We did not mention hyperammonemia as a reflection of the ammonia levels itself because it is not true. Not every child that has high ammonia levels will have clinical hyperammonemia too. As ammonia is a direct product of the reaction catalyzed by asparaginase, it is expected that with enzymatic activity, there will be an increase in ammonia,[7] which will not always be related to the hyperammonemia reaction.

We considered the good practices for the processing of samples. It was obtained immediately before and after the infusion and analyzed at the hospital's laboratory, located in the same building and very close to the collection site.

We did not perform multivariate analysis. Table 1 is a 2×2 table that shows the investigation of the individual factors risks (univariate analysis). The National Cancer Institute (NCI) criteria mentioned in the letter received are for risk analyses of event-free survival (EFS) and overall survival (OS), not for an infusion reaction. We do not know if there is a cut-off point for age as risk factor for infusion reaction.

We thank the statistical analysis suggestions, but we do not think it necessary to use them. We applied techniques appropriate to our objective of assessing the post and pre-infusion ammonia level ratio.

Finally, the differential diagnosis of infusion reactions to asparaginase, using ammonia dosage as a tool to identify enzymatic inactivation in this context, may contribute to a safer making decision as to whether or not to continue treatment due to the non-availability of alternative asparaginase formulations in some countries, considered the objective and limitations of this strategy. Unfortunately, infusions reactions to native E. coli asparaginase are frequent and severe in some cases.[5] Even though there is a gold standard for identifying inactivation, especially the silent one, the discussion of asparaginase monitoring alternatives in the pharmacovigilance scope keeps relevant to oncology practice.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Amanda Cabral dos Santos https://orcid.org/0000-0003-0056-0262

References

1. Santos AC, Land MGP and Lima EC. Ammonia level as a proxy of asparaginase inactivation in children: a strategy for classification of infusion reactions. *J Oncol Pharm Pract* 2021; 0: 1–9. https://doi.org/10.1177%2F1078155221998738
2. Kloos RQH, Uyl-de Groot CA, van Litsenburg RR, et al. A cost analysis of individualized asparaginase treatment in pediatric acute lymphoblastic leukemia. *Pediatr Blood Cancer* 2017; 64: e26651.
3. Baruchel A, Brown P, Rizzari C, et al. Increasing completion of asparaginase treatment in childhood acute lymphoblastic leukaemia (ALL): summary of an expert panel discussion. *ESMO Open* 2020; 5: e000977.
4. Cecconelo DK, Magalhães MR, Werlang ICR et al. Asparaginase: an old drug with new questions. *Hematol Transfus Cell Ther* 2020; 42: 275–282.
5. Santos AC, Land MGP, Silva NP, et al. Reactions related to asparaginase infusion in a 10-year retrospective. *Cohort Rev Bras Hematol Hemoter* 2017; 39: 337–342.
6. Tong WH, van der Sluis IM and Pieters R. Ammonia levels should not be used as a surrogate marker of levels of asparaginase activity in acute lymphoblastic leukemia patients [Letter to Editor]. *Pediatr Blood Cancer* 2015; 63: 561–562.
7. Czogała M, Balwierz W, Sztefko K, et al. Utility of ammonia concentration as a diagnostic test in monitoring of the treatment with l-asparaginase in children with acute lymphoblastic leukemia. *Biomed Res Int* 2014; 2014: 1–6.

Amanda C Santos[1], Marcelo G P Land[1] and Elisangela C Lima[2]

[1]Instituto de Puericultura e Pediatria Martagão Gesteira, PPGCM – FM (Graduate program in medical clinic – medical school) Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

[2]School of Pharmacy, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Corresponding author:
Amanda Cabral dos Santos, Instituto de Puericultura e Pediatria Martagão Gesteira, Federal University of Rio de Janeiro, University City, Rio de Janeiro, Brazil.
Email: amandacabral1986@gmail.com

Comment on Ammonia level as a proxy of asparaginase inactivation in children: A strategy for classification of infusion reactions

I read the article of Santos *et al.*[1] They found that the ammonia levels served as a proxy of asparaginase inactivation in children with acute lymphoblastic leukemia (ALL) using native *E. coli* asparaginase.

Asparaginase is a non-human enzyme that catalyzes the hydrolysis of asparagine into aspartic acid and ammonia.[2] The efficacy of asparaginase can be evaluated by measuring the levels of asparaginase activity.[3–6] Although the most direct way of assessing asparaginase efficacy is the measurement of asparagine from the blood.[7] The evaluation of asparagine depletion is, however, technically difficult.[8,9] Measuring asparagine levels in cerebrospinal fluid (CSF) is also studied to evaluate its efficacy.[10,11] In the last decade, also the role of assessing asparaginase antibodies during asparaginase therapy has been, extensively, studied. However, different results were published with sometimes controversy.[6,12–16]

Previously, ammonia levels have been suggested to reflect the asparaginase activities.[17,18] It has been suggested in case reports that ammonia release could lead to encephalopathy.[19,20] Moreover in a previous prospective study, it was shown that the ammonia level was not related to central neurotoxicity.[21] Given the unclear role of the clinical utility of ammonia levels in daily practice, I would like to comment on the paper of Santos *et al*. It should be mentioned that the current standard of practice to evaluate asparaginase efficacy is therapeutic drug monitoring (TDM) with measuring asparaginase activities which is now used world-wide.[22] Also some important statistical questions can be raised, which I address below.

First, in the paper of Santos *et al*. the upper limit of normal of the ammonia levels is unclear. The authors had only defined the corresponding ammonia levels according to grades 1 or 2 using the Common Terminology Criteria for Adverse Events (CTCAE) 3/4.03 version.[1] If the upper limit of normal of the ammonia levels was used according to a previous paper[18], there would be no (cor)relation, as currently shown in Figure 3 of their paper.

Second, in my opinion, the relationship shown in panel B of this Figure 3 seems not correct. Santos *et al*. should not mention hyperammonemia as reflection of the ammonia levels itself. My suggestion is that by using the Fisher Exact test is the correct way to analyze this relationship. For example: low/high ammonia levels *versus* no hypersensitivity/or reaction in a 2-by-2 contingency tables. Also, I suggest to use a Violin plot rather than a box plot, as a Violin plot also show the probability density of the data at different values.[23]

Third, two laboratory issues. To avoid the ongoing production of ammonia by asparaginase *ex vivo* did the authors adhere to the following procedure: were the blood samples put in an ice bath and were these samples immediately processed at their laboratory? The authors also obtained blood samples immediately after the asparaginase courses, why did not the authors measured ammonia trough levels?

Fourth, some statistical issues. The authors studied 245 infusions in 32 patients, and 19 reactions were observed in 17 children. I was wondering if the authors noticed that given this information only two risk factors should be studied. The authors chose to use a logistic regression model. By using more than two risk factors, this model could be overfitted. More importantly, why did these authors chose to use a logistic regression model? Their study group was rather small, hence a descriptive statistical approach, to present the data, would be more appropriate.

Lastly, in their Table 1, they authors present the odds ratio of age for each year of life. Why did not the authors use the National Cancer Institute (NCI) criteria for age, for example: age less than 10 years and age at least 10 years?

To conclude, in the past decade monitoring of asparaginase efficacy has proven to be very successful, mainly by implementing asparaginase activities to monitor asparaginase pharmacokinetics.[22] Other (surrogate) measurements are available, including ammonia measurements. However, the pharmacology of asparaginase is rather difficult and some controversies do exist. Challenges herein are still to be solved.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Wing H Tong https://orcid.org/0000-0002-5462-6012

References

1. Santos AC, Land MGP and Lima EC. Ammonia level as a proxy of asparaginase inactivation in children: a strategy for classification of infusion reactions. *J Oncol Pharm Pract* 2021: 1078155221998738. DOI: 10.1177/1078155221998738.
2. Bussolati O, Belletti S, Uggeri J, et al. Characterization of apoptotic phenomena induced by treatment with L-asparaginase in NIH3T3 cells. *Exp Cell Res* 1995; 220: 283–291.
3. Kloos RQH, Pieters R, Jumelet FMV, et al. Individualized asparaginase dosing in childhood acute lymphoblastic leukemia. *J Clin Oncol* 2020; 38: 715–724.
4. Salzer W, Bostrom B, Messinger Y, et al. Asparaginase activity levels and monitoring in patients with acute lymphoblastic leukemia. *Leuk Lymphoma* 2018; 59: 1797–1806.

5. Asselin B and Rizzari C. Asparaginase pharmacokinetics and implications of therapeutic drug monitoring. *Leuk Lymphoma* 2015; 56: 2273–2280.
6. Tong WH, Pieters R, Kaspers GJ, et al. A prospective study on drug monitoring of PEGasparaginase and erwinia asparaginase and asparaginase antibodies in pediatric acute lymphoblastic leukemia. *Blood* 2014; 123: 2026–2033.
7. van der Sluis IM, Vrooman LM, Pieters R, et al. Consensus expert recommendations for identification and management of asparaginase hypersensitivity and silent inactivation. *Haematologica* 2016; 101: 279–285.
8. Gentili D, Zucchetti M, Conter V, et al. Determination of L-asparagine in biological samples in the presence of L-asparaginase. *J Chromatogr B Biomed Appl* 1994; 657: 47–52.
9. Lanvers-Kaminsky C, Westhoff PS, D'incalci M, et al. Immediate cooling does not prevent the ex vivo hydrolysis of L-asparagine by asparaginase. *Ther Drug Monit* 2014; 36: 549–552.
10. Henriksen LT, Nersting J, Raja RA, et al.; Nordic Society of Paediatric Haematology and Oncology (NOPHO) Group. Cerebrospinal fluid asparagine depletion during pegylated asparaginase therapy in children with acute lymphoblastic leukaemia. *Br J Haematol* 2014; 166: 213–220.
11. Rizzari C, Lanvers-Kaminsky C, Valsecchi MG, et al. Asparagine levels in the cerebrospinal fluid of children with acute lymphoblastic leukemia treated with pegylated-asparaginase in the induction phase of the AIEOP-BFM ALL 2009 study. *Haematologica* 2019; 104: 1812–1821.
12. Tong WH, Pieters R, Tissing WJ, et al. Desensitization protocol should not be used in acute lymphoblastic leukemia patients with silent inactivation of PEGasparaginase. *Haematologica* 2014; 99: e102–e104.
13. Liu Y, Smith CA, Panetta JC, et al. Antibodies predict pegaspargase allergic reactions and failure of rechallenge. *J Clin Oncol* 2019; 37: 2051–2061.
14. Swanson HD, Panetta JC, Barker PJ, et al. Predicting success of desensitization after pegaspargase allergy. *Blood* 2020; 135: 71–75.
15. Willer A, Gerß J, König T, et al. Anti-Escherichia coli asparaginase antibody levels determine the activity of second-line treatment with pegylated E coli asparaginase: a retrospective analysis within the ALL-BFM trials. *Blood* 2011; 118: 5774–5782.
16. Khalil A, Würthwein G, Golitsch J, et al. Pre-existing antibodies against polyethylene glycol reduce asparaginase activities on first administration of pegylated *E. coli* asparaginase in children with acute lymphocytic leukemia. *Haematol* 2020. DOI: 10.3324/haematol.2020.258525.
17. Czogała M, Balwierz W, Sztefko K, et al. Clinical utility of ammonia concentration as a diagnostic test in monitoring of the treatment with L-asparaginase in children with acute lymphoblastic leukemia. *Biomed Res Int* 2014; 2014: 1–8.
18. Tong WH, Pieters R and van der Sluis IM. Ammonia levels should not be used as a surrogate marker of levels of asparaginase activity in acute lymphoblastic leukemia patients. *Pediatr Blood Cancer* 2016; 63: 564–565.
19. Pound CM, Keene DL, Udjus K, et al. Acute encephalopathy and cerebral vasospasm after multiagent chemotherapy including PEG-asparaginase and intrathecal cytarabine for the treatment of acute lymphoblastic leukemia. *J Pediatr Hematol Oncol* 2007; 29: 183–186.
20. Jaing TH, Lin JL, Lin YP, et al. Hyperammonemic encephalopathy after induction chemotherapy for acute lymphoblastic leukemia. *J Pediatr Hematol Oncol* 2009; 31: 955–956.
21. Tong WH, Pieters R, de Groot-Kruseman HA, et al. The toxicity of very prolonged courses of PEGasparaginase or erwinia asparaginase in relation to asparaginase activity, with a special focus on dyslipidemia. *Haematologica* 2014; 99: 1716–1721.
22. Lanvers-Kaminsky C. Asparaginase pharmacology: challenges still to be faced. *Cancer Chemother Pharmacol* 2017; 79: 439–450.
23. Thrun MC, Gehlert T and Ultsch A. Analyzing the fine structure of distributions. *PLoS One* 2020; 15: e0238835.

Wing H Tong[1],[2]
[1]Department of Public Health and Primary Care (PHEG), Leiden University Medical Center, Leiden, the Netherlands
[2]Argos Zorggroep "DrieMaasStede", Center for Specialized Geriatric Care, Schiedam, the Netherlands

Corresponding author:
Wing H Tong, Department of Public Health and Primary Care (PHEG), Leiden University Medical Center, Building 3, Hippocratespad 21, PO Box 9600, 2300 RC, Leiden, the Netherlands.
Email: w.h.tong@lumc.nl