Dosage of high-dose methotrexate as CNS prophylaxis in DLBCL - a detailed analysis of toxicity and impact on CNS relapse

Matthew R. Wilson[1], Amy A Kirkwood[2], Nicole Wong Doo[3], Carole Soussain[4], Sylvain Choquet[5], Charlotte Lees[6], Christopher Fox[7], Gavin Preston[8], Matthew Ahearne[9], Tim Strüßmann[10], Aline Clavert[11], Chiara Rusconi[12], Matthew Ku[13], Jahanzaib Khwaja[14], Mayur Narkhede[15], Katharine Lewis[16], Eric Durot[17], Jeffery Smith[18], Loic Renaud[19], Andrés J. M. Ferreri[20], Tarec el-Galaly[21], Kate Cwynarski[14], Pam McKay[1], Toby A. Eyre[6]

Affiliations:

1. Beatson West of Scotland Cancer Centre, Glasgow, United Kingdom
2. Cancer Research UK and UCL Cancer Trials Centre, UCL Cancer Institute, London, United Kingdom
3. Concord Clinical School, Concord Hospital University of Sydney, Sydney, Australia
4. Institut Curie Hôpital René Huguenin, Saint-Cloud, France
5. La Pitie Salpetriere Hospital, APHP-Sorbonne Universite, Paris, France
6. Oxford University Hospitals NHS Trust, Churchill Cancer Center, Oxford, United Kingdom
7. Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom
8. Aberdeen Royal Infirmary, Aberdeen, United Kingdom
9. University Hospitals of Leicester NHS Trust, Leicester, United Kingdom
10. University Medical Center Freiburg, Freiburg, Germany
11. Service des Maladies du Sang, CHU Angers, Angers, France
12. Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy
13. St Vincent's Hospital Melbourne, Melbourne
14. University College London Hospitals NHS Foundation Trust, London, United Kingdom
15. University of Alabama, Birmingham, USA
16. Linear Clinical Research and Sir Charles Gairdner Hospital, WA, Australia
17. Hôpital Robert Debré CHU de Reims, France
18. Liverpool University Hospitals Foundation Trust, Liverpool, United Kingdom
19. Hôpital Saint-Louis, Assistance Publique-Hôpitaux de Paris, France
20. IRCCS San Raffaele Scientific Institute, Milano, Italy
21. Aalborg University Hospital, Aalborg, Denmark

Corresponding Author:

Dr Matthew R. Wilson

Department of Haematology, Beatson West of Scotland Cancer Centre

1053 Great Western Road

Glasgow G12 0YN

matthew.wilson@ggc.scot.nhs.uk

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To the Editor,

Central nervous system (CNS) relapse in diffuse large B-cell lymphoma (DLBCL) is a rare event, occurring in 2-5% and is associated with a poor prognosis.[1] Certain patient and disease characteristics significantly increase this risk.[2] CNS-directed prophylaxis has often been incorporated into first-line therapy in patients at highest risk. In light of cumulative evidence suggesting that intrathecal (IT) therapy is ineffective[3], high-dose intravenous methotrexate (HD-MTX) has become widely used as prophylaxis, based largely on retrospective, underpowered analyses suggesting a potential benefit.[4]

We published an analysis of 1,384 patients receiving HD-MTX prophylaxis either intercalated between R-CHOP (i-HD-MTX) or at 'end-of-treatment' (EOT), demonstrating increased R-CHOP delays with i-HD-MTX and, crucially, similar rates of CNS relapse between the approaches.[5] EOT HD-MTX is now considered the optimal approach. The overall rate of CNS relapse seen in patients with a high CNS-IPI (9.1%), despite the use of HD-MTX, raised the question as to whether it has any benefit, irrespective of delivery time.

Several additional studies have addressed this question[6-9], with the largest being a recent retrospective analysis of 2,418 patients.[10] There was no clinically significant reduction in CNS relapse in patients in first complete remission who received HD-MTX (n=356), nor any clear benefit in ultra-high risk subgroups. Accepting the limitations of retrospective analyses, there is now significant uncertainty about the role of HD-MTX as CNS prophylaxis in DLBCL. However, given the lack of alternative strategies, and concern that the aforementioned studies were underpowered to demonstrate benefit in ultra high-risk subgroups, it is likely that HD-MTX will still be used for selected patients. One such group is testicular DLBCL, where prospective IELSG trial data suggests a potential benefit of HD-MTX, albeit at doses of 1.5g/m2 and in combination with IT therapy.[11]

There remains a lack of consensus regarding the optimal dosage and HD-MTX cycle number when used as prophylaxis, with international guidelines lacking consensus on this matter.[4,12,13] In our prior international study, we found huge variation in practice, with 25% of patients having ≥3 cycles and some having up to 6.[5] Given the potential significant toxicity of HD-MTX and the uncertainty around its efficacy, we performed an analysis of the impact of HD-MTX dosage on both toxicity and patient outcome (survival and specifically CNS relapse).

The details of the HD-MTX database including inclusion/exclusion criteria, patient baseline characteristics and treatments are previously described.[5] 1,384 patients were included, n=635 receiving EOT HD-MTX and n=749 i-HD-MTX; a total of 3111 HD-MTX cycles were analysed. A landmark cohort of patients alive and in CR 8 months from diagnosis was used for all outcome analyses (CNS relapse, PFS and OS) to control for immortality bias and included n=1217 (EOT n=587, i-HD-MTX n=630). Statistical methodology is described in **Appendix S1.**

Baseline characteristics are described previously[5] (**Table S1).** The median follow-up from 8-month landmark was 31.3 months (IQR 15.6-52.6). Details of number and dose of HD-MTX cycles (cumulative and peak [maximum individual dose]) are displayed in **Table S2**.

Although the median number of HD-MTX cycles and median cumulative dose were equal in the two groups (2 cycles, 6 g/m^2 respectively), significantly more patients received ≥3 cycles (37% vs 12%, p<0.0001) or had a cumulative dose >9 g/m^2 in the i-HD-MTX group. More patients had a peak HD-MTX dose of <3 g/m^2 in the EOT group (23% vs 9%, p<0.001): these patients were older, had lower baseline creatinine clearance, higher ECOG performance status, higher CNS-IPI, and were more likely to receive fewer HD-MTX cycles (**Table S2**). Analyses of factors influencing first HD-MTX dose are described in **Appendix S3.**

Numerically higher rates of cycle 1 and 2 toxicities were recorded with i-HD-MTX (**Figure 1A**). However, due to the potential confounding effect of recent R-CHOP, only toxicities following EOT HD-MTX were analysed in further detail (**Tables S3/S4)**. 252/635 (40%) experienced toxicity thought related to HD-MTX, with 44/635 (7%) grade ≥3. The most common were mucositis, hepatic, infection and renal with 14% experiencing renal toxicity (grade ≥2, 6; grade ≥3, 2%).

Higher doses in cycle 1 were associated with an increased risk of mucositis, but no other toxicities. In cycle 2, higher dose was associated with an increased risk of hepatic toxicities, in all patients, and those given at least 90% of the first cycle dose. No significant difference was seen for grade ≥3 events, however, numbers were small for cycle 2 (N=16) and not analysable by type. Patients were less likely to be given a second cycle if they experienced toxicity in cycle 1; 26% vs 5%, p<0.001. This difference was greatest for renal toxicity; 51% vs 7%, p<0.001 with no patients experiencing grade ≥3 continuing; 100% vs 10.3%, p <0.001. Similar findings were observed for mucositis (p<0.001, any and grade ≥3) and hepatic toxicity (grade ≥3 only, p<0.001).

Patients who experienced toxicity in cycle 1 were at higher risk of another event in cycle 2, this was significant for all events analysed and included a 58% risk of a hepatic event compared to 5% risk in those who had not experienced one in cycle 1 (p<0.0001). Patients without grade ≥3 events in cycle 1 were at very low risk of having a grade ≥3 event in cycle 2 even when treated with ≥90% of the dose (1.7%).

In the landmark cohort, 47 CNS relapse events occurred (n=45 with complete covariate data), 36 were isolated and 11 synchronous with systemic relapse. Twelve CNS relapse events occurred before the 8-month landmark (8 isolated, 4 synchronous). Full details of analyses on CNS relapse, PFS and OS are in **Table S5**. There was no significant reduction in CNS relapse with increasing HD-MTX dose, considering dose either cumulatively (HR 0.69 (95% CI 0.39-1.22), p=0.20) (total dose: ≤6 g/m^2 vs >6 g/m^2, **Figure 1C**) or as peak dose (HR 0.99 (95% CI 0.38-2.55), p=0.98). Similarly, there was no significant difference in PFS for either cumulative HD-MTX dose (HR 1.04 (95%CI 0.77-1.41), p=0.80) **(Figure 1C)** or peak dose (HR 1.06 (95%CI 0.63-1.77), p=0.83). Non-relapse mortality (NRM) was reported in 55/1384 (4.0%) of patients overall, and 44 in the landmark cohort. There was no association between NRM and cumulative HD-MTX dose (**Appendix S2).**

We present the largest study of its kind, analysing 1,384 patients receiving a total of 3,111 HD-MTX cycles, specifically assessing the impact of HD-MTX dose on toxicity, CNS relapse and survival. We demonstrated no reduction in CNS relapse with higher cumulative or peak

doses of HD-MTX. We used a multivariable landmark analysis to mitigate for immortality bias and to account for potential early events, preventing HD-MTX completion.

We limited our detailed analysis of HD-MTX toxicity to the EOT group, given the potential impact of concurrent R-CHOP with i-HD-MTX. However, it is noteworthy that the i-HD-MTX group had significantly more patients with ≥3 cycles and higher cumulative dosage, and we did observe numerically greater toxicity in the i-HD-MTX group. Although we did not record toxicities occurring with R-CHOP alone in the EOT arm to serve as a comparator, the rates of infection (16.4%) and mucositis (15%) recorded with i-HD-MTX are higher than that described with R-CHOP alone in previous phase 3 trials.[14]

In the EOT group, toxicity was still relatively frequent (40%, 7% grade ≥3). We demonstrated a low (2%) rate of grade ≥3 renal toxicity in the EOT group which provides some reassurance, however, there were clearly age based adjustments made, and it is also possible that physicians made judgements on risk of renal toxicity and implemented additional precautions, which are not recorded. Although increasing cumulative or peak HD-MTX dose did not significantly increase the overall risk of HD-MTX toxicity, we found an increased risk of mucositis with higher dose in cycle 1 and increased liver toxicity with higher doses in cycle 2.

Our dataset provides valuable insight into prescribing patterns with HD-MTX. Patients experiencing any toxicity were more likely to stop after 1 HD-MTX cycle, with renal toxicity showing the strongest association. If patients continued to cycle 2, those who had experienced toxicity in cycle 1 were much more likely to do so again. Although we did not see any evidence that the grade was likely to increase, this needs to be caveated by the fact clinicians may have already stopped for patients they felt were at higher risk of worsening toxicity.

We observed that most patients received doses of HD-MTX of either 3 or 3.5g/m^2. The evidence for this practice is derived from PCNSL studies, where pharmacokinetic analyses determined that HD-MTX doses of ≥3g/m^2 are required to reach CNS tumoricidal concentrations.[15] Our sub-analyses showed some evidence of increased toxicity with 3.5g/m^2 vs 3g/m^2 (renal, mucositis), in keeping with our overall observation that toxicity increases with higher doses **(Table S6)**. However, the event number was small and dose choices are potentially subject to clinician bias.

Our data do not allow determination of a clear cut-off for HD-MTX dose which significantly minimises toxicity, especially considering that clinicians made dose decisions based on patient characteristics. It was reassuring to observe that patients who did not experience toxicity with cycle 1 HD-MTX were highly unlikely to have a toxicity event with cycle 2. However, considering the clear association between increased dosage and toxicity observed, and the intention to deliver an effective HD-MTX dose, it appears reasonable to deliver doses of no more than 3-3.5g/m2 for a maximum of 2 cycles.

The strengths of this study are the multicentre design, large sample size and granularity of the HD-MTX data. The main limitations pertain to its retrospective, non-randomised design which leaves potential for selection bias, particularly when considering patients who were

retrospectively identified as having received EOT HD-MTX. We had no data on patients who were intended to receive EOT HD-MTX but ultimately did not receive it due to toxicity with R-CHOP or disease progression. We acknowledge that some toxicities may have occurred but were not recorded in case-notes. We also recognise that, although the sample is large, the number of CNS relapses remained relatively small and despite multivariable adjustments there may have been other factors affecting dose which may confound the treatment effects.

In summary, we found no evidence for increased efficacy with higher doses of HD-MTX when used as CNS prophylaxis in DLBCL, and demonstrated greater risk of toxicity with increased dose. Patients who experienced toxicity with cycle 1 HD-MTX were much more likely to do so again if they continued to cycle 2. Therefore, in the increasingly uncommon scenario where HD-MTX is used as CNS prophylaxis, our recommendation would be that a maximum of 2 cycles should be given at doses no higher than 3-3.5 g/m^2 following R-CHOP. Where toxicity is encountered with first HD-MTX delivery, there does not appear to be rationale in continuing with subsequent cycles.

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Authorship Contributions:

MRW, TAE, AAK, KC and PM designed the original HD-MTX timing study. AAK performed all statistical analyses. MRW, AAK and TAE analysed data and wrote the paper. All other authors participated in collection of data and in writing/reviewing the manuscript.

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