%) died during follow-up compared to 225 (39.7%) in the control group. The meta-analysis conducted on mortality data found a non-significant RR of death of 0.70 (95% CI: 0.44-1.11; *P*=0.13).

Exploration of heterogeneity

The clinical heterogeneity is present most noticeably in

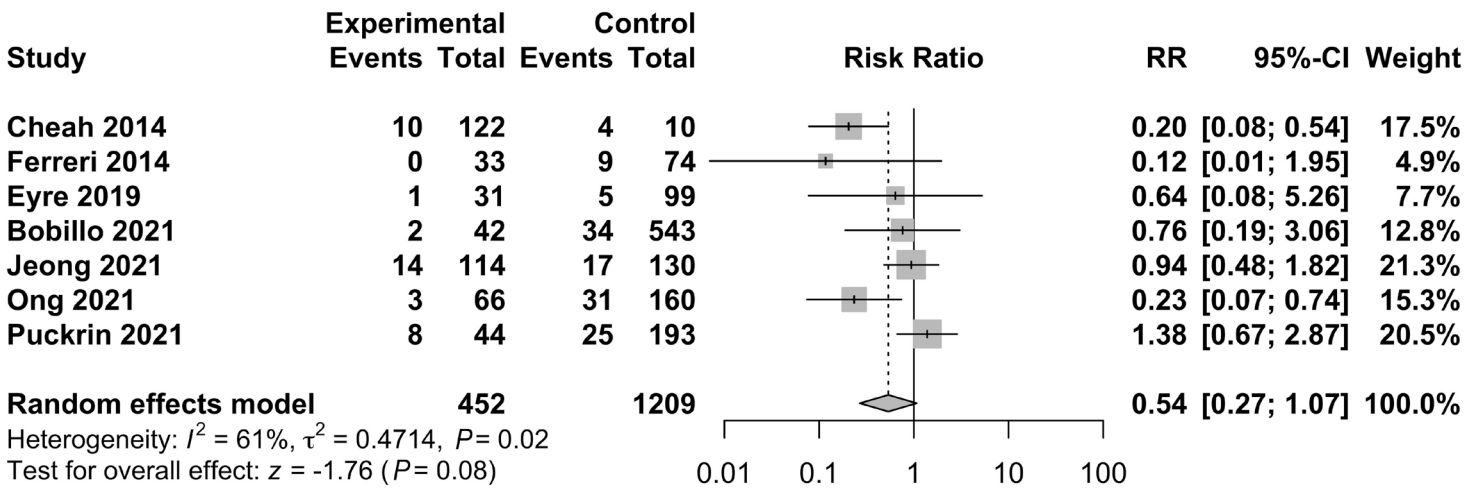


Figure 2. Meta-analysis of relative risk of central nervous system relapse. RR: risk ratio; 95% CI: 95% confidence interval.

Table 4. Frequency of central nervous system relapse and mortality.

Study	Follow up in months	Frequency of CNS relapse	Time to CNS relapse in months	Overall survival
Cheah *et al.*[29]	Median: 41 Range: 2.4-223	HD-MTX: 10/122 (8.1%) Controls: 4/10 (40%)*	Median: 10.8 Range: 4-109.6	5 years: HD-MTX: 96/122 (78%) Controls: 5/10 (50%)*
Ferreri *et al.*[33]	Median: 60 Range: 24-156	HD-MTX: 0/33 (0%) Controls: 9/74 (12%)	Median: 12 Range: 7-55	5 years: HD-MTX: 29/33 (87%) Controls: 40/74 (54%)
Eyre *et al.*[32]	Median: 33.6 Range: 4.8-106.8	HD-MTX: 1/31 (3%) Controls: 5/99 (5%)	Median: 9.4 Range: 1.8-70.8	NP
Bobillo *et al.*[12]	Median: 81.6 Range: NP	HD-MTX: 2/42 (4.8%) Controls: IT or no prophylaxis: 12/253 (4.7%); and 22/290 (7.6%), respectively.	Median: 9 Range: 6-110	NP
Jeong *et al.*[30]	Median: 50.2 Range: NP 95% CI: 45.6-53.1	HD-MTX: 14/114 (12%) Controls: 17/130 (13%)*	Median: 8.4 Range: NP 95% CI: 5.7-10.7	5 years: HD-MTX: 79/144 (69.2%) Controls: 80/130 (61.9%)*
Ong *et al.*[11]	Median: 20 Range: 10-96	HD-MTX: 3/66 (5%) Controls: 31/160 (19%).	Isolated CNS relapse: 7 Range: 4-50 Concomitant CNS and systemic relapse: 8 Range: 4-80	3 years: HD-MTX: 46/66 (69.1%) Controls: 101/160 (63.2%)
Puckrin *et al.*[31]	Median: 35.3 Range: 0.29-105.7	HD-MTX: 8/44 (18%) Controls: 25/193 (13%)*	Median: 7.4 Range: 0.9-49.3	5 years: HD-MTX: 22/44 (50%) Controls: 116/193 (60%)*

*Supplementary data provided by the authors. CNS: central nervous system; HD-MTX: high-dose methotrexate; NP: not provided; IT: intrathecal prophylaxis; 95% CI: 95% confidence interval.

the differential approach to high-risk classification (Table 1) and pre-diagnostic work-up (Table 2). As for methodological heterogeneity, the studies are estimated to be comparable with regard to both design (retrospective study design) and execution (chart review conducted by a small group of researchers) but divergent in regard to follow-up time (Table 4). As the calculated statistical heterogeneity of 61% among studies investigating the risk of CNS relapse may represent substantial heterogeneity, we conducted a sensitivity analysis for our primary endpoint including the studies by Ong,[11] Bobillo,[12] and Jeong[30] that had applied CNS-IPI risk stratification to the full cohort and the study by Puckrin[31] that had done so partially (Figure 4A). This did not alter the direction of the results but reduced the efficacy of CNS prophylaxis to prevent CNS relapse from a RR of 0.54 to 0.77 (95% CI: 0.38-1.56; *P*=0.46) while statistical heterogeneity decreased from 61% to 55%.

To test our hypothesis that IT prophylaxis and no prophylaxis can be equated, we performed a sensitivity analysis excluding the studies by Cheah[29] and Eyre[32] in which the controls only received IT prophylaxis (Figure 4B). This reduced the heterogeneity from 61% to 54% and altered the RR of CNS relapse from 0.54 to 0.68 (95% CI: 0.33-1.42; *P*=0.31).

Discussion

This meta-analysis attempts to estimate the benefit HD-MTX CNS prophylaxis confers to DLBCL patients, at high risk of CNS relapse, treated with frontline R-CHOP(-like) chemoimmunotherapy. A non-significant trend toward HD-MTX reducing CNS relapse with a RR of 0.54 (95% CI:

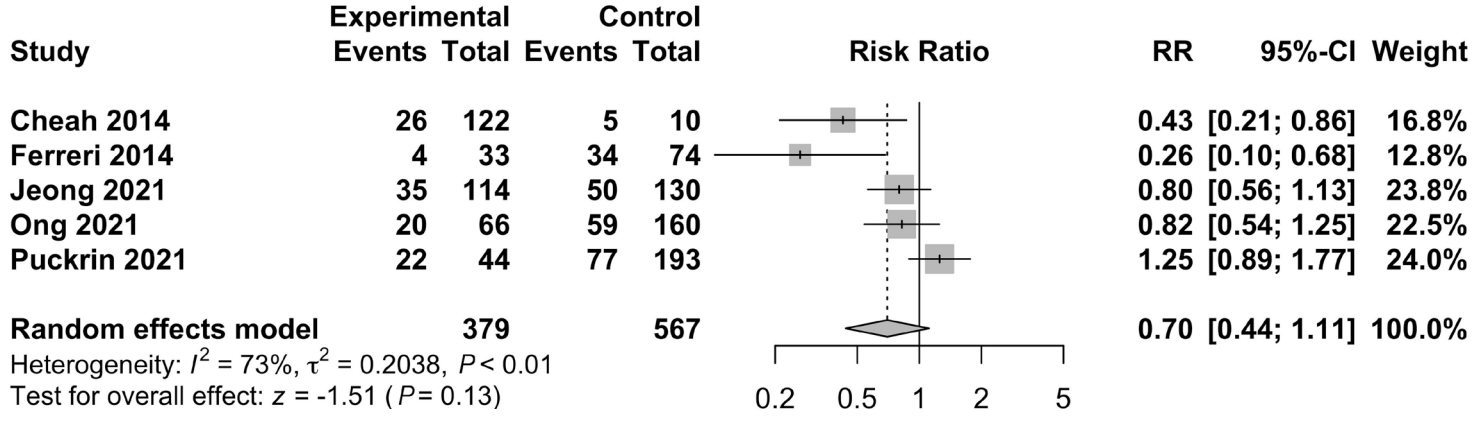
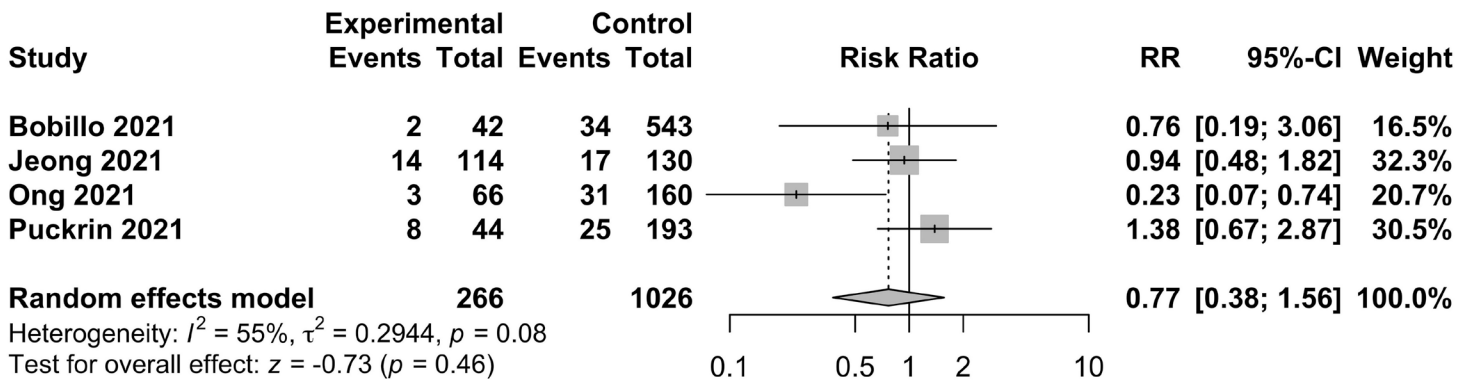


Figure 3. Meta-analysis on mortality of studies reporting on death. RR: risk ratio; 95% CI: 95% confidence interval.

A



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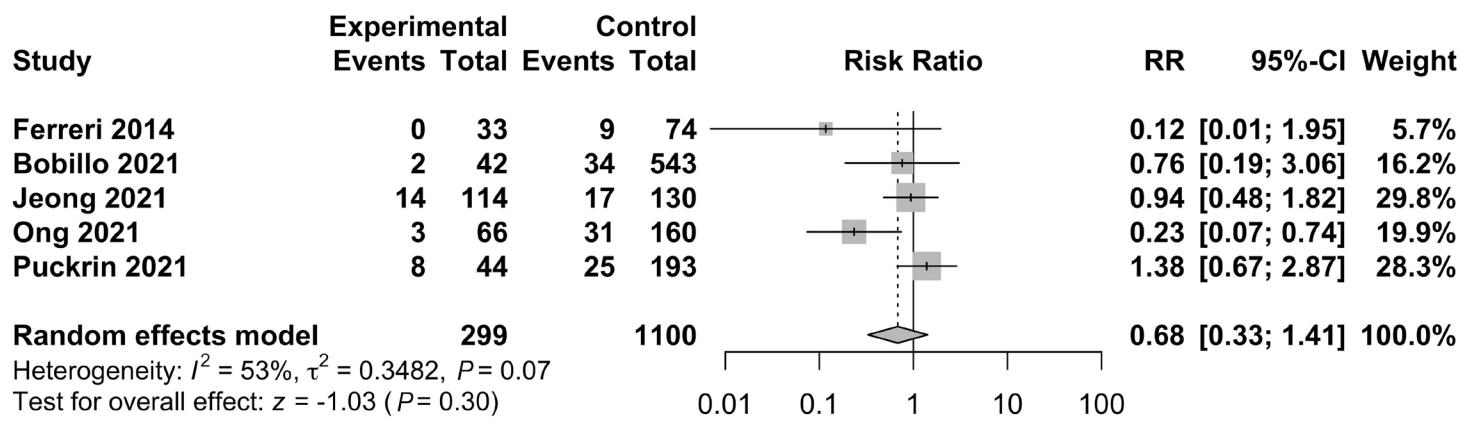

Figure 4. Sensitivity analyses. (A) Sensitivity analysis excluding studies not using the Central Nervous System International Prognostic Index as the risk stratification tool. (B) Sensitivity analysis excluding studies with cohorts consisting of patients only receiving intrathecal prophylaxis. RR: risk ratio; 95% CI: 95% confidence interval.

0.27-1.07; *P*=0.08) was found. No difference in the RR of death, regardless of HD-MTX treatment, was demonstrated. These results are based on a cohort of 1,661 patients from seven studies.

A sensitivity analysis (Figure 4A) on studies using the CNS-IPI for high-risk classification reduced the calculated RR from 0.54 to 0.77 (95% CI: 0.38-1.56; *P*=0.46). While we expected a larger reduction in heterogeneity when stringently defining the criteria for administration of CNS prophylaxis, the RR from the sensitivity analysis is in line with data from the largest retrospective study conducted on 2,418 high-risk patients (CNS-IPI 4-6) receiving CNS prophylaxis.[15] A sub-analysis of 1,616 patients achieving complete remission found no difference in CNS relapse rates between the patients who received high-dose prophylaxis (5%) and those who did not (6.5%) (adjusted hazard ratio=0.74, 95% CI: 0.4-1.3; *P*=0.30). We also conducted a sensitivity analysis excluding studies in which controls exclusively received IT prophylaxis (Figure 4B). Although this reduced the heterogeneity from 61% to 54%, it had no effect on the risk of CNS relapse. This indicates that the choice of control group (± IT prophylaxis or no prophylaxis) does not alter the direction of the outcome. Results from recent meta-analyses[17,18] and a network meta-analysis[19] have been contrasting. Ho *et al.*[17] examined patients at intermediate to high risk of CNS relapse and found no statistically significant benefit of CNS prophylaxis in their cohort of 3,770 patients from ten studies, of which three studies employed IT prophylaxis and seven HD-MTX ± IT prophylaxis. A sub-analysis comparing studies using HD-MTX (n=1,826 patients) against studies using IT prophylaxis (n=1,944 patients) found no difference between the subgroups (*P*=0.67). In contrast, Zhang *et al.*[18] found a protective effect of CNS prophylaxis. They analyzed the risk of CNS relapse in patients given CNS prophylaxis with HD-MTX ± IT (n=1,124) *versus* no prophylaxis or only IT (n=3,856) showing a RR of 0.70 (95% CI: 0.55-0.88; *P*=0.002). The network meta-analysis[19] included 6,614 patients from 24 studies receiving five different interventions. None of the listed regimens was shown to reduce CNS relapse rate compared with no prophylaxis.

Zhang *et al.*[18] also found an improved 3-year overall survival based on three studies of 244 patients receiving HD-MTX ± IT prophylaxis *versus* 255 patients receiving no prophylaxis or only IT prophylaxis with a RR of survival of 1.17 (95% CI: 1.03-1.32). In two other studies, no benefit was found on 2-year overall survival (RR=1.04, 95% CI: 0.92-1.17). The contradictory results of the meta-analyses may be due to the inclusion of subgroups of patients treated with more aggressive regimens known to penetrate the blood-brain barrier or patients who received additional high-dose chemotherapy with autologous stem cell support, which may reduce the risk of CNS relapse.[16] All three meta-analyses also included studies in which a proportion of enrolled patients had not received rituximab. Rituximab is thought to affect the risk of CNS relapse through better overall disease control.[35] All three meta-analyses included studies with cohort overlap (references [26,30] and [12,14,29], respectively), reducing the transparency of the actual number of events each analysis is based upon. Thus, the benefit of prophylactic strategies remains debatable. A recent study retrospectively investigating individual patient-level data from several registries came to a similar conclusion as our meta-analysis, namely that there was no statistically significant effect of HD-MTX prophylaxis on the risk of CNS relapse.[15] This is reassuring as there was considerable overlap in the cohorts providing data for both reports (Bobillo,[12] Cheah,[29] Eyre,[32] and Puckrin[31]).

The present meta-analysis was conducted on DLBCL patients with a high risk of CNS relapse. Patients receiving frontline R-CHOP form the largest subgroup among these patients and addition of HD-MTX increases the treatment-related toxicity considerably.[36] The combination of uncertain benefit with additional toxicity is the reason we considered the investigation of HD-MTX in this particular group of special interest. All included patients had received R-CHOP(-like) treatment and were considered at high risk of CNS relapse, either by listed risk factors or based on the treating physician's administration of CNS prophylaxis. The robustness of the study design was explored by conducting sensitivity analyses demonstrating the consistency of the results obtained. Our meta-analysis is limited by the fact that it is based solely on retrospective cohort studies, as no randomized controlled trials have been conducted in this setting. The anti-lymphoma chemotherapy backbone varied across included studies with Cheah,[29] Eyre,[32] Jeong,[30] and Ong[11] enrolling R-CHOP-treated patients exclusively while Ferreri,[33] Puckrin,[31] and Bobillo[12] also included patients treated with R-EPOCH, R-COPE and R-CHOP followed by R-ICE. This heterogeneity in chemotherapy backbone may have contributed to the heterogeneity seen in our meta-analysis. The selection of patients was based on a high-risk classification, but as the risk estimation comprises variations of clinical and molecular features, an interstudy difference in inclusion criteria was present. The range of follow-up varied from 20 to 60 months. As CNS relapses are more prevalent within the first 2 years, a shorter follow-up is justifiable but Ong,[11] Cheah,[29] and Ferreri[33] included historic cohorts where the difference in follow-up time may influence outcomes, as it has been suggested that the effect of HD-MTX is primarily one of delaying, rather than preventing, CNS relapse.[12] The majority of patients in the intervention arm received 3-3.5 g/m^2 HD-MTX, but consensus regarding the optimal dose and number of cycles of prophylaxis is lacking. There was significant heterogeneity in the timing of HD-MTX administration (Table 3) which could introduce bias; however, a recent publication did not find that timing had an impact on efficacy.[37]

Pre-diagnostic work-up of CNS involvement varied. Ong[11] excluded four patients in whom CNS relapse presented within the first 4 months, while time to relapse was as short as 0.9 and 1.8 months in the studies by Puckrin[31] and Eyre, respectively.[32]

A further ten studies could potentially have been included if all requests for supplementary data had been successful. Of these, six found no beneficial effect of HD-MTX prophylaxis, two studies did find a benefit from HD-MTX while for the remaining two, efficacy was not an outcome and thus not reported. Given that the majority of omitted studies came to a similar conclusion as that of our meta-analysis, the risk of them influencing the overall result, had we been able to include all studies, is considered negligible (see *Online Supplementary Table S5* for excluded studies).

All studies included in the meta-analysis carried a "high" risk of bias according to ROBINS-I. The confidence in the evidence was estimated to be "low", as assessed by the GRADE approach.

Our data indicate that HD-MTX does not prevent or, at best, only slightly reduces the incidence of later CNS relapse. We were also unable to demonstrate an impact of HD-MTX on survival. Conventional designs of meta-analyses have difficulties in fully accommodating and comparing the diversity of data in non-randomized studies of a retrospective nature due to the low incidence of CNS relapse, uncertainty about the target group for CNS prophylaxis, and the diversity of current first-line and prophylactic treatment strategies. For the same reasons, a direct comparison in a prospective randomized trial aimed at addressing CNS prophylaxis efficacy with current stratification and treatment modalities no longer seems to be advisable. Instead, efforts should be focused on designing more effective prophylactic interventions together with improving the risk assessment or detection of subclinical CNS involvement at the time of primary diagnosis by more sensitive assays. A recent study from New Zealand[38] tried to reduce the bias of subclinical CNS involvement by performing multiparametric flow cytometry evaluation on pre-diagnostic cerebrospinal fluid on all patients enrolled. Despite these efforts, thorough diagnostic work-up did not seem to affect the incidence of early CNS relapses. More sensitive diagnostic assays may improve the detection of subclinical CNS involvement. Analyzing circulating tumor DNA has shown promising preliminary results.[39-41] Prospective studies are needed to evaluate such new therapeutic and diagnostic interventions in precision medicine-based clinical practice.

Disclosures

No conflicts of interest to disclose.

Contributions

LMP, THN, CL, and ERT designed the study. ERT and THN screened the literature, extracted the data, and estimated the risk of bias and quality of evidence. CL performed the statistical analyses. All authors contributed to interpreting the data. ERT drafted the manuscript and all authors read, revised, and approved the final draft.

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Data-sharing statement

Requests for data can be made to the corresponding author.

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