TITLE PAGE

Comment on: “Successful use of nitrous oxide during lumbar punctures: A call for nitrous oxide in pediatric oncology clinics” *Pediatr Blood Cancer.* 2017;**64**:e26610.

Authors: Dr Victoria Forster[1], Dr Gabriele Escherich[2], Dr Christina Halsey[3]

1: The Arthur and Sonia Labatt Brain Tumour Research Centre, The Hospital for Sick Children, Toronto, ON, Canada

2: Clinic for Pediatric Hematology and Oncology, University Medical Center, Hamburg, Germany

3: Wolfson Wohl Cancer Research Centre, Institute of Cancer Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, Garscube Estate, Switchback Road, Bearsden, Glasgow, United Kingdom.

Corresponding Author: Dr Christina Halsey, Wolfson Wohl Cancer Research Centre, Institute of Cancer Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1QH UK. Tel +44 141 330 8135, Fax +44 141 330 8094

Word Count Main Text: 486, Tables/Figures: 0

Running Title: Nitrous oxide use in paediatric oncology. Keywords: Acute lymphoblastic leukemia, anesthesia, late effects of cancer treatment, chemotherapy, methotrexate, neurotoxicity of chemotherapy.

Abbreviations Key

MRI	Magnetic resonance imaging
NMDA	N-methyl-D-aspartate

Dear Editor,

In response to the recent “call for nitrous oxide in pediatric oncology clinics”[1], we urge extreme caution. Although this relatively small study did not record significant acute toxicity, we draw reader’s attention to a serious potential safety concern regarding use of nitrous oxide in combination with methotrexate, the most commonly administered intrathecal drug in paediatric oncology. Two recent case reports describe severe neurotoxicity following the use of nitrous oxide anaesthesia in paediatric leukaemia patients receiving intrathecal methotrexate[2,3]. Although these do not prove a causal relationship, there is a body of experimental and clinical data that support the theory that nitrous oxide may increase the toxicity of anti-folate medications such as methotrexate[4]. Indeed, the British National Formulary states “nitrous oxide increases antifolate effect of methotrexate – avoid concomitant use”[5].

Methotrexate related neurotoxicity is common, with 4-12% of children experiencing neurological adverse events during therapy[2]. Importantly, adverse events often have delayed presentation several days or longer after methotrexate administration. In addition, up to 20% of children have subclinical leukoencephalopathy on MRI scanning, and both clinical neurotoxicity and subclinical leukoencephalopathy are associated with adverse long-term neurocognitive outcomes[6]. It is crucial that all possible measures are taken to reduce this significant side effect of methotrexate, and the absence of immediate side effects from nitrous oxide anaesthesia does not necessarily equate to an absence of long-term impact on neurocognition.

Preclinical studies in rats have shown that intraperitoneal methotrexate combined with pre-treatment nitrous oxide administration, decreased the 50% lethal dose of

methotrexate by 6-fold from 60mg/kg to 10mg/kg, with the authors recommending against use of nitrous oxide before or during methotrexate administration in humans based on their findings[7]. In addition, a clinical study in patients with breast cancer showed that severe unpredictable toxic effects from methotrexate were probably due to an interaction with nitrous oxide[8].

Mechanistically, methotrexate inhibits the enzyme dihydrofolate reductase, leading to disruption of one-carbon metabolism important for nucleotide synthesis and amino acid metabolism. One consequence of reduced bioavailability of folates in the one-carbon cycle is a reduction in methionine synthase activity leading to increased homocysteine levels and reduced methionine levels. Downstream metabolites of homocysteine act at the NMDA receptor to cause neurotoxicity in preclinical models[9] and human data confirm the link between homocysteine, NMDA activation and clinical methotrexate-associated neurotoxicity[10]. Nitrous oxide also inhibits methionine synthase via irreversibly inactivating its essential cofactor – vitamin B12[7]. The same pathway is implicated in the pathogenesis of the serious neurological syndrome subacute combined degeneration of the cord, seen following nitrous oxide use in patients with untreated vitamin B12 deficiency. As a second cautionary note, patients with haematological malignancies may be at increased risk for subclinical vitamin B12 deficiency due to chronic proton pump inhibitor use, enteropathy and/or malnutrition[2].

Therefore, given the potential for enhanced neurotoxicity, we urge all paediatric oncology units to take a precautionary principle and avoid the use of nitrous oxide in patients undergoing lumbar puncture for administration of intrathecal methotrexate.

Dr Victoria Forster, Dr Gabriele Escherich & Dr Christina Halsey

Conflict of Interest Statement: The authors have no conflicts of interest to declare

Acknowledgements: we would like to acknowledge funding from Children with Cancer UK (2014/170) and the Glasgow Children's Hospital Charity (GCHC/PSG/2016/11) which support our work on mechanisms of neurotoxicity in acute lymphoblastic leukaemia.

References:

1. Livingston M, Lawell M, McAllister N. Successful use of nitrous oxide during lumbar punctures: A call for nitrous oxide in pediatric oncology clinics. Pediatric blood & cancer 2017:64(11).
2. Forster VJ, van Delft FW, Baird SF, et al. Drug interactions may be important risk factors for methotrexate neurotoxicity, particularly in pediatric leukemia patients. Cancer chemotherapy and pharmacology 2016:78(5):1093-1096.
3. Lobel U, Trah J, Escherich G. Severe neurotoxicity following intrathecal methotrexate with nitrous oxide sedation in a child with acute lymphoblastic leukemia. Pediatric blood & cancer 2015:62(3):539-541.
4. Ueland PM, Refsum H, Wesenberg F, et al. Methotrexate therapy and nitrous oxide anesthesia. The New England journal of medicine 1986:314(23):1514.
5. Paediatric Formulary Committee. BNF for Children 2009. London: BMJ Group, Pharmaceutical Press, and RCPCH Publications; 2009.
6. Cheung YT, Sabin ND, Reddick WE, et al. Leukoencephalopathy and long-term neurobehavioural, neurocognitive, and brain imaging outcomes in survivors of childhood acute lymphoblastic leukaemia treated with chemotherapy: a longitudinal analysis. The Lancet Haematology 2016:3(10):e456-e466.

7. Ermens AA, Schoester M, Spijkers LJ, et al. Toxicity of methotrexate in rats preexposed to nitrous oxide. Cancer research 1989:49(22):6337-6341.
8. Goldhirsch A, Gelber RD, Tattersall MN, et al. Methotrexate/nitrous-oxide toxic interaction in perioperative chemotherapy for early breast cancer. Lancet 1987:2(8551):151.
9. Li Y, Vijayanathan V, Gulinello M, et al. Intrathecal methotrexate induces focal cognitive deficits and increases cerebrospinal fluid homocysteine. Pharmacol Biochem Behav 2010:95(4):428-433.
10. Kishi S, Griener J, Cheng C, et al. Homocysteine, pharmacogenetics, and neurotoxicity in children with leukemia. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2003:21(16):3084-3091.