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, 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. 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.

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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. 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. The efficacy of asparaginase can be evaluated by measuring the levels of asparaginase activity. Although the most direct way of assessing asparaginase efficacy is the measurement of asparagine from the blood. The evaluation of asparaginase depletion is, however, technically difficult. Measuring asparagine levels in cerebrospinal fluid (CSF) is also studied to evaluate its efficacy. 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.

Previously, ammonia levels have been suggested to reflect the asparaginase activities. It has been suggested in case reports that ammonia release could lead to encephalopathy. Moreover in a previous prospective study, it was shown that the ammonia level was not related to central neurotoxicity. 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. 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. If the upper limit of normal of the ammonia levels was used according to a previous paper, 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.

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. 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.

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