Check for updates

RESEARCH ARTICLE

REVISED Retrospective cohort study monitoring PEG-asparaginase activity in acute lymphoblastic leukemia patients with and without premedication [version 2; peer review: 1 approved, 2 approved with reservations]

Michael Losasso[1], Bruce Bostrom [ID]2, Yoav Messinger [ID]2

[1]University of St. Thomas, 2115 Summit Avenue, St. Paul, Minnesota, 55105, USA
[2]Cancer and Blood Disorders, Children's Minnesota, 2525 Chicago Avenue South, Minneapolis, Minnesota, 55404, USA

Abstract

Background: PEG-L-asparaginase (pegaspargase) is a critical component of therapy for children and adults with acute lymphoblastic leukemia (ALL). Allergic reactions, which may occur in up to one third of patients, are the major cause for discontinuation. One study reported lower rates of allergic reactions with premedication. Besides allergy, an unknown number of patients develop silent neutralizing antibodies not associated with allergic reactions. The purpose of this retrospective cohort study was to determine the incidence of silent inactivation of pegasparaginase and compare incidence of allergic reactions with and without premedication.

Methods: Using a commercial assay, asparaginase activity was monitored following pegaspargase (2500 units/m) in newly diagnosed children and young adults with B- and T-cell ALL from February 2013 to May 2017. The incidence of allergic reactions before and after initiation of premedication in May 2015 was compared.

Results: One patient out of 59 (1.7%) had silent inactivation after the second dose. No patient had silent inactivation after the first pegaspargase dose and no standard risk B-cell ALL patients, who received only two pegaspargase doses in combination with oral dexamethasone, had silent inactivation. The incidence of grade 3 or 4 allergic reactions was 3.7% per dose with premedication (methylprednisolone, acetaminophen and diphenhydramine) versus 5.2% without. The incidence per patient with premedication given for most of the doses was 8.3% versus 17% without. These values are not statistically significant. Premedication did not affect pegaspargase activity.

Conclusions: Due to the low incidence of silent inactivation with intravenous pegaspargase and the unlikely event patients receiving only two doses of pegasparaginase would receive erwinase for this possible

Open Peer Review

Reviewer Status ? ? ✓

	Invited Reviewers		
	1	**2**	**3**
version 2 (revision) 17 Jan 2020			✓ report
version 1 04 Jul 2019	? report	? report	? report

1 **Bernard L. Marini** [ID], University of Michigan College of Pharmacy, Ann Arbor, USA

2 **Vinod Pullarkat**, City of Hope Medical Center, Duarte, USA

3 **David J. Young** [ID], National Institutes of Health (NIH), Bethesda, USA

Any reports and responses or comments on the article can be found at the end of the article.

transient silent inactivation, we recommend routine monitoring of pegaspargase activity only in patients scheduled to receive more than two doses.

Keywords

Pegasparginase Activity, Premedication, Silent Inactivation, Allergy, Anaphylaxis

Corresponding author: Bruce Bostrom (Bruce.Bostrom@childrensmn.org)

Author roles: Losasso M: Data Curation, Investigation, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Bostrom B**: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Messinger Y**: Conceptualization, Methodology, Project Administration, Resources, Supervision, Visualization, Writing – Review & Editing

Introduction

PEG-L-asparaginase (pegaspargase) is a critical component of therapy for children and adults with acute lymphoblastic leukemia (ALL). Its use is hampered by many issues including allergic reactions, silent inactivation, thrombosis, hyperbilirubinemia and pancreatitis[1]. Other common toxicities, such as hyperglycemia and hypertriglyceridemia, may be mitigated with the use of metformin[2] and omega-3[3] and fenofibrate[4]. There is also ongoing interest in the use of carnitine to treat, and possibly prevent, hepatic toxicity, manifested by a severe increase in direct bilirubin, among other findings[5].

The optimal dose, dose interval and target asparaginase level for pegaspargase is not completely established[6]. In pediatrics, a dose of 2500 units/m² is the norm, whereas for adult patients, doses are often reduced due to increased toxicity at the pediatric dose. Some investigators have suggested using a pharmacokinetic driven model to individualize pegaspargase dosing[7].

The use of premedication (acetaminophen, diphenhydramine and a corticosteroid) has been suggested as a possible means of reducing allergic reactions. In a multi-center study testing the use of pediatric-based regimens in young adults, the rate of grade 3 or 4 allergic reactions was reduced from 10% to 4% after premedication was mandated[8]. A study in adults with ALL reported allergic reactions in 7.2% of patients when pegaspargase was given concurrently with, or followed by, one week of prednisone[9]. Using a novel mouse model of asparaginase hypersensitivity, pretreatment with seven days of oral dexamethasone was the only agent capable of mitigating the severity of hypersensitivity and partially restoring asparaginase activity[10]. Dexamethasone given at the time of, or for one week following, asparaginase was not as effective.

The presence of antibodies against asparaginase may be found, from as early as the end of induction therapy[11,12]. The presence of asparaginase antibodies had sensitivity of 87–88% and specificity of 68–69% for clinical reactions[11]. The presence of asparaginase antibodies at end of induction did not appear to alter prognosis in a large multi-center study[12]. This suggests that measuring asparaginase activity is more useful than looking for the presence of antibodies.

Silent inactivation of pegaspargase activity by anti-asparaginase antibodies or other immune-mediated mechanisms are potentially of greater concern than bone-fide allergic reactions, as patients with grade 3–4 allergic reactions to pegaspargase will be switched to erwinase, which theoretically will improve outcome. The true incidence of silent inactivation is unknown, as there are no reports of a comprehensive screening program for silent inactivation in a large multi-institutional trial. The largest published study found silent inactivation in 7/89 (8%) of patients[13]. However, these patients received induction with native *Escherichia coli* asparaginase before switching to pegaspargase, which is not current practice. The authors also report in the same group of patients that silent antibodies may spontaneously resolve with continued pegaspargase[14]. Notably, lower silent inactivation with pegaspargase than native *Escherichia coli* asparaginase have been reported[15–17] Prudence suggests that patients who receive premedications should have pegaspargase activity monitored after every dose, due to the possible but unproven concern that premedication will mask allergic reactions and silent inactivation. In fact, a consensus panel of experts recommends screening for silent inactivation in all patients undergoing therapy for ALL with asparaginase[18]. Additionally, the low grade 1–2 allergic reactions that are more common than silent inactivation do require pegaspargase activity monitoring to ensure a switch to erwinase if confirmed as true inactivation.

Methods

Ethical statement

As the use of premedications and measurement of pegaspargase activity was considered by the leukemia provider group at Children's Minnesota to be necessary for optimal care, no informed consent was obtained. Parents/adult patients were not informed of results unless intervention was indicated, which did not occur. This retrospective review study was approved by the institutional review board of Children's Minnesota (IRB# 1606-062).

Patients

This retrospective study occurred in a large pediatric oncology center that diagnoses and treats approximately 40 new cases of ALL yearly in children and young adults up to age 30. If there are open studies, the patients are enrolled on Children's Oncology Group protocols. Otherwise, patients are treated according to the most recent risk adapted protocols for standard risk B, high risk B and T-ALL. In order to reduce acquisition bias, charts of every patient in first remission who received pegaspargase from December 2013 to September 2016 were abstracted (N=99). As this was a pilot study and the expected reduction of grade 3 or 4 allergic reactions with premedications was unknown at the time, sample sizes calculations could not be calculated. Data from all 99 patients were used to estimate the incidence of grade 3 or 4 allergic reactions by patient and by dose. For the detailed pharmacokinetic analysis, we used a subgroup of all patients from May 2014 to September 2016 (N=46) who had pegaspargase levels drawn. This number was sufficient to define the confidence intervals of the pegaspargase activity.

Pegaspargase administration

A total of 112 blood samples from these 46 patients were collected from a central venous portacath in conjunction with scheduled clinical visits from 3 to 12 days following pegaspargase administration at the standard dose of 2500 mg/m^2. Pegaspargase was given by intramuscular injection or intravenously per Children's Oncology Group protocols on an intermittent schedule starting with induction and completed prior to starting maintenance therapy. Because the distribution of the collection days clustered in ranges from day 3–5, 6–8 and 10–12, for analyses, pegaspargase activity was grouped in these categories. One data point was omitted from analysis in this version of the manuscript because it was and extreme outlier that was not congruent with other values from the patient or group (UPN 11; day 7 after 3rd dose; value 2.86; greater than 99.9th percentile). Two data points were removed because they were drawn after anaphylaxis and as expected undetectable (UPN 38 and 42 after 2nd dose). These values have been retained in the online dataset. To better estimate the incidence of silent inactivation, pegaspargase levels lower than 0.01 units/ml were looked for in the data from an additional 13 patients making a total of 59 evaluated. No evidence of silent inactivation was found in these 13 patients.

These patients were all treated according to Children's Oncology Group protocols, using either intramuscular or intravenous pegaspargase as the only form of asparaginase. Intramuscular asparaginase was the standard of care until 2010 when intravenous administration became the new standard of care based on the Children's Oncology Group AALL0932 protocol[19]. A comprehensive review of published studies concluded that the risk of grade 3 or 4 allergic reactions is independent of the pegaspargase route of administration[19].

Premedication administration

We became aware of an abstract showing a decrease in grade 3 or 4 allergic reactions in a multi-institutional study employing pegaspargase in young adults with ALL[20]. This prompted us to institute in May 2015 strict manadatory premedication with acetaminophen (10–15 mg/kg orally), diphenhydramine (1 mg/kg orally or intravenously), and methylprednisolone (1 mg/kg intravenously), within the hour prior to administering pegaspargase. Every subsequent patient was to receive with all three of the premedication drugs without exception. The numbers with and without premedication are listed in Table 2 (per pegaspargase dose) and Table 3 (per patient).

Assessment of allergic reactions

Allergic reactions to were graded per CTC 4.0 toxicity scales. We compared the incidence of grade 3 or 4 allergic reaction in patients with and without premedication, both per pegaspargase dose and per patient.

Pharmacokinetic analysis

Routine monitoring of pegaspargase activity in patients with ALL was initiated in 2013 after the 'asparaginase activity analysis' test approved by Clinical Laboratory Improvement Amendments was introduced by AIBioTech, Richmond, VA 23225 US. Subsequent to the introduction in 2015 of a quantitatively identical test by Next Molecular Analytics, Chester, VA, samples were exclusively sent there.

Statistical analyses

SPSS version 23 was used for graphing and analyses. Grouped data were displayed with box graphs depicting the ~1st, 25th, 50th (median), 75th and ~99th percentiles. The data was normally distributed by visual inspection of the normal curve and Kolmogorov-Smirnov test, with the exception of the day 6–8 pegaspargase activity level. Removal of one extreme outlier as described above rendered the day 6–8 pegaspargase activity level normally distributed. The comparison of pegaspargase activity with and without premedication was done by independent sample t-test. The comparison of grade 3 or 4 allergic reactions by patient and pegaspargase dose with and without the use of premedication was done by chi-squared analysis. As some premedication doses were missed due to omission by the treating physician, an additional analysis of the incidences of those who received premedication after every dose or most doses were compared to those who received no premedication before any dose. Missed pegaspargase activity samples were omitted from analysis (Table I).

Results

Pharmacokinetic analyses were done on 100 specimens from 46 patients. The 46 patients included 12 standard risk B-cell patients, 21 high risk B-cell ALL patients, and 13 T-cell ALL patients. There were 25 males and 21 females. The ages ranged from one to 29 years with a median of 8.3 years. The number of

Table 1. Percent of pegaspargase activity specimens collected following doses one to nine.

	One	Two	Three	Four	Five	Six	Seven	Eight	Nine	Total
Collected	8	33	18	19	18	6	7	2	1	112
Missed	38	12	16	10	7	6	3	3	1	96
Total	46	45	34	29	25	12	10	5	2	208
% Collected	17%	73%	53%	66%	72%	50%	70%	40%	50%	54%

Table 2. Grade 3–4 allergic reactions by use of premedication per dose of pegaspargase.

Premeds used with the dose	Total doses	Doses with allergic reaction	Percent of doses with grade 3–4 allergic reaction
no	155	8	5.2%
yes	185	7	3.7%

Table 3. Grade 3–4 allergic reactions by use of premedication per patient.

	Grade 3–4 allergic reactions	None	Total
No premedication	7 (17%)	35 (83%)	42
Some premedication	4 (6%)	58 (94%)	62
Every dose premedication	4 (12%)	30 (88%)	34

specimens and missed specimens per pegaspargase dose number are shown in Table 1. These include all patients who had pegaspargase activity collected which included those eliminated from other analyses as they were not in first remission. First dose specimens were frequently missed, whereas specimens on doses two to seven were collected at least half the time.

Figure 1 is a box and whisker graph of pegaspargase activity on days 3–5, 6–8 and 10–12. The mean, standard error of the mean, standard deviation and number of data points are: 1.37, 0.21 0.76 units/mL and 13, respectively, for day 3–5; 0.88, 0.04, 0.31 units/mL and 61 for day 6–8; 0.89, 0.06, 0.28 units/mL and 26 for day 10–12. These values are similar to those previously reported in pediatric patients with ALL[21,22].

Figure 2 is box and whisker graph of the pegaspargase activity on day 6–8, following doses with or without premedication. This time ranged was used for the comparison as it is the most common time for checking asparaginase activity. The mean and standard deviation for the no premedication group is 0.69 and 0.21 units/mL (N=12 samples), respectively, and for the premedication group is 0.93 and 0.32 units/mL (N=49 samples). These were significantly different by independent samples t-tests for equal variances not assumed (p = 0.003). The day 3–5 data had only one patient in the no premedication group so was not analyzed. The day 10–12 data showed higher median value in the group that received premedications (0.92 vs 0.83) which was not statistically significant due to insufficient power.

Only one patient had silent inactivation with the following activity levels by dose number and day following pegaspargase activity was checked: dose 1, day 24 - 0.11 units/mL; dose 2, day 8 - 0.05 units/mL; dose 3 day 6 - 0.01 units/mL; dose 4 day 8 - 0.33 units/mL; dose 5, day 8 - 0.82 units/mL; and dose 6, day 10 - 0.62 units/mL The low values after doses 2 and 3 were not reviewed due to a clerical error until after dose 4, which showed adequate activity, so pegaspargase was continued until the end of treatment.

For the analysis of the role of premedication in preventing grade 3–4 allergic reactions, the data was analyzed per pegaspargase dose and per patient. In the analysis per pegaspargase dose, premedication did not significantly reduce grade 3–4 allergic reactions. However due to insufficient numbers the power was only 0.24. With premedication, 7/185 (3.7%) had grade 3–4 allergic reactions compared to 8/155 (5.2%) without premedication (Table 2).

Table 3 shows the incidence of grade 3–4 allergic reactions per patient. Without premedication, 7/42 (17%) had grade 3–4 allergic reactions. When premedication was given most of the time (usually the first dose was missed), 4/62 (6%) had grade 3–4 allergic reactions. When premedication was given for every dose, 4/34 (12%) had allergic reactions. There was no significant effect of premedication on grade 3–4 allergic reactions by dose when the premedication group (8/96; 8.3%) was compared to the no premedication group (7/42; 17%) (chi square = 2.09; p = 0.15) (Table 3). However due to insufficient numbers the power was only 0.54. There was no difference in the distribution of patients who did or did not receive premedication by risk group.

Discussion

Compared with historical controls that received similar therapy, premedication did reduce the incidence of grade 3 or 4 allergic reactions when measured per patient or per dose of pegaspargase. The power calculation was only 0.54 per patient and 0.24 per pegaspargase dose, thus our study was underpowered to show statistical significance due to insufficient numbers. As premedication does not negatively affect pegaspargase activity levels, and other studies using historical comparisons have suggested premedication may reduce allergic reactions, we are continuing the practice[8,9].

The interesting observation by Tong *et al.* that asparaginase antibodies generated after native *E. coli* asparaginase may resolve while on pegaspargase continuation therapy needs to be confirmed in patients who receive only pegaspargase during
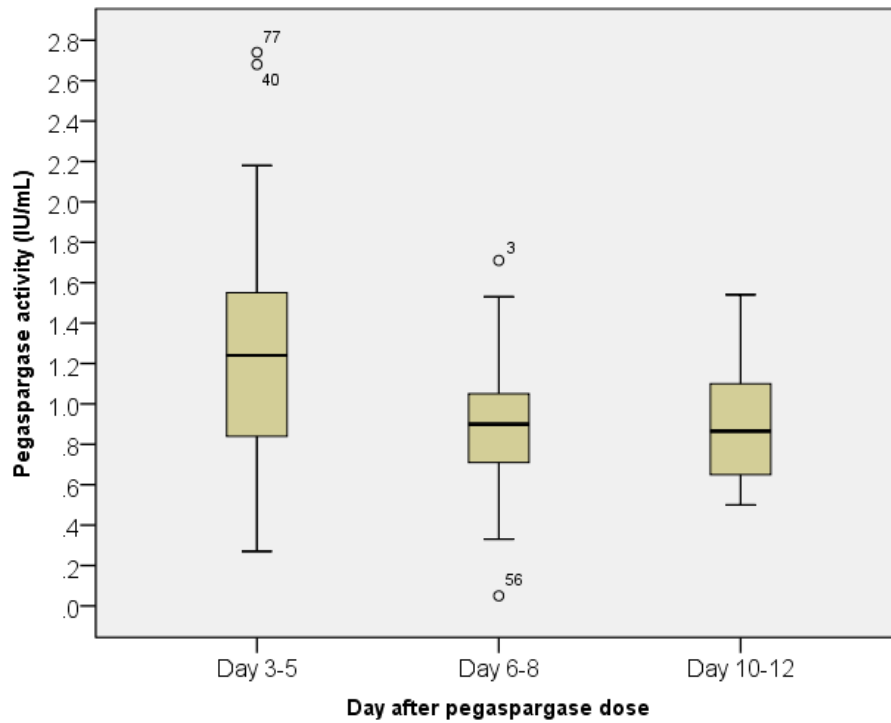
Figure 1. Box plot of pegaspargase activity following 2500 units/m² on day 3–5, 6–8 and 10–12. The data point below the line is from the patient with silent inactivation. Data points outside of the whiskers of the ~1st and ~99th percentiles are represented by a circle (outlier more than 1.5 times the interquartile range). The attached number is a data point and not a value.

Figure 2. Box plot of pegaspargase activity day 6–8 after 2500 units/m² with and without premedication. Patients who received premedication has a significantly greater value (p=0.003). The mean and standard deviation for the premedication group is 0.93 and 0.32 units/mL and for the no premedication group is 0.69 and 0.21 units/mL. Data points outside of the whiskers of the ~1st and ~99th percentiles are represented by a circle (outlier more than 1.5 times the interquartile range). The attached number is a data point and not a value.
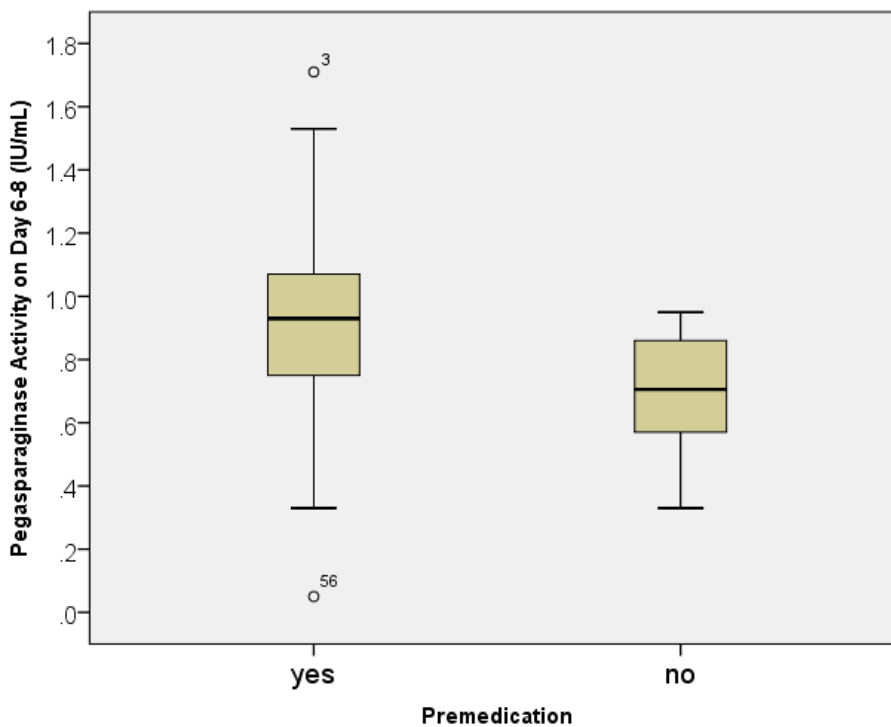
induction and beyond[14,19]. We noted a transient decrease in pegaspargase activity, likely due to silent inactivating antibodies in 1/59 patients (1.7%). This decrease of pegaspargase activity occurred after the second dose in a high-risk B-cell ALL patient and resolved with continuation of pegaspargase dosing. No decrease in pegaspargase activity was seen in standard risk patients who received only two doses of pegaspargase in combination with oral dexamethasone. This observation is limited by small numbers of patients and multiple missed levels after the first pegaspargase dose.

Limitations of the study include multiple missed activity levels that may have found additional patients with silent inactivation. The sample size also makes it difficult to estimate the true incidence of silent inactivation and if premedication reduces the incidence of grade 3 or 4 allergic reactions. Additional studies are needed to clarify this. Another limitation is the patients had only one activity level per pegaspargase dose, limiting evaluation of true pharmacokinetics especially in patients with higher or lower than expected levels.

We found low incidence of silent inactivation with intravenous pegaspargase. Our study suggests that patients treated with regimens that include only two doses of pegaspargase, given with dexamethasone, may not need asparaginase levels due to even lower silent inactivation, but this would need confirmation by larger studies. We do suggest that patients who have questionable allergic reactions (grade 1–2) would benefit from asparaginase levels that will direct switch to erwinase if confirmed as true inactivation.

Our data showed a statistically significantly greater pegaspargase activity of the day 6–8 pegaspargase level with the use of premedication. Analysis of the day 3–5 and 10–12 pegaspargase levels did not show a significant difference likely due to the small sample sizes.

The finding of difference in the pegaspargase level with premedication has not been previously described to our knowledge. This may be of clinical significance and should, if possible confirmed in other retrospective studies. Given the increased acceptance of premedication for pegaspargase it is unlikely and perhaps unethical to confirm in a prospective randomized trial, in our opinion.

Addendum
A recent publications by 2 other groups also reported reduction of infusion reactions and need for erwinase using premedication. They also found a low incidence of silent inactivation with intravenous pegaspargase of 1.5% in one report and < 1% in another report similar to our finding of one in 59 patients (1.7%)[23,24].

Data availability
Underlying data
Underlying data available at https://doi.org/10.6084/m9.figshare.8281826.v1

References

1. Stock W, Douer D, DeAngelo DJ, et al.: **Prevention and management of asparaginase/pegasparaginase-associated toxicities in adults and older adolescents: recommendations of an expert panel.** Leuk Lymphoma. 2011; **52**(12): 2237–53.
   PubMed Abstract | Publisher Full Text

2. Bostrom B, Uppal P, Chu J, et al.: **Safety and efficacy of metformin for therapy-induced hyperglycemia in children with acute lymphoblastic leukemia.** J Pediatr Hematol Oncol. 2013; **35**(7): 504–8.
   PubMed Abstract | Publisher Full Text

3. Bostrom B: **Successful management of extreme hypertriglyceridemia from pegaspargase with omega-3.** Pediatr Blood Cancer. 2012; **59**(2): 350.
   PubMed Abstract | Publisher Full Text

4. Seah J, Lin K, Tai D, et al.: **Conservative management of L-asparaginase-induced hypertriglyceridemia in an adult patient: a case report and review of the literature.** Onkologie. 2012; **35**(10): 596–8.
   PubMed Abstract | Publisher Full Text

5. Alshiekh-Nasany R, Douer D: **L-Carnitine for Treatment of Pegasparaginase-Induced Hepatotoxicity.** Acta Haematol. 2016; **135**(4): 208–10.
   PubMed Abstract | Publisher Full Text

6. Salzer W, Bostrom B, Messinger Y, et al.: **Asparaginase activity levels and monitoring in patients with acute lymphoblastic leukemia.** Leuk Lymphoma. 2017; **18**: 1–10.
   PubMed Abstract | Publisher Full Text

7. Douer D, Aldoss I, Lunning MA, et al.: **Pharmacokinetics-based integration of multiple doses of intravenous pegaspargase in a pediatric regimen for adults with newly diagnosed acute lymphoblastic leukemia.** J Clin Oncol. 2014; **32**(9): 905–11.
   PubMed Abstract | Publisher Full Text

8. Stock W, Luger SM, Advani AS, et al.: **A pediatric regimen for older adolescents and young adults with acute lymphoblastic leukemia: results of CALGB 10403.** Blood. 2019; **133**(14): 1548–1559.
   PubMed Abstract | Publisher Full Text | Free Full Text

9. Aldoss I, Douer D, Behrendt CE, et al.: **Toxicity profile of repeated doses of PEG-asparaginase incorporated into a pediatric-type regimen for adult acute lymphoblastic leukemia.** Eur J Haematol. 2016; **96**(4): 375–80.
   PubMed Abstract | Publisher Full Text

10. Fernandez CA, Smith C, Karol SE, et al.: **Effect of premedications in a murine model of asparaginase hypersensitivity.** J Pharmacol Exp Ther. 2015; **352**(3): 541–551.
    PubMed Abstract | Publisher Full Text | Free Full Text

11. Liu C, Kawedia JD, Cheng C, et al.: **Clinical utility and implications of asparaginase antibodies in acute lymphoblastic leukemia.** Leukemia. 2012; **26**(11): 2303–9.
    PubMed Abstract | Publisher Full Text | Free Full Text

12. Ko RH, Jones TL, Radvinsky D, et al.: **Allergic reactions and antiasparaginase antibodies in children with high-risk acute lymphoblastic leukemia: A children's oncology group report.** Cancer. 2015; **121**(23): 4205–11.
    PubMed Abstract | Publisher Full Text | Free Full Text

13. 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**(13): 2026–2033.
PubMed Abstract | Publisher Full Text | Free Full Text

14. 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**(7): e102–4.
PubMed Abstract | Publisher Full Text | Free Full Text

15. Place AE, Stevenson KE, Vrooman LM, *et al.*: **Intravenous pegylated asparaginase versus intramuscular native *Escherichia coli* L-asparaginase in newly diagnosed childhood acute lymphoblastic leukaemia (DFCI 05-001): a randomised, open-label phase 3 trial.** *Lancet Oncol.* 2015; **16**(16): 1677–90.
PubMed Abstract | Publisher Full Text

16. Park JH, Ritchie EK, Rao AV, *et al.*: **A Pediatric-Inspired Regimen Containing Multiple Doses of Intravenous Pegylated Asparaginase Appears Safe and Effective in Newly Diagnosed Adult Patients with Ph-Negative Acute Lymphoblastic Leukemia in Adults up to Age 60: Results of a Multi-Center Phase II Clinical Trial.** *Blood.* 2016; **128**(22): 1629–29.
Publisher Full Text

17. Schore RJ, Devidas M, Bleyer A, *et al.*: **Plasma asparaginase activity and asparagine depletion in acute lymphoblastic leukemia patients treated with pegaspargase on Children's Oncology Group AALL07P4.** *Leuk Lymphoma.* 2019; **60**(7): 1740–48.
PubMed Abstract | Publisher Full Text | Free Full Text

18. 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**(3): 279–85.
PubMed Abstract | Publisher Full Text | Free Full Text

19. Beaupin LK, Bostrom B, Barth MJ, *et al.*: **Pegaspargase hypersensitivity reactions: intravenous infusion versus intramuscular injection - a review.** *Leuk Lymphoma.* 2017; **58**(4): 766–772.
PubMed Abstract | Publisher Full Text

20. Advani AS, Sanford B, Luger S, *et al.*: **Frontline-Treatment Of Acute Lymphoblastic Leukemia (ALL) In Older Adolescents and Young Adults (AYA) Using a Pediatric Regimen Is Feasible: Toxicity Results of the Prospective US Intergroup Trial C10403 (Alliance).** *Blood.* 2013; **122**(21): 3903.
Publisher Full Text

21. Silverman LB, Supko JG, Stevenson KE, *et al.*: **Intravenous PEG-asparaginase during remission induction in children and adolescents with newly diagnosed acute lymphoblastic leukemia.** *Blood.* 2010; **115**(7): 1351–3.
PubMed Abstract | Publisher Full Text | Free Full Text

22. Angiolillo AL, Schore RJ, Devidas M, *et al.*: **Pharmacokinetic and pharmacodynamic properties of calaspargase pegol *Escherichia coli* L-asparaginase in the treatment of patients with acute lymphoblastic leukemia: results from Children's Oncology Group Study AALL07P4.** *J Clin Oncol.* 2014; **32**(34): 3874–82.
PubMed Abstract | Publisher Full Text | Free Full Text

23. Cooper SL, Young DJ, Bowen CJ, *et al.*: **Universal premedication and therapeutic drug monitoring for asparaginase-based therapy prevents infusion-associated acute adverse events and drug substitutions.** *Pediatr Blood Cancer.* 2019; **66**(8): e27797.
PubMed Abstract | Publisher Full Text

24. Marini BL, Brown J, Benitez L, *et al.*: **A single-center multidisciplinary approach to managing the global Erwinia asparaginase shortage.** *Leuk Lymphoma.* 2019; **60**(12): 2854–2868.
PubMed Abstract | Publisher Full Text

Open Peer Review

Current Peer Review Status: ❓ ❓ ✔

Version 2

Reviewer Report 04 February 2020

https://doi.org/10.5256/f1000research.24084.r58717

✔ **David J. Young** (ID)

Translational Stem Cell Biology Branch of the National Heart, Lung, and Blood Institute, National Institutes of Health (NIH), Bethesda, MD, USA

The authors have responded to each of the comments by all three reviewers, making the suggested corrections, providing acceptable alternative approaches, or explaining why the changes would not be possible. These revisions have not raised any new concerns.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pediatrics, pediatric hematology/oncology, drug development, clinical trials, leukemia

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 01 October 2019

https://doi.org/10.5256/f1000research.21156.r53445

? **David J. Young** (iD)

Translational Stem Cell Biology Branch of the National Heart, Lung, and Blood Institute, National Institutes of Health (NIH), Bethesda, MD, USA

This report by Losasso, Bostrom and Messinger expand upon our growing understanding of the appropriate treatment with and management of asparaginase-based therapies in lymphoblastic leukemia. In this retrospective study, the authors have reviewed the single center experience of therapeutic drug monitoring, examining patient drug levels to determine the incidence of drug inactivation and looked at the relationship between pre-treatment and serious adverse events.

In their review, the authors found a 1.7% rate of drug inactivation. Of note, this rate is comparable to several different prior studies which the authors have referred here. Although the authors did not measure directly for inactivating antibodies, the use of drug activity levels to impute the presence of antibodies is now considered an acceptable approach as it is generally the primary determinant of drug activity for most patients, and as such is not a limitation in this work.

Their observation of transient inactivation and then recovery of drug efficacy is an important finding, and bears future examination as this strongly argues, as others have, for rechallenging patients with prior history, especially in high-risk patients where receiving multiple doses of asparaginase has been conclusively linked to better outcomes.

Contrary to other studies, the authors remark that they did not observe a significant decrease in clinically significant (grade 3-4) reactions with predication. Although statistically correct, it is difficult to support this statement for several reasons. First, although the authors do not see a ***statistically*** significant effect, there is without a doubt a ***clinically important*** trend of 50% reduction in events. Indeed, this would represent an absolute risk reduction of 8.7% with a number needed to treat of about 11, and is almost identical to other studies. *Post hoc* power analysis, although an admitted abuse of statistics, suggests that this data may have reached significance with 30-40 additional patients.

Furthermore, the authors themselves continue to use and argue for premedication, which would argue that they agree that there is benefit to pre-medication despite the lack of statistical findings. Yet, the only reason for pre-medication, given the historic concerns regarding silent inactivation, is to reduce severe adverse reactions. These contradictions need to be resolved.

Finally, the authors suggest that monitoring of drug levels is not necessary in low-risk patients, given that they did not identify inactivation during the first two doses. This statement is too strong. The authors themselves admit that there was a large number (83%) of missed levels during the first administration, likely a consequence of the admittedly chaotic logistics of early induction therapy experienced at many institutes. Although their data do suggest that silent inactivation following one dose would likely still be detectable at the second dose, this has not been rigorously tested, and is based primarily upon the experience of a single patient. Therefore, to include the recommendation of not testing low-risk patients, especially its inclusion within the abstract that (regrettably) may be all that some read, and without mentioning the rate of missed levels in the same abstract, is difficult to support.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pediatrics, pediatric hematology/oncology, drug development, clinical trials, leukemia

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 19 Dec 2019
Bruce Bostrom, Children's Minnesota, 2525 Chicago Avenue South, Minneapolis, USA

David Young Reviewer 3
This report by Losasso, Bostrom and Messinger expand upon our growing understanding of the appropriate treatment with and management of asparaginase-based therapies in lymphoblastic leukemia. In this retrospective study, the authors have reviewed the single center experience of therapeutic drug monitoring, examining patient drug levels to determine the incidence of drug inactivation and looked at the relationship between pre-treatment and serious adverse events.

In their review, the authors found a 1.7% rate of drug inactivation. Of note, this rate is comparable to several different prior studies which the authors have referred here. Although the authors did not measure directly for inactivating antibodies, the use of drug activity levels to impute the presence of antibodies is now considered an acceptable approach as it is generally the primary determinant of drug activity for most patients, and as such is not a limitation in this work.

Their observation of transient inactivation and then recovery of drug efficacy is an important finding, and bears future examination as this strongly argues, as others have, for re-challenging patients with prior history, especially in high-risk patients where receiving multiple doses of asparaginase has been conclusively linked to better outcomes.

Contrary to other studies, the authors remark that they did not observe a significant decrease in clinically significant (grade 3-4) reactions with predication. Although statistically correct, it is difficult to support this statement for several reasons. First, although the authors do not see a ***statistically*** significant effect, there is without a doubt a ***clinically important*** trend of 50% reduction in events. Indeed, this would represent an absolute risk reduction of 8.7% with a number needed to treat of about 11 and is almost identical to other studies. *Post hoc* power analysis, although an admitted

abuse of statistics, suggests that this data may have reached significance with 30-40 additional patients.

Furthermore, the authors themselves continue to use and argue for premedication, which would argue that they agree that there is benefit to pre-medication despite the lack of statistical findings. Yet, the only reason for pre-medication, given the historic concerns regarding silent inactivation, is to reduce severe adverse reactions. These contradictions need to be resolved.

Response: we agree with the reviewer points and his astute observations. We would have included his *Post hoc* analysis if we could but were concerned like him about those who would view these statistics unkindly. Several things were altered: In Discussion 1st paragraph this sentence is now changed: "Compared with historical controls that received similar therapy, premedication did reduce the incidence of grade 3 or 4 allergic reactions when measured per patient or per dose of pegaspargase. The power calculation was only 0.54 per patient and 0.24 per pegasparaginase dose thus our study was underpowered to show statistical significance." The 4th paragraph this whole section was removed: "Contrary to the findings of a large multi-institutional trial, where the introduction of premedication significantly reduced the incidence of high-grade allergic reactions, our study did not show a statistically significant reduction with the use of premedication. [7] Despite this, we continue to use premedication in all patients receiving pegasparaginase."

Finally, the authors suggest that monitoring of drug levels is not necessary in low-risk patients, given that they did not identify inactivation during the first two doses. This statement is too strong. The authors themselves admit that there was a large number (83%) of missed levels during the first administration, likely a consequence of the admittedly chaotic logistics of early induction therapy experienced at many institutes. Although their data do suggest that silent inactivation following one dose would likely still be detectable at the second dose, this has not been rigorously tested, and is based primarily upon the experience of a single patient. Therefore, to include the recommendation of not testing low-risk patients, especially its inclusion within the abstract that (regrettably) may be all that some read, and without mentioning the rate of missed levels in the same abstract, is difficult to support.

Response: we do agree with the reviewer completely that the number of missed levels after the 1st dose is very high. We also agree that our numbers are too small for this strong conclusion. We have altered the abstract to include this sentence in the results: "Asparaginase dose levels were mostly missed after the first asparaginase dose." We eliminated this sentence: "No patient had silent inactivation after the first pegaspargase dose." And the conclusions: "We found low incidence of silent inactivation with intravenous pegaspargase. Future studies may confirm if patients treated with regimens with only two doses of pegasparaginase, given with dexamethasone, may not need asparaginase levels due to even lower silent inactivation."

In discussion 2nd paragraph this was altered: "No decrease in pegaspargase activity was seen in standard risk patients who received only two doses of pegaspargase in combination with oral dexamethasone. This observation is limited by small numbers of patients and multiple missed levels after the first pegaspargase dose." The 4th paragraph was altered also: "We found low incidence of silent inactivation with intravenous pegaspargase. Our study suggests that patients treated with regimens that include only two doses of pegasparaginase, given with dexamethasone, may not need asparaginase levels due to even lower silent inactivation, but this would need confirmation by larger studies."

- **Is the work clearly and accurately presented and does it cite the current literature?**
Yes
- **Is the study design appropriate and is the work technically sound?**Yes
- **Are sufficient details of methods and analysis provided to allow replication by others?**Yes
- **If applicable, is the statistical analysis and its interpretation appropriate?**Yes
- **Are all the source data underlying the results available to ensure full reproducibility?**Yes
- **Are the conclusions drawn adequately supported by the results?**Partly

Competing Interests*No competing interests were disclosed.*

Competing Interests: No competing interests

Reviewer Report 30 September 2019

? **Vinod Pullarkat**

Department of Hematology and Hematopoietic Cell Transplantation, Gehr Family Center for Leukemia Research, City of Hope Medical Center, Duarte, CA, USA

In this manuscript the authors have retrospectively examined the incidence of allergic reactions in a cohort of patients with ALL receiving multiple doses of PEGylated aspraginase. They show that premedication did not have an impact on allergic reactions. The study has drawbacks inherent in a retrospective analysis of a heterogeneously treated population with non standardized blood sampling as acknowledged by the authors. My comments are as follows:

1. A concern with premedication is whether it will mask a true antibody mediated allergy. A major question therefore is whether the allergic reactions observed were actually antibody mediated allergic reactions or infusion reactions that do not affect asparaginase activity. Therefore authors should show the asparaginase levels of patients who had a reaction and compare to those who did not, in order to show whether the reactions observed are due to drug neutralizing antibodies.

2. In their conclusion (in the abstract) the authors focus on patients who receive only 2 doses of PEG asparaginase. In most regimens patients will receive more doses of PEG asparaginase and hence it is unclear why authors focus on patients who receive only 2 doses. The correct conclusion in my opinion is that premedication did not impact the incidence of allergic reaction and premedication did not mask silent inactivation.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
No

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: Speakers Bureau and Advisory board for Servier and Jazz pharmaceuticals

Reviewer Expertise: Treatment of acute lymphoblastic leukemia

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 19 Dec 2019

Bruce Bostrom, Children's Minnesota, 2525 Chicago Avenue South, Minneapolis, USA

Vinod Pullarkat: Department of Hematology and Hematopoietic Cell Transplantation, Gehr Family Center for Leukemia Research, City of Hope Medical Center, Duarte, CA, USA

In this manuscript the authors have retrospectively examined the incidence of allergic reactions in a cohort of patients with ALL receiving multiple doses of PEGylated asparaginase. They show that premedication did not have an impact on allergic reactions. The study has drawbacks inherent in a retrospective analysis of a heterogeneously treated population with non-standardized blood sampling as acknowledged by the authors. My comments are as follows:

1. A concern with premedication is whether it will mask a true antibody mediated allergy. A major question therefore is whether the allergic reactions observed were actually antibody mediated allergic reactions or infusion reactions that do not affect asparaginase activity. Therefore authors should show the asparaginase levels of patients who had a reaction and compare to those who did not, in order to show whether the reactions observed are due to drug neutralizing antibodies.

Response: It would have been wonderful to be able to do this, but we cannot. Unfortunately, when patients had a reaction (be it allergic reaction or infusion reaction) no further pegaspargase was given. Therefore, these patients had no subsequent asparaginase activity measured. Additionally, this is an inherent difficulty with these patients – whose infusion is stopped right away and they never receive full dose of pegaspargase rendering subsequent asparaginase activity meaningless.

1. In their conclusion (in the abstract) the authors focus on patients who receive only 2 doses of PEG asparaginase. In most regimens patients will receive more doses of PEG asparaginase and hence it is unclear why authors focus on patients who receive only 2 doses. The correct conclusion in my opinion is that premedication did not impact the incidence of allergic reaction and premedication did not mask silent inactivation.

Response: We agree completely. The abstract and the paper has changes that completely address this. To the Abstract conclusion we added this sentence: "Though premedication resulted in lower allergic reactions the difference was not statistically different due to insufficient power." In discussion the 1st paragraph completely addresses this: "Compared with historical controls that received similar therapy, premedication did reduce the incidence of grade 3 or 4 allergic reactions when measured per patient or per dose of pegaspargase. The power calculation was only 0.54 per patient and 0.24 per pegasparaginase dose, thus our study was underpowered to show statistical significance due to insufficient numbers."

The comment suggesting not monitoring levels in patients receiving only two doses of pegasparaginase is to potentially minimize unnecessary testing in this group of patients. In COG protocols only two doses of pegasparaginase are given to lower risk children who make up the majority of patients under the age of 18. However we have removed this recommendation due to large number of first dose pegaspargase levels missed in these patients.

- **Is the work clearly and accurately presented and does it cite the current literature?** Yes
- **Is the study design appropriate and is the work technically sound?** Yes
- **Are sufficient details of methods and analysis provided to allow replication by others?** Yes
- **If applicable, is the statistical analysis and its interpretation appropriate?** Partly
- **Are all the source data underlying the results available to ensure full reproducibility?** No
- **Are the conclusions drawn adequately supported by the results?** Partly

Competing Interests: *Speakers Bureau and Advisory board for Servier and Jazz pharmaceuticals*

Reviewer Expertise: Treatment of acute lymphoblastic leukemia

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests

Reviewer Report 26 September 2019

? **Bernard L. Marini** iD

Department of Pharmacy Services and Clinical Sciences, Michigan Medicine, University of Michigan College of Pharmacy, Ann Arbor, MI, USA

Drs. Losasso, Bostrom, and Messinger should be applauded for their work in characterizing asparaginase activity levels in ALL patients. The results and research are clearly presented and accurately reported. The conclusions of the study are appropriate, and the limitations of this retrospective cohort study are

well-described.

Some specific suggestions/comments/recommendations:

Abstract:
- I would include the n in the incidence of hypersensitivity reactions, or acknowledge that the study may not have been powered to assess this outcome. Readers who only look at the abstract may wrongly conclude pre-medication is not effective, when the analysis may have been underpowered to detect a statistically significant difference in hypersensitivity reactions in the pre-medication vs. no pre-medication group.

- The paper, methods, and title are focused on the impact of pre-medication on activity levels; however, the conclusion primarily talks about the low incidence of silent inactivation with two doses; thus monitoring only being necessary in patients that receive >2 doses. I would consider a sentence re: pre-medication here as well.

Intro:
- Not essential, but could consider mentioning fibrates in the treatment of hypertriglyceridemia, which is another first line option.

- Would cite more recent data on the impact of pre-medication on preventing hypersensitivity reactions - Cooper SL, Young DJ, Bowen CJ, Arwood NM, Poggi SG, Brown PA. Universal premedication and therapeutic drug monitoring for asparaginase-based therapy prevents infusion-associated acute adverse events and drug substitutions. Pediatr Blood Cancer. 2019 Aug;66(8):e27797.[1] and Marini BL, Brown J, Benitez L, Walling E, Hutchinson RJ, Mody R, Jasty Rao R, Slagle L, Bishop L, Pettit K, Bixby DL, Burke PW, Perissinotti AJ. A single-center multidisciplinary approach to managing the global Erwinia asparaginase shortage. Leuk Lymphoma. 2019 May 17:1-15[2].

- I would be careful with stating asparaginase antibodies are always predictive of future allergic reactions, as not all antibodies inactivate asparaginase or lead to clinical hypersensitivity reactions (ex: CCG01961). Consider rewording to soften this language.

- In paragraph 5, the statement is made that silent inactivation is of greater concern than allergic reactions. However, one could argue that allergic reactions are more common than silent inactivation with asparaginase (~1%), and monitoring activity levels in the setting of grade 1-2 allergic reactions is essential to determine which patients with clinical hypersensitivity reactions are having true antibody mediated reactions vs. other, non-antibody mediated (possibly ammonia vs. other immune-mediated) allergic reactions that would not warrant a switch to another asparaginase preparation.

- Other large scale studies assessing the rate of silent inactivation and finding the rate to be <8% could also be included in this discussion (or cited). These include, AALL07P4, Schore, et al. Leuk Lymph 2019 [3], CCG1962, and Park JH, et al. Blood. 2016;128:1629[4].

Methods:
- While a sample size was not calculated a priori, could the authors conduct a sensitivity analysis on the data determined the detectable effect size at 80% power in the primary outcome of interest (rate of grade 3/4 allergic reactions?)?

- Were dose reductions in PEG ever conducted in this data set for toxicity/age/risk factors for hepatotoxicity?

- It is unclear how silent inactivation is defined in this data-set from the methods. It may also be clinically relevant to capture "accelerated clearance", which also may be clinically relevant. Not all anti-asparaginase antibodies lead to full inactivation of the drug, but some lead to accelerated clearance and lower than expected values at early time points. A patient with silent inactivation would have a peak level that is undetectable, whereas a patient with accelerated clearance may clear the PEG by day ~7 when an expected duration of asparagine depletion is 21-28 days with a full pediatric dose.

- Were any levels obtained in patients with low-grade hypersensitivity reactions? If so, did any of these patients have accelerated clearance/"loud" inactivation?

- Were the activity levels normally distributed? If not, perhaps medians in the box plot and a Mann Whitney U for comparison would be more accurate. Similarly, if the values in the 2x2 contingency table are small, consider a Fisher's exact test rather than chi-square for the incidence of hypersensitivity reaction comparison (although it likely won't make a significant difference).

- Was there a protocol to say every patient should have a level with every dose, or were levels obtained at random/provider discretion? Could there then be a selection bias in the patients who have activity levels obtained since so many were missed? I'm assuming first dose levels were "missed" because the risk of inactivation with the first dose should theoretically be zero, so they were perhaps more accurately intentionally omitted.

Results:
- Is there any possibility that the level on day 6-8 of 2.8 was spurious? Were there any concomitant levels in this patient with this dose to make sense of such a high level?

- The one patient being described as having silent inactivation - I do not think I would characterize this as silent inactivation technically, since they had detectable activity levels at several time points, and no peak level is ever undetectable. I would consider this a transient accelerated clearance with dose 2 and 3. If silent inactivation was truly present, a patient should not have activity present for a full week and then have full activity with later doses. Patients with activity levels even as low as ~0.02 may still have asparagine depletion (AALL07p4).

- It may be interesting to graph levels over time in some of these patients with very low levels. Was there ever more than one level obtained per dose? Were repeat levels obtained to verify the low activity levels or follow the activity level trend over time (as is suggested in some guidelines?). This would help characterize the PK better in these patients.

- Was the choice to pre-medicate done at a strict, uniform point in time (i.e., was this a quasi-experimental design where a protocol to pre-medicate all patients was mandated?), or was pre-medication at the choice of the treating provider? If this was selected by the provider and not systematic, could there have been some slight selection bias where patients perceived to be at higher risk of hypersensitivity reactions were given pre-medication? This could have diminished the impact of pre-medication on reducing the rate of hypersensitivity reactions. Consider this in the discussion section if deemed relevant.

Discussion:

- I would more accurately call the silent inactivation here "accelerated clearance" as the patient had asparagine depletion for at least a week, even with the low level.

- Consider discussing the possible value of obtaining activity levels in patients with questionable allergic reactions (grade 1/2).

- Could add as a limitation that it appears patients only had one activity level obtained per dose, which makes it challenging to interpret PK, especially in patients with higher or lower than expected activity levels.

Overall, a very well-done paper with logical conclusions. Thank you for allowing me to review the manuscript and for your hard work in this research.

References

1. Cooper SL, Young DJ, Bowen CJ, Arwood NM, Poggi SG, Brown PA: Universal premedication and therapeutic drug monitoring for asparaginase-based therapy prevents infusion-associated acute adverse events and drug substitutions.*Pediatr Blood Cancer*. 2019; **66** (8): e27797 PubMed Abstract | Publisher Full Text

2. Marini BL, Brown J, Benitez L, Walling E, Hutchinson RJ, Mody R, Jasty Rao R, Slagle L, Bishop L, Pettit K, Bixby DL, Burke PW, Perissinotti AJ: A single-center multidisciplinary approach to managing the global Erwinia asparaginase shortage.*Leuk Lymphoma*. 2019. 1-15 PubMed Abstract | Publisher Full Text

3. Schore RJ, Devidas M, Bleyer A, Reaman GH, Winick N, Loh ML, Raetz EA, Carroll WL, Hunger SP, Angiolillo AL: Plasma asparaginase activity and asparagine depletion in acute lymphoblastic leukemia patients treated with pegaspargase on Children's Oncology Group AALL07P4.*Leuk Lymphoma*. 2019; **60** (7): 1740-1748 PubMed Abstract | Publisher Full Text

4. Park JH, Ritchie EK, Rao AV, Devlin S, Hsu M, Khawaja TT, O'Donnell B, Cazacu MI, Stopka DM, Tallman MS, Douer D: A Pediatric-Inspired Regimen Containing Multiple Doses of Intravenous Pegylated Asparaginase Appears Safe and Effective in Newly Diagnosed Adult Patients with Ph-Negative Acute Lymphoblastic Leukemia in Adults up to Age 60: Results of a Multi-Center Phase II Clinical Trial. *Blood*. 2016; **128** (1629).

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: Servier (consultant)

Reviewer Expertise: Asparaginase, leukemia treatment, therapeutic drug monitoring

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 19 Dec 2019

Bruce Bostrom, Children's Minnesota, 2525 Chicago Avenue South, Minneapolis, USA

Bernard L. Marini, Department of Pharmacy Services and Clinical Sciences, Michigan Medicine, University of Michigan College of Pharmacy, Ann Arbor, MI, USA

Drs. Losasso, Bostrom, and Messinger should be applauded for their work in characterizing asparaginase activity levels in ALL patients. The results and research are clearly presented and accurately reported. The conclusions of the study are appropriate, and the limitations of this retrospective cohort study are well-described.

Some specific suggestions/comments/recommendations:

Abstract:
- I would include the n in the incidence of hypersensitivity reactions, or acknowledge that the study may not have been powered to assess this outcome. Readers who only look at the abstract may wrongly conclude pre-medication is not effective, when the analysis may have been underpowered to detect a statistically significant difference in hypersensitivity reactions in the pre-medication vs. no pre-medication group.Response: done. Number of patients was added. Dr Marini is correct that the study was underpowered and we have added statements to that effect in the abstract and first paragraph of the discussion.
- The paper, methods, and title are focused on the impact of pre-medication on activity levels; however, the conclusion primarily talks about the low incidence of silent inactivation with two doses; thus monitoring only being necessary in patients that receive >2 doses. I would consider a sentence re: pre-medication here as well. Response: done. Sentence added: "Though premedication resulted in lower allergic reactions the difference was not statistically different due to small numbers."**Intro:**
- Not essential but could consider mentioning fibrates in the treatment of hypertriglyceridemia, which is another first line option.Response: done and referenced.
- Would cite more recent data on the impact of pre-medication on preventing hypersensitivity reactions - Cooper SL, Young DJ, Bowen CJ, Arwood NM, Poggi SG, Brown PA. Universal premedication and therapeutic drug monitoring for asparaginase-based therapy prevents infusion-associated acute adverse events and drug substitutions. Pediatr Blood Cancer. 2019 Aug;66(8):e27797.[1] and Marini BL, Brown J, Benitez L, Walling E, Hutchinson RJ, Mody R, Jasty Rao R, Slagle L, Bishop L, Pettit K, Bixby DL, Burke PW, Perissinotti AJ. A single-center multidisciplinary approach to managing the global Erwinia asparaginase shortage. Leuk Lymphoma. 2019 May 17:1-15[2]. Response: Reference 1 is already there in the addendum and added the reference 2 to the addendum.
- I would be careful with stating asparaginase antibodies are always predictive of future allergic reactions, as not all antibodies inactivate asparaginase or lead to clinical hypersensitivity reactions (ex: CCG01961). Consider rewording to soften this

language.Response we now quote exactly the sensitivity and specificity reported by the St. Jude group [Liu et al. Leukemia. 2012 Nov;26(11):2303-9] which addresses your concern.

- In paragraph 5, the statement is made that silent inactivation is of greater concern than allergic reactions. However, one could argue that allergic reactions are more common than silent inactivation with asparaginase (~1%), and monitoring activity levels in the setting of grade 1-2 allergic reactions is essential to determine which patients with clinical hypersensitivity reactions are having true antibody mediated reactions vs. other, non-antibody mediated (possibly ammonia vs. other immune-mediated) allergic reactions that would not warrant a switch to another asparaginase preparation. Response: we altered the paragraph somewhat and specified: "…potentially of greater concern than bone-fide allergic reactions, as patients with grade 3-4 allergic reactions to pegaspargase will be switched to erwinase, which theoretically will improve outcome."As you suggested at the end of the paragraph we also added this sentence: "Additionally, the low grade 1-2 allergic reactions that are more common than silent inactivation do require pegaspargase activity monitoring to ensure a switch to erwinase if confirmed as true inactivation."

- Other large scale studies assessing the rate of silent inactivation and finding the rate to be <8% could also be included in this discussion (or cited). These include, AALL07P4, Schore, et al. Leuk Lymph 2019 [3], CCG1962, and Park JH, et al. Blood. 2016;128:1629[4].Response: Done. Also quoted Place et al. "Notably, lower silent inactivation with pegaspargase than native Escherichia coli asparaginase have been reported. [15-17]" **Methods:**

- While a sample size was not calculated a priori, could the authors conduct a sensitivity analysis on the data determined the detectable effect size at 80% power in the primary outcome of interest (rate of grade 3/4 allergic reactions?)?Response: A sentence in first paragraph of the discussion was changed as follows: "Compared with historical controls that received similar therapy, premedication did reduce the incidence of grade 3 or 4 allergic reactions when measured per patient or per dose of pegaspargase. The power calculation was only 0.54 per patient and 0.24 per pegasparaginase dose thus our study was underpowered to show statistical significance."

- Were dose reductions in PEG ever conducted in this data set for toxicity/age/risk factors for hepatotoxicity? Response: No none. All PEG doses were given at a dose of 2500 units/m2, as documented in methods.

- It is unclear how silent inactivation is defined in this data-set from the methods. It may also be clinically relevant to capture "accelerated clearance", which also may be clinically relevant. Not all anti-asparaginase antibodies lead to full inactivation of the drug, but some lead to accelerated clearance and lower than expected values at early time points. A patient with silent inactivation would have a peak level that is undetectable, whereas a patient with accelerated clearance may clear the PEG by day ~7 when an expected duration of asparagine depletion is 21-28 days with a full pediatric dose. Response: we would suggest that prior studies defined silent inactivation similarly to our study. Here none of the measurements in the current study have included peak levels. Thus, like others we would suggest that accelerated clearance without noted clinical symptoms is the same as silent inactivation.

- Were any levels obtained in patients with low-grade hypersensitivity reactions? If so, did any of these patients have accelerated clearance/"loud" inactivation?Response: As noted – we did not collect low-grade (level 1-2) reactions therefore we cannot answer this question unfortunately.

- Were the activity levels normally distributed? If not, perhaps medians in the box plot and a Mann Whitney U for comparison would be more accurate. Similarly, if the values in the 2x2 contingency table are small, consider a Fisher's exact test rather than chi-square for the

incidence of hypersensitivity reaction comparison (although it likely won't make a significant difference).Response: The data was normally distributed by visual inspection of the normal curve and Kolmogorov-Smirnov test of normality, WITH THE EXCEPTION of the day 6-8 pegasparaginase activity level.   As Dr Marini pointed out this contained an extreme outlier which we could not explain. Thus we elected to remove this outlier and the distribution was normal. The removal of this was discussed in the methods.Data was reanalyzed and graphs redone after this change. Analysis showed a statistically significant difference in the day 6-8 pegaspargase level with and without premeds (lower without premed. Of note a significant difference was also seen with the dataset containing the outlier. Also analysis of the day 3-5 and 10-12 pegaspargase levels did not show a significant difference with premeds perhaps due to the small sample sizes.The finding of difference in the pegasparaginase level with premeds has not been previously described to our knowledge. This may be of clinical significance and should, if possible confirmed in other retrospective studies. Given the increased acceptance of premeds for PEG it is unlikely and perhaps unethical to confirm in a prospective randomized trial, in our opinion. Text relating to this new finding has been added to the manuscript in various places.

- Was there a protocol to say every patient should have a level with every dose, or were levels obtained at random/provider discretion? Could there then be a selection bias in the patients who have activity levels obtained since so many were missed? I'm assuming first dose levels were "missed" because the risk of inactivation with the first dose should theoretically be zero, so they were perhaps more accurately intentionally omitted. Response: our protocol did instruct that all pegaspargase doses will be followed by levels, as outlined in the methods. Compliance with this instruction was not complete at all as we outlined in the results. We think that there was no collection bias but rather non-compliance. It is true also that there is a misconception that risk of inactivation after the first dose is zero. Please note the recent report from Mary Relling group suggesting that many allergic reactions are actually to the PEG and may occur after the 1st dose of pegaspargase. We in fact have seen patients develop anaphylaxis right after the 1st dose. (Liu Y, Smith CA, Panetta JC, Yang W, Thompson LE, Counts JP, Molinelli AR, Pei D, Kornegay NM, Crews KR, Swanson H, Cheng C, Karol SE, Evans WE, Inaba H, Pui CH, Jeha S, Relling MV. Antibodies Predict Pegaspargase Allergic Reactions and Failure of Rechallenge. J Clin Oncol. 2019 Aug 10;37(23):2051-2061. doi:10.1200/JCO.18.02439. Epub 2019 Jun 12. PubMed PMID: 31188727.)**Results:**

- Is there any possibility that the level on day 6-8 of 2.8 was spurious? Were there any concomitant levels in this patient with this dose to make sense of such a high level?Response: this patient (UPN 11) after dose 3 of PEG had level of 2.8 on day 7. The scanned faxed report from AI Biotech was checked and confirmed the result of 2.8 as reported. We have no other levels after dose 3 to validate this result. After the preceding dose (#2) the result on day 7 was 0.78 and after the subsequent dose (#)4 the result on day 11 was 0.97. Based on the extreme deviance from a normal distribution we elected to remove this from the graphs and data analysis as discussed above.

- The one patient being described as having silent inactivation - I do not think I would characterize this as silent inactivation technically, since they had detectable activity levels at several time points, and no peak level is ever undetectable. I would consider this a transient accelerated clearance with dose 2 and 3. If silent inactivation was truly present, a patient should not have activity present for a full week and then have full activity with later doses. Patients with activity levels even as low as ~0.02 may still have asparagine depletion (AALL07p4).Response: It is our suggestion that accelerated clearance occurs through immune mechanism – and as we suggested above is similar to silent inactivation. Though it

is possible that the patient had transient accelerated clearance with dose 2 and 3 as an explanation, it remains possible that that patient had transient silent inactivation after dose 3 and had become tolerant PEG when continued dose administered as we explain in the discussion paragraph 2 noting to Tong papers.

- It may be interesting to graph levels over time in some of these patients with very low levels. Was there ever more than one level obtained per dose? Were repeat levels obtained to verify the low activity levels or follow the activity level trend over time (as is suggested in some guidelines?). This would help characterize the PK better in these patients.Response: the patient UPN 28 with the silent activation did have level 0.05 day 8 then 0.00 day 16. It verified the very low levels. No others had confirmatory low levels.

- Was the choice to pre-medicate done at a strict, uniform point in time (i.e., was this a quasi-experimental design where a protocol to pre-medicate all patients was mandated?), or was pre-medication at the choice of the treating provider? If this was selected by the provider and not systematic, could there have been some slight selection bias where patients perceived to be at higher risk of hypersensitivity reactions were given pre-medication? This could have diminished the impact of pre-medication on reducing the rate of hypersensitivity reactions. Consider this in the discussion section if deemed relevant.As we suggested above these were mandated premedications given at a specified time. It was not at the choice of the treating provider; therefore bias seems unlikely.

Discussion:

- I would more accurately call the silent inactivation here "accelerated clearance" as the patient had asparagine depletion for at least a week, even with the low level.We respectfully disagree as we consider accelerated clearance equal to silent inactivation, since they actually are the same mechanism.

- Consider discussing the possible value of obtaining activity levels in patients with questionable allergic reactions (grade 1/2). Response: Thank you. This was added to the last paragraph of the discussion.

- Could add as a limitation that it appears patients only had one activity level obtained per dose, which makes it challenging to interpret PK, especially in patients with higher or lower than expected activity levels. Response: Thank you, added to the limitation paragraph.Overall, a very well-done paper with logical conclusions. Thank you for allowing me to review the manuscript and for your hard work in this research.

- **Is the work clearly and accurately presented and does it cite the current literature?** Yes

- **Is the study design appropriate and is the work technically sound?**Partly

- **Are sufficient details of methods and analysis provided to allow replication by others?**Yes

- **If applicable, is the statistical analysis and its interpretation appropriate?**Partly

- **Are all the source data underlying the results available to ensure full reproducibility?**Yes

- **Are the conclusions drawn adequately supported by the results?**Yes

References

1. Cooper SL, Young DJ, Bowen CJ, Arwood NM, Poggi SG, Brown PA: Universal premedication and therapeutic drug monitoring for asparaginase-based therapy prevents infusion-associated acute adverse events and drug substitutions.*Pediatr Blood Cancer*. 2019; **66** (8): e27797 PubMed Abstract | Publisher Full Text

2. Marini BL, Brown J, Benitez L, Walling E, Hutchinson RJ, Mody R, Jasty Rao R, Slagle L, Bishop L, Pettit K, Bixby DL, Burke PW, Perissinotti AJ: A single-center multidisciplinary approach to

managing the global Erwinia asparaginase shortage.*Leuk Lymphoma*. 2019. 1-15 PubMed Abstract | Publisher Full Text

3. Schore RJ, Devidas M, Bleyer A, Reaman GH, Winick N, Loh ML, Raetz EA, Carroll WL, Hunger SP, Angiolillo AL: Plasma asparaginase activity and asparagine depletion in acute lymphoblastic leukemia patients treated with pegaspargase on Children's Oncology Group AALL07P4.*Leuk Lymphoma*. 2019; **60** (7): 1740-1748 PubMed Abstract | Publisher Full Text

4. Park JH, Ritchie EK, Rao AV, Devlin S, Hsu M, Khawaja TT, O'Donnell B, Cazacu MI, Stopka DM, Tallman MS, Douer D: A Pediatric-Inspired Regimen Containing Multiple Doses of Intravenous Pegylated Asparaginase Appears Safe and Effective in Newly Diagnosed Adult Patients with Ph-Negative Acute Lymphoblastic Leukemia in Adults up to Age 60: Results of a Multi-Center Phase II Clinical Trial. *Blood*. 2016; **128** (1629).

Competing Interests: No competing interests