original report

Antibodies Predict Pegaspargase Allergic Reactions and Failure of Rechallenge

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abstract

PURPOSE Pegaspargase (PEG-ASP) has largely replaced native *Escherichia coli* asparaginase (L-ASP) in the treatment of acute lymphoblastic leukemia because of its longer half-life and lower immunogenicity. Risk factors for allergic reactions to PEG-ASP remain unclear. Here, we identify risk factors for reactions in a front-line acute lymphoblastic leukemia trial and assess the usefulness of serum antibodies for diagnosing allergy and predicting rechallenge outcome.

PATIENTS AND METHODS PEG-ASP was administered to 598 patients in St Jude's Total XVI study. Results were compared with Total XV study (ClinicalTrials.gov identifiers: NCT00549848 and NCT00137111), which used native L-ASP. Serum samples (n = 5,369) were analyzed for anti–PEG-ASP immunoglobulin G by enzyme-linked immunosorbent assay. Positive samples were tested for anti–polyethylene glycol (PEG) and anti–L-ASP. We analyzed potential risk factors for reactions and associations between antibodies and reactions, rechallenge outcomes, and PEG-ASP pharmacokinetics.

RESULTS Grade 2 to 4 reactions were less common in the Total XVI study with PEG-ASP (81 [13.5%] of 598) than in the Total XV study with L-ASP (169 [41.2%] of 410; $P = 1.4 \times 10^{-23}$). For Total XVI, anti-PEG, not anti–L-ASP, was the predominant component of anti–PEG-ASP antibodies (96%). In a multivariable analysis, more intrathecal therapy (IT) predicted fewer reactions ($P = 2.4 \times 10^{-5}$), which is consistent with an immunosuppressant contribution of IT. Anti–PEG-ASP was associated with accelerated drug clearance ($P = 5.0 \times 10^{-6}$). Failure of rechallenge after initial reactions was associated with anti–PEG-ASP ($P = .0078$) and was predicted by the occurrence of angioedema with first reaction ($P = .01$).

CONCLUSION Less IT therapy was the only independent clinical risk factor for reactions to PEG-ASP. PEG, and not L-ASP, is the major antigen that causes allergic reactions. Anti–PEG-ASP has utility in predicting and confirming clinical reactions to PEG-ASP as well as in identifying patients who are most likely to experience failure with rechallenge.

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INTRODUCTION

Asparaginase is used to treat acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphoma. It exploits the insufficiency of asparagine synthesis in lymphoid blasts by depleting asparagine.[1] There is increasing interest in its use for treating disease other than leukemia—for example, breast cancer.[2]

PEGylation, conjugating drugs to polyethylene glycol (PEG), has been used widely in recent drug development.[3] PEGylated drugs have longer half-lives and are less immunogenic. PEGylated *Escherichia coli* L-asparaginase (PEG-ASP) is replacing native *E coli* L-asparaginase (L-ASP) in ALL treatment regimens[4,5] and causes less hypersensitivity.[6-9] Nonetheless, reactions to PEG-ASP are not uncommon.[10] These reactions are problematic because they cause morbidity[11] and inadequate asparaginase activity,[12] which necessitates the switch to *Erwinia* asparaginase (Erwinase), a formulation that requires frequent administration at great expense.[13] It is often unclear whether clinical symptoms are indicative of true allergy or some other acute reaction. There is increasing recognition that PEG itself can serve as an allergen,[14] but its contributions to PEG-ASP reactions remain poorly characterized, as do risk factors for allergy to PEG-ASP.

We evaluated serum antibodies to PEG-ASP, L-ASP, and PEG itself as well as their performance characteristics in identifying allergic reactions, predicting the success of rechallenge, and predicting asparaginase pharmacokinetics in a front-line clinical trial, the Total XVI study. We also evaluated other risk factors for allergy and compared findings with the predecessor trial Total XV, which used L-ASP instead of PEG-ASP (Data Supplement).

ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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Clinical trial information: NCT00549848.

PATIENTS AND METHODS

Patients and Treatment

Children (N = 598) with newly diagnosed ALL were enrolled in St Jude Children's Research Hospital Total XVI protocol (TXVI) study from September 2007 to March 2017 and were evaluable for reactions to PEG-ASP. Administration of PEG-ASP (as Oncaspar; Enzon Pharmaceuticals, Cranford, NJ) and intrathecal therapy (IT) was confirmed in the research database and the medical record. Use of H1-antihistamines (diphenhydramine and cetirizine), H2-antihistamines (ranitidine), and glucocorticoids (dexamethasone, prednisolone, and hydrocortisone) as premedication was retrieved for patients who experienced reactions to PEG-ASP. Asparaginase-related reactions were prospectively graded using the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0. Data from 410 patients in the St Jude Total XV protocol (TXV) were revisited.[15] Patients on both protocols were assigned to either the low-risk (LR) arm or standard/high-risk (SHR) arm (Data Supplement). Treatment in TXVI differed from that in TXV mainly by the inclusion of a higher number of IT injections during remission induction for higher-risk patients (Data Supplement) and by use of PEG-ASP instead of L-ASP. Informed consent from parents or guardians and patient assent were obtained with oversight by the institutional review board.

Anti-Asparaginase Antibodies

Serum for anti–L-ASP antibodies (TXV)[15] and anti–PEG-ASP antibodies (TXVI) was drawn at multiple time points (Data Supplement). Anti–PEG-ASP immunoglobulin G was detected using a modified enzyme-linked immunosorbent assay (ELISA) to avoid Tween-20 (Data Supplement)[15,16] and analyzed as a continuous variable (optical density) and dichotomous variable (negative or positive). Samples that were positive for anti–PEG-ASP underwent reflex testing against L-ASP (BioVendor, Brno, Czech Republic) and PEG-bovine catalase (Sigma-Aldrich, St Louis, MO), to determine whether the anti–PEG-ASP was directed against L-ASP or PEG.

Asparaginase Activity, Pharmacokinetics, and Ex Vivo Neutralization

Serum asparaginase activity in TXVI was measured (Data Supplement).[17] Pharmacokinetic parameters were estimated with the induction day 3 and continuation week 7 dose (Data Supplement) and the asparaginase activity on day 14 (trough) after a dose of PEG-ASP 2,500 U/m^2 was estimated for 582 patients for induction and 495 patients for continuation. Fifty-three samples, of which 36 were positive for anti–PEG-ASP, were used for ex vivo neutralization assay to test whether antibodies inhibited serum asparaginase activity.

Genotyping and Genetic Ancestry

We used genome-wide genotyping of germline DNA to estimate ancestry and assign patients to one of three groups as described (Data Supplement): white; black; and Hispanic, Asian, or other.

Statistical Analysis

We used Wilcoxon rank sum or χ^2 tests to identify risk factors for reactions or antibody positivity, to compare antibody positivity by reaction status or age, and to assess predictors of rechallenge outcome. General logistic regression models were used for multivariable analysis, with no model selection procedure.

Sensitivity, specificity, positive predictive value, and negative predictive value of antibodies for reactions were estimated (Data Supplement). Analyses were performed using R3.5.0 (http://www.r-project.org).[18] No adjustments to P values were made for multiple comparisons.

RESULTS

Reactions to PEG-ASP Differ From Those to L-ASP

Overall, 81 patients (13.5%) in TXVI developed at least one grade 2 to 4 reaction to PEG-ASP (Fig 1). This percentage is much lower ($P = 1.4 \times 10^{-23}$) than that for L-ASP reactions in TXV (41.2%),[15] but a higher proportion of those who experienced a reaction (71.6%) were grade 3 or 4 in TXVI compared with TXV (14.2%; $P = 1.5 \times 10^{-19}$). For TXVI, PEG-ASP reactions predominantly occurred with the first few doses after the 11- to 18-week hiatus after induction, with somewhat differing timing of reactions for TXV (Data Supplement). Patient characteristics at baseline in the two protocols were comparable with the exception of ancestry and a higher number of patients who were classified as high risk for CNS relapse in TXVI than TXV (Data Supplement).

Risk Factors for PEG-ASP Reactions

All features previously associated with allergy[15,19,20] were examined as possible risk factors in addition to the number of intrathecal injections, because it was higher in the TXVI study than in the TXV study and because it was confounded with T-cell immunophenotype, previously identified as protecting against reactions.[15,20] Two clinical features, assignment to the LR—as opposed to SHR—therapy arm and non–American Indian ancestry, were associated with L-ASP allergies in TXV.[15,19] In contrast, in TXVI, PEG-ASP reactions differed neither by risk arm ($P = .84$), nor ancestry ($P = .30$; Table 1). Ancestry also had no association with PEG-ASP reactions when analyses were confined to the SHR arm ($P = .71$) or to patients with B-cell ALL ($P = .21$).

Cranial irradiation was omitted and replaced by systemic and intrathecal therapy (IT) in TXV[21] and TXVI, which included more IT than TXV for those who were at highest risk of CNS relapse (Data Supplement). During induction, TXV patients received two to four ITs, whereas TXVI patients received one to seven ITs (Data Supplement). The number of induction ITs was negatively associated with PEG-ASP reactions in TXVI ($P = 4.2 \times 10^{-6}$; Table 1), which was also true among patients with B-cell ALL only ($P = 4.8 \times 10^{-5}$). With the lower number of ITs and the lower proportion of patients in TXV who were classified as

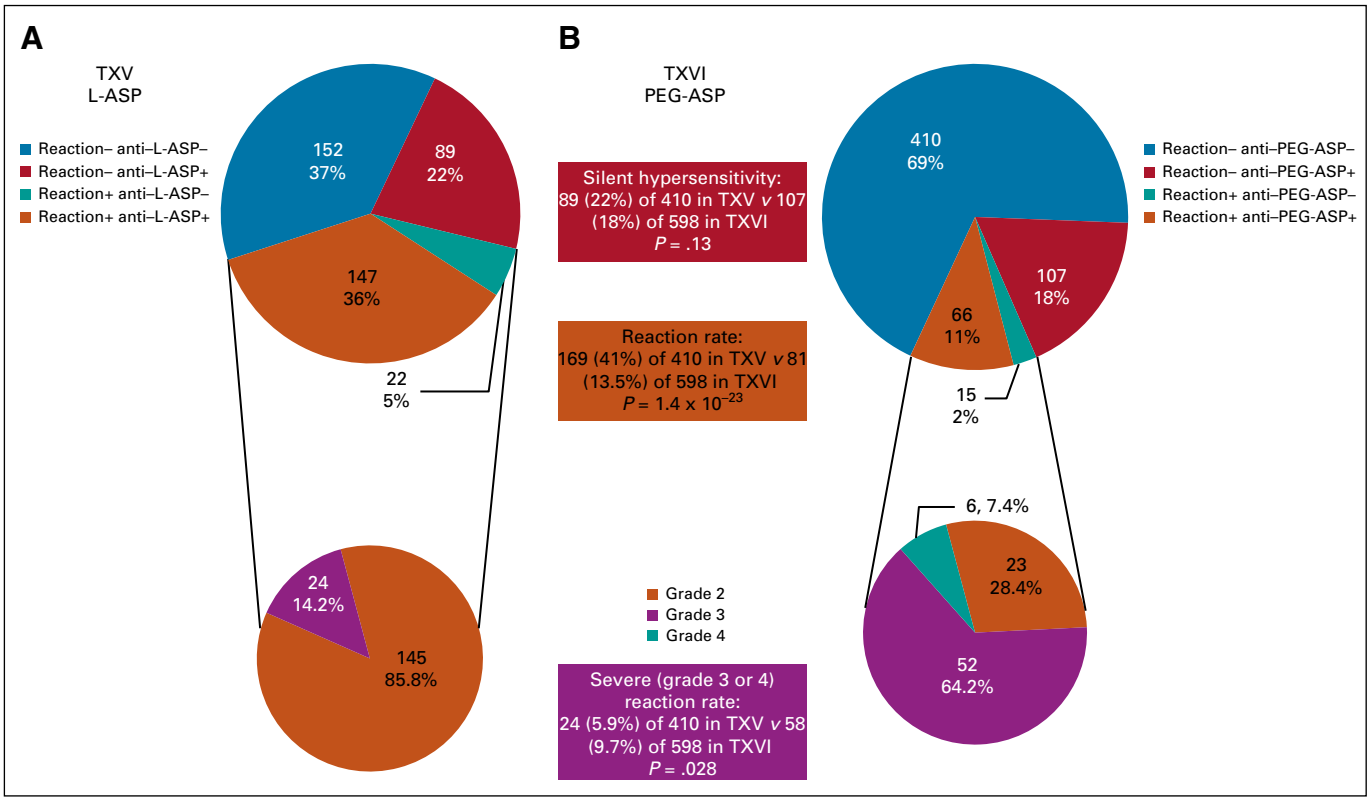


FIG 1. Reactions by antibody status, preparation, and protocol. (A) Frequency of patients by their anti–*Escherichia coli* asparaginase (L-ASP) status and reaction to L-ASP in the Total XV study (TXV; upper chart). Distribution of patients who experienced reactions to L-ASP by their reaction grade (lower chart). (B) Frequency of patients by their anti-pegaspargase (PEG-ASP) status and reaction to PEG-ASP in Total XVI study (TXVI; upper chart). Distribution of patients who experienced reactions to PEG-ASP by their reaction grade (lower chart). All reactions are grade 2 or greater. Silent hypersensitivity (antibody positive but reaction negative) occurred in 89 (22%) of 410 and 107 (18%) of 598 patients on TXV versus TXVI ($P = .13$). Grade 2 to 4 reactions were less common (81 [13.5%] of 598 patients) in TXVI (to PEG-ASP) than in TXV (169 [41%] of 410 patients; to L-ASP; $P = 1.4 \times 10^{-23}$), but a higher proportion were grade 3 or 4 (24 [14.2%] of 169 *v* 58 [71.6%] of 81; $P = 1.5 \times 10^{-19}$). Overall, there were more grade 3 or 4 reactions with PEG-ASP than L-ASP ($P = .028$). All *P* values were generated from χ^2 test. Reaction+, patients with allergic reactions to L-ASP (TXV) or PEG-ASP (TXVI); Reaction−, patients who received L-ASP (TXV) or PEG-ASP (TXVI) but did not have reactions to asparaginase.

CNS2 or CNS3 (Data Supplement), there was no significant association between the number of ITs and L-ASP reactions in TXV ($P = .082$).

Risk Factors for Antibodies and Their Association With Reactions

As described in Patients and Methods and the Data Supplement, the antibody assay for TXVI differed from that of TXV, with Tween-20 removed from all buffers as a result of structural similarity to PEG. Using the modified assay, 618 (11.5%) of 5,369 samples from 598 patients tested positive for anti–PEG-ASP in TXVI. Of the positive samples, 96 (15.5%) were positive for both anti-PEG and anti–L-ASP, 495 (80.1%) were positive for anti-PEG only, and nine (1.5%) were positive for anti–L-ASP only.

Of 81 patients who experienced PEG-ASP reactions, 66 (81.5%) had at least one sample that was positive for anti–PEG-ASP (Fig 1B). This percentage is similar to TXV for anti–L-ASP with L-ASP as the antigen (87.0%),[15] indicating similar sensitivity of the modified assay for PEG-ASP as had been true for the prior assay for L-ASP antibodies ($P = .25$). Furthermore, 79.3% (410 of 517) of non-allergic patients never had a positive sample (Fig 1B), which indicated improved specificity to detect allergy of the modified assay for PEG-ASP antibodies compared with the original assay for L-ASP used in TXV (63.1%; $P = 2.0 \times 10^{-6}$).[15] Modifying the assay reagents for use with PEG-ASP instead of L-ASP improved the clinical performance of the ELISA to detect clinical allergic reactions compared with the original assay developed for L-ASP antibodies (Data Supplement).

In multivariable analyses, the absence of Down syndrome ($P = .030$) and a lower number of ITs ($P = 4.2 \times 10^{-9}$) were associated with anti–PEG-ASP positivity (Data Supplement).

In the TXVI study as a whole, antibody status was significantly associated with risk for reaction—past or future—at most time points, except for induction day 8 (Fig 2A and Data Supplement), although associations differed in LR versus SHR patients. Thirty patients had positive day 1

TABLE 1. Association Between Patient- and Treatment-Related Categorical Variables and Allergic Reactions to PEG-ASP at Any Time During Therapy in the Total XVI Study

Characteristic	No.	Reaction+, No. (%)	Reaction−, No. (%)	P_{uni}*	P_{multi}†
Age at diagnosis, years					
≤ 10	437	55 (12.6)	382 (87.4)	.27	—
> 10	161	26 (16.0)	135 (84.0)		
Sex					
Male	350	48 (13.7)	302 (86.3)	.90	—
Female	248	33 (13.3)	215 (86.7)		
Ancestry‡					
Black	80	11 (13.6)	69 (86.4)	.30	—
White	396	60 (15.2)	336 (84.8)		
Other	120	10 (8.3)	110 (91.7)		
ALL lineage					
B cell	495	73 (14.7)	422 (85.3)	.056	.96
T cell	103	8 (7.8)	95 (92.2)		
Risk arm					
LR	260	36 (13.8)	224 (86.2)	.84	—
SHR	338	45 (13.3)	293 (86.7)		
Down syndrome					
Absent	585	81 (13.8)	504 (86.2)	.30	.16
Present	13	0 (0.0)	13 (100.0)		
Induction IT, No. of doses§‖					
1-3	250	50 (20.0)	200 (80.0)	1.9×10^{-5}	2.4×10^{-5}
4-7	302	23 (7.6)	279 (92.4)		
Induction PEG-ASP, No. of doses§					
0	2	0 (0.0)	2 (100.0)	.72	—
1	400	51 (12.8)	349 (87.2)		
2	150	22 (14.7)	128 (85.3)		

NOTE. Although not significant in univariable analysis, ALL lineage was used in multivariable analysis because of its significant association with *Escherichia coli* asparaginase reactions in the Total XV study.[15] Down syndrome was used in multivariable analysis because of its significant association with anti–PEG-ASP in the Total XVI study and its association with allergy in the Children's Oncology Group AALL0232 study.[20] Lineage, Down syndrome, and induction IT were analyzed in multivariable analysis.

Abbreviations: ALL, acute lymphoblastic leukemia; IT, intrathecal therapy; LR, low-risk arm; PEG-ASP, pegaspargase; reaction+, patients with allergic reactions to PEG-ASP at any time during therapy; reaction−, patients without allergic reactions and received at least one dose of PEG-ASP; SHR, standard/high-risk arm.

*P_{uni} values were generated from χ^2 test.

†P_{multi} values were generated from multiple logistic regression among patients who received PEG-ASP after remission induction and were evaluable postinduction.

‡Genetically determined with STRUCTURE as described in Patients and Methods. Of 598 evaluable patients, 596 had germline DNA available for genotyping.

§Analysis of association between these variables and reaction was restricted to patients who received PEG-ASP after remission induction and were evaluable postinduction.

‖When analyzed as a continuous variable rather than a categorical variable, the number of induction IT doses was still negatively associated with postinduction reactions ($P = 4.2 \times 10^{-6}$).

samples for anti–PEG-ASP (5.1%; Data Supplement) before the first exposure to PEG-ASP, and antibody positivity correlated with the risk of subsequent reaction (P = .026; Data Supplement). Associations between antibodies and the risk of future reactions (only) are described (Data Supplement).

The most common time for reactions was during reinduction I for LR and continuation weeks 1 to 6 for SHR patients. In LR patients, anti–PEG-ASP positivity at several time points predicted reinduction I reactions (Data Supplement), with receiver operating characteristic (ROC) curves having area under the curve (AUC) greater than 0.8 at consolidation day 1

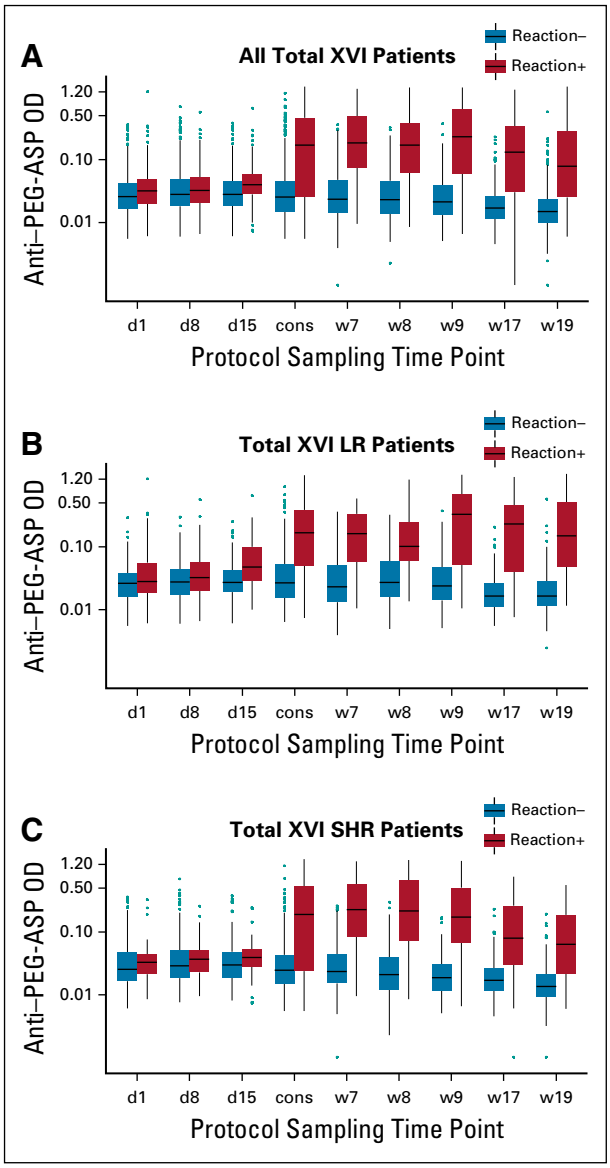


FIG 2. Association between anti-pegaspargase (PEG-ASP) antibody levels at different protocol time points and allergic reactions to PEG-ASP at any time during therapy in all Total XVI study (TXVI) patients (A; *P* values are .047, .098, 1.1E-4, 3.2E-13, 5.7E-24, 2.8E-25, 1.8E-25, 5.3E-21, and 3.9E-22 comparing reactive patients and nonreactive patients); TXVI low-risk arm (LR) patients (B; *P* values are .17, .14, 2.5E-4, 3.1E-7, 6.5E-12, 1.5E-10, 1.7E-11, 7.9E-13, and 5.0E-13 comparing reactive and nonreactive patients); and TXVI standard/high-risk arm (SHR) patients (C; *P* values are .19, .36, .066, 2.6E-7, 1.6E-13, 8.6E-16, 2.5E-15, 6.8E-10, and 1.6E-11 comparing reactive patients and nonreactive patients). cons, consolidation day 1; d1, induction day 1; d8, induction day 8; d15, induction day 15; OD, optical density; Reaction+, patients with allergic reactions at any time during therapy; Reaction−, patients who received PEG-ASP and never developed allergy; w7, continuation week 7; w8, continuation week 8; w9, continuation week 9; w17, continuation week 17; w19, continuation week 19.

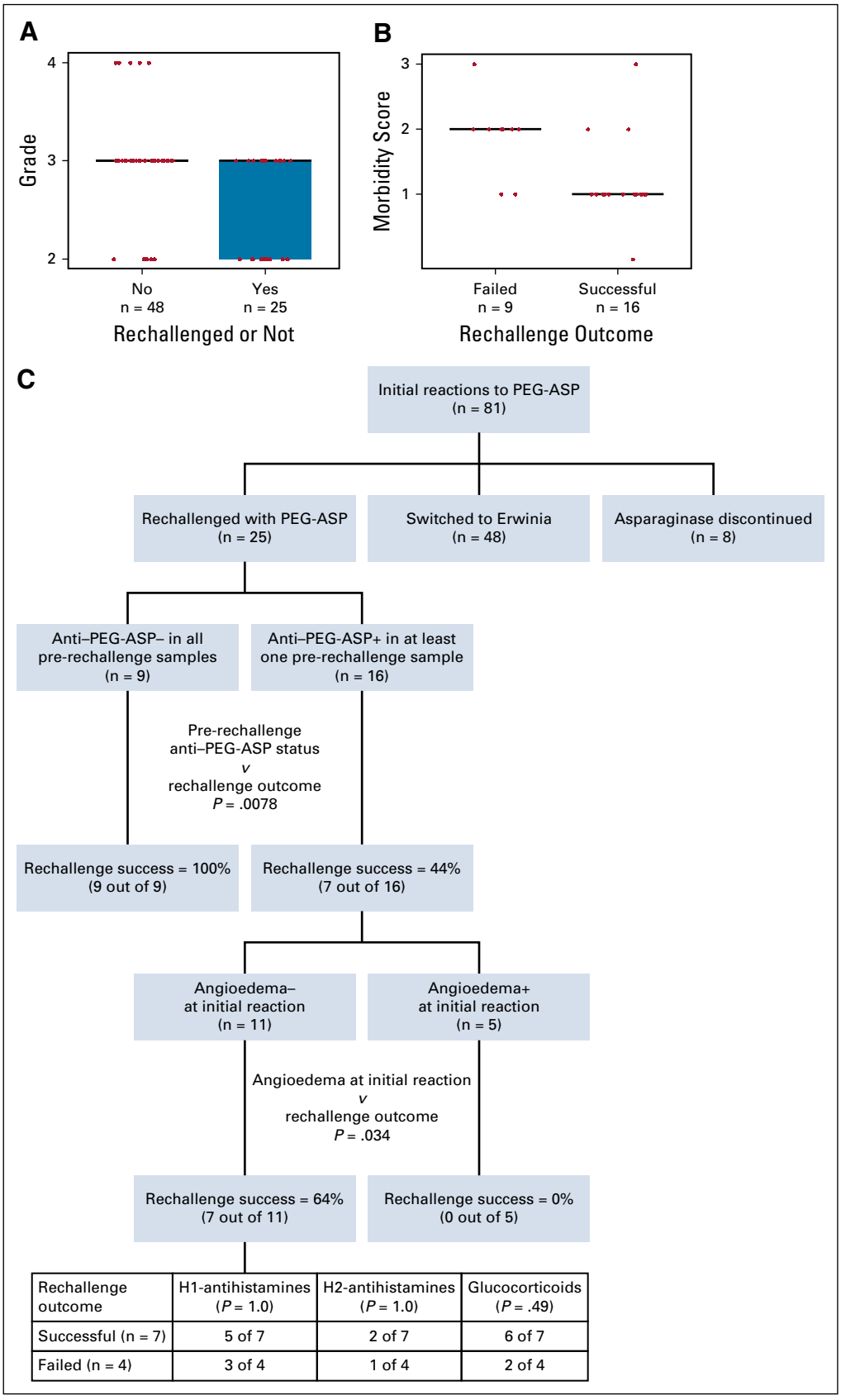
(Data Supplement). In SHR patients, only antibody positivity on consolidation day 1 was a significant predictor of continuation weeks 1 to 6 reactions ($P = 1.6 \times 10^{-17}$; Data Supplement), and ROC curve AUCs were not greater than 0.8 until week 7 (Data Supplement), just after the reactions at weeks 1 to 6. The predictive utility of anti-PEG, but not anti–L-ASP, was similar to that of anti–PEG-ASP (Data Supplement).

How well did postreaction antibody tests confirm past reactions? Among LR patients, sensitivity of anti–PEG-ASP exceeded 70% and specificity exceeded 90% until week 19, approximately 10 weeks after the reactions during reinduction I (weeks 7 to 9; Data Supplement), and with AUCs of ROC curves greater than 0.9 for weeks 17 and 19 (Data Supplement). Anti-PEG and anti–L-ASP were also confirmative in LR patients, albeit not as markedly (Data Supplement). Among SHR patients, sensitivity of anti–PEG-ASP exceeded 70% and specificity exceeded 90% at weeks 7, 8, and 9, after reactions during continuation weeks 1 to 6 (Data Supplement), and with AUCs for ROC curves greater than 0.9 at weeks 8 and 9 (Data Supplement). Anti-PEG and anti–L-ASP were also confirmative in SHR patients (Data Supplement).

On the basis of the time course of anti–PEG-ASP in those patients who did experience reactions (Data Supplement), the highest anti–PEG-ASP levels occurred approximately 37 days after reactions compared with approximately 50 days for anti–L-ASP after L-ASP reactions in the TXV study.[15]

Rechallenge

When patients experienced reactions during PEG-ASP, clinicians were often unsure if the reaction was a true allergy. Thus, of the 81 patients who experienced reactions to PEG-ASP, 25 were rechallenged with PEG-ASP instead of switching to Erwinase. This was a biased group: patients with less severe reactions were more likely to be rechallenged (grade 0 to 2 *v* grade 3 to 4; $P = .0041$; Fig 3A). Of those who were rechallenged, 16 tolerated rechallenge, whereas nine experienced failure and had another reaction. What predicted the success or failure of rechallenge in this group? The grade of initial reactions did not (grade 0 to 2 *v* grade 3 to 4; $P = .41$). Clinical symptoms were classified into five categories: urticarial (flushing, rash, hives, erythema, and pruritus), facial angioedema, respiratory (dyspnea, coughing, wheezing, and bronchospasm), hypotension, and GI symptoms (nausea, vomiting, diarrhea, and abdominal pain), to explore whether categories of symptoms could predict rechallenge outcome. A morbidity score on the basis of the number of symptom categories was assigned and higher morbidity scores were associated with rechallenge failure ($P = .0091$; Fig 3B). Angioedema ($P = .012$) and GI symptoms ($P = .040$) were associated with rechallenge failure (Data Supplement). Anti–PEG-ASP immediately after the reaction ($P = .041$) or positive anti–PEG-ASP before rechallenge ($P = .0078$) were associated with rechallenge failure. In multivariable analysis, angioedema and pre-rechallenge anti–PEG-ASP positivity



Rechallenge outcome	H1-antihistamines (*P* = 1.0)	H2-antihistamines (*P* = 1.0)	Glucocorticoids (*P* = .49)
Successful (n = 7)	5 of 7	2 of 7	6 of 7
Failed (n = 4)	3 of 4	1 of 4	2 of 4

FIG 3. Association between anti-pegaspargase (PEG-ASP) status, reaction severity and clinical symptoms, and PEG-ASP rechallenge. (A) Association between the grade of the initial reaction and whether the patient was rechallenged or not (*P* = .0041). (B) Association between morbidity score assigned from clinical symptoms and rechallenge outcome (*P* = .0091). (C) Classification and regression tree of rechallenge outcome prediction.

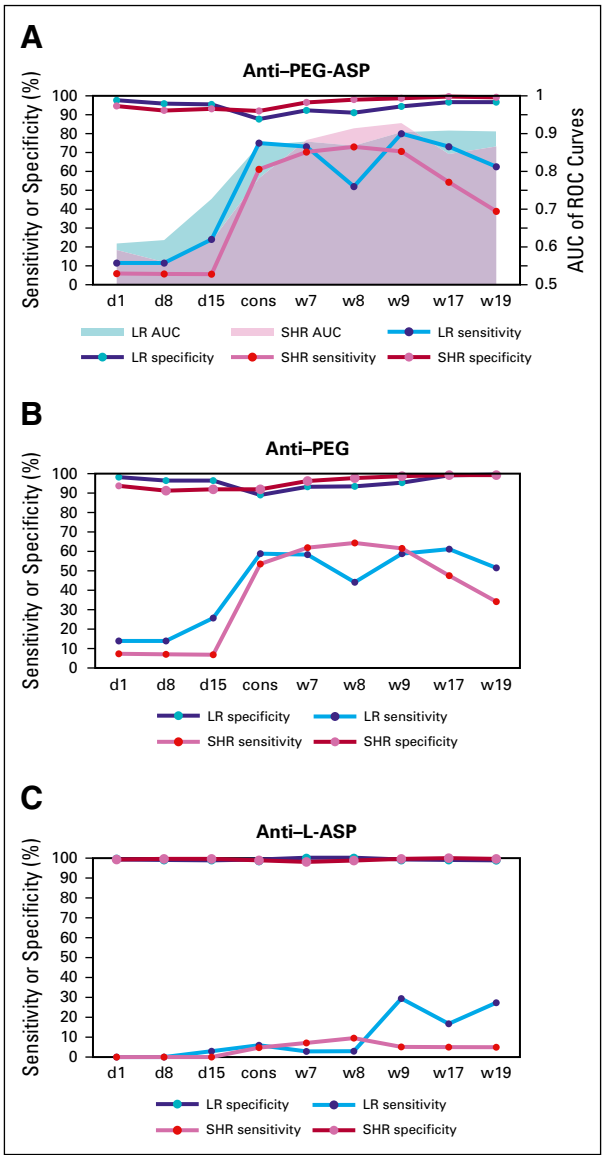


FIG 4. Sensitivity and specificity of antibodies for allergic reactions by risk arm. Sensitivity, specificity and area under the curve (AUC) of receiver operating characteristic (ROC) curves for the association between (A) anti-pegaspargase (PEG-ASP) antibody, (B) anti-PEG antibody, (C) and anti–*Escherichia coli* asparaginase (L-ASP) antibody measured at different protocol time points and allergic reactions to PEG-ASP at any time during therapy. cons, consolidation day 1; d1, induction day 1; d8, induction day 8; d15, induction day 15; LR, low-risk arm; SHR, standard/high-risk arm; w7, continuation week 7; w8, continuation week 8; w9, continuation week 9; w17, continuation week 17; w19, continuation week 19.

remained associated with rechallenge failure ($P = .010$ and $P = .027$; Data Supplement). No association was found with the use of H1-antihistamines ($P = 1.0$), H2-antihistamines ($P = 1.0$), or glucocorticoids ($P = .20$; Fig 3C).

It is not possible to evaluate the impact of rechallenge on serum asparaginase activity with rechallenge doses for those patients who reacted again, because the second reactions tended to occur so early during infusions that only a small percentage of the dose was administered: nine of nine patients who experienced failure with rechallenge received no more than 12% of the planned dose when they rereacted; thus, serum asparaginase activity was not evaluable. For 13 of 25 rechallenged patients who received the full PEG-ASP dose for continuation week 7 and had serum activity measured within 14 days, median estimated trough serum asparaginase activity was 0.38 U/mL, which was lower than among the 481 patients who did not have any reaction and were evaluable at week 7 (0.54 U/mL; $P = .042$), but still above the putative threshold for desired activity of 0.1 U/mL for most patients. In fact, only two of 13 rechallenged patients and 18 of 481 non-rechallenged patients had asparaginase levels less than 0.1 U/mL.

Impact of Antibody on Asparaginase Activity

Among the 20 samples that were tested, no association was found between antibody levels—anti–PEG-ASP, anti–L-ASP, or anti-PEG—and neutralization of spiked PEG-ASP activity (Data Supplement). Anti–PEG-ASP did not neutralize L-ASP either (Data Supplement). In contrast, there was an association between the neutralization of spiked L-ASP activity and levels of anti–L-ASP ($P < .001$ Data Supplement).

In vivo, antibody status was not associated with asparaginase levels during induction ($P = .20$ for anti–PEG-ASP and anti-PEG; $P = .27$ for anti–L-ASP; Data Supplement; n = 582), but there was an association during continuation (n = 495; $P = 5.0 \times 10^{-6}$ for anti–PEG-ASP; $P = 1.2 \times 10^{-8}$ for anti–L-ASP; and $P = 7.5 \times 10^{-6}$ for anti-PEG; Data Supplement). The activity-lowering effect of anti–L-ASP was stronger than that of anti–PEG-ASP ($P = .0032$) during continuation.

DISCUSSION

Antibodies Were Useful at Diagnosing Allergic Reactions and Predicting Rechallenge Failure

There has been controversy over what constitutes PEG-ASP allergic reactions, and it is difficult to make the diagnosis on the basis of symptoms alone.[22] The diagnosis is important, because relapse can be minimized by detecting asparaginase allergy and promptly switching to another preparation—for example, Erwinase.[23] However, Erwinase requires more frequent administration, has unfavorable pharmacokinetic properties, and is more expensive; therefore, avoiding an incorrect diagnosis of PEG-ASP allergy is also important. Criteria proposed to differentiate true allergy from other reactions have included timing,[24] severity and type of symptoms, and low serum asparaginase activity.[22] There are few modern trials using PEG-ASP upfront that have included measurements of serum antibodies optimized for PEG-ASP. Studies with native L-ASP followed by PEG-ASP have increased rates of allergy and antibodies that do not reflect the current findings using PEG-ASP upfront.[12,25] The strongest clinical evidence of a true allergy is likely to be the recurrence

of reactions upon rechallenge. Here, using a modified ELISA assay for anti–PEG-ASP antibodies, antibodies were associated with reaction recurrence upon rechallenge (Fig 3), and they also displayed better sensitivity, specificity, and positive and negative predictive value for the first reaction to PEG-ASP than was true for anti–L-ASP antibodies for the first reaction to L-ASP (Fig 4 and Data Supplement).

Rechallenge would also be considered a failure if serum asparaginase activity after rechallenge was too low. Although it is not possible to evaluate serum asparaginase activity in those who experience failure with rechallenge and thus stop infusions early, we did compare serum asparaginase activity (estimated after 2,500 U/m^2) in those who were rechallenged versus patients at an identical time point who had not suffered reaction. We found that although serum asparaginase was marginally lower (P = .042), it remained greater than a putative desired trough concentration of 0.1 U/mL in 84.6% of rechallenged and 96.3% of nonreacting patients.

Although anti–PEG-ASP was associated with lower serum asparaginase later in therapy, anti–L-ASP was more inhibitory of asparaginase activity, both in vivo and ex vivo, than anti–PEG-ASP (Data Supplement). Thus, anti–PEG-ASP may not directly inhibit the enzymatic activity of PEG-ASP, possibly because of the poor accessibility of the enzyme active site created by PEGylation. It is also possible that anti–PEG-ASP antibodies or accompanying immunologic changes increased the clearance of PEG-ASP without neutralizing PEG-ASP activity, similar to findings from the pegloticase trials.[26,27] Because infusions are usually stopped early in the case of a reaction, and because anti-PEG antibodies are not always neutralizing, use of serum asparaginase activity as a method to detect real allergy is not ideal.

Taken together, our data indicate that neither clinical reactions nor the presence of anti–PEG-ASP antibodies can be assumed to indicate inadequate serum asparaginase activity in this setting in which PEG-ASP is used in upfront ALL regimens.

Antibodies Were Primarily Directed at PEG

Consistent with the modest neutralization of PEG-ASP activity in vivo, antibodies against PEG-ASP were primarily directed against PEG, not L-ASP. Although PEGylation decreased immunogenicity compared with native L-ASP (Fig 1), PEG itself is becoming increasingly recognized as an antigen.[14,28] Anti-PEG was the predominant component (96%) of anti–PEG-ASP (Fig 5 and Data Supplement), as was reported for pegloticase for gout.[29] Prevalence of anti-PEG in patients with ALL has not been extensively reported, but in the general population it has been reported to be as high as 10% to 30%.[30,31] In fact, pretreatment anti-PEG were at higher risk of subsequent reactions to PEG-ASP (P = .026; Data Supplement). We reported two patients who experienced reactions to PEG-ASP and were successfully switched to L-ASP without additional reaction, which indicates that their reactions were likely mediated by anti-PEG and not anti–L-ASP.[32] At the time of that report, the ELISA used had not been optimized for the detection of anti–PEG-ASP. One of those two patients was enrolled in the TXVI study. In retrospect, using our modified ELISA, the patient was positive for anti–PEG-ASP and anti-PEG, but negative for anti–L-ASP, which explains the success of L-ASP substitution. The success of the L-ASP substitution in this single patient as well as the underlying antibody profile support the idea that it would be helpful if L-ASP or alternative non-PEGylated formulations were commercially available for use in selected patients who have become sensitized to PEG itself. These findings also support the importance of our assay, which can distinguish antibodies to PEG versus those to the asparaginase formulation. Patients who are allergic to PEG-ASP experienced failure with PEGylated Erwinase and suffered cross-reactions, likely to PEG.[33] Of note, our patient with the highest preexisting levels of anti–PEG-ASP and anti-PEG in the TXVI study experienced a reaction to his or her first dose of PEG-ASP. Similar cases have been reported for pegloticase and pegnivacogin.[34,35] Of interest, we found that age was associated with higher preexisting anti–PEG-ASP levels ($P = 8.6 \times 10^{-10}$; Data Supplement), possibly because as children mature, there is increasing exposure to PEG-containing products, such as laxatives (Miralax), eye drops, tablet coatings, topicals, and food.

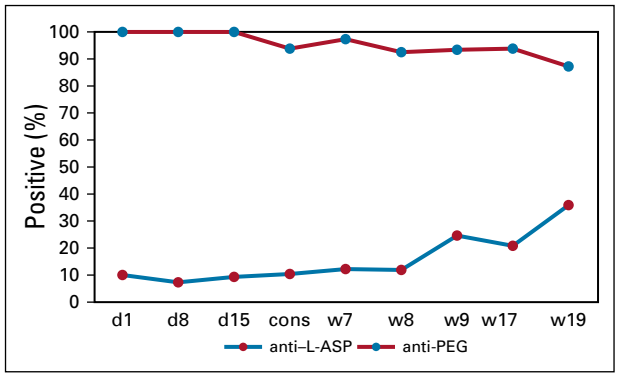


FIG 5. Of samples that were positive for anti-pegaspargase, shown are the percent positive for anti-PEG and anti–*Escherichia coli* asparaginase (L-ASP) at each protocol time point in the Total XVI study. cons, consolidation day 1; d1, induction day 1; d8, induction day 8; d15, induction day 15; w7, continuation week 7; w8, continuation week 8; w9, continuation week 9; w17, continuation week 17; w19, continuation week 19.

Consistent with our previous analysis from the TXV study with L-ASP,[15] asparaginase activity was lower in those few samples from PEG-ASP–treated patients with higher anti–L-ASP levels. Thus, it may be important to differentiate anti–PEG-ASP from anti–L-ASP in patients treated with PEG-ASP.

Clinical Features Predicted Rechallenge Failure

Although we acknowledge that patients with the most severe reactions to PEG-ASP were not rechallenged, of those who were rechallenged, anti–PEG-ASP positivity associated with rechallenge failure, which provides additional evidence that our antibody assay distinguished real PEG-ASP allergy from nonallergy infusion reactions. Of interest, angioedema and GI reactions were the symptom classes associated with rechallenge failure, whereas reaction severity grade was not predictive. In multivariable analysis, angioedema and anti–PEG-ASP were the strongest predictors of rechallenge failure (Data Supplement). All nine rechallenged patients without anti–PEG-ASP positive samples before rechallenge were successfully rechallenged and tolerated all subsequent PEG-ASP doses. Furthermore, eight of these nine patients never developed any anti–PEG-ASP antibodies. Premedication did not affect rechallenge outcome, similar to some studies,[36,37] although we acknowledge that rechallenge was biased against those with the most impressive initial reactions in our group and that the immunosuppression of our ALL regimen may differ from that of others.

Some Clinical Features Predicted Allergy, and Allergy to PEG-ASP Differed From That to L-ASP

We reported fewer reactions to L-ASP among patients with T-cell ALL.[15] Here, we observed fewer reactions in patients with T-cell ALL in the TXVI study in univariable analysis among SHR patients ($P = .044$), but this association disappeared ($P = .49$) in a multivariable analysis that included the number of ITs, a novel risk factor reported herein. IT number was the only factor associated with reactions in all patients ($P = 2.4 \times 10^{-5}$; Table 1). Among the three drugs in ITs, methotrexate is immunosuppressive and IT methotrexate can reach cytotoxic levels in serum, which has systemic effects.[38-40] Admittedly, other systemic chemotherapy administered during induction was also immunosuppressive, but it did not differ significantly from patient to patient as much as the number of ITs (Data Supplement); therefore, other chemotherapy was unlikely to differentiate those at higher versus lower risk of reactions. We hypothesize that IT use may not have been a risk factor in other trials because it was not evaluated, and most trials do not have as many ITs, nor as much variability in the number of ITs among patients.[41] In addition, a higher proportion of patients in the TXVI study (Data Supplement) were CNS positive and thus received more ITs compared with prior St Jude trials and many other ALL trials. Representing a small subset (13 of 598), Down syndrome was associated with the absence of anti–PEG-ASP ($P = .030$; Data Supplement), which is consistent with our previous report.[20]

Although reactions to PEG-ASP were less common than reactions to L-ASP, which was consistent with a previous report,[1] they were more severe (Fig 1), similar to other trials.[42-44] Unlike in the TXV study with L-ASP, neither the risk nor the timing of reaction differed by risk arm in TXVI using PEG-ASP. Patients who experienced reactions received a median of only three doses of PEG-ASP before their reaction (Data Supplement), similar to other studies.[43,44]

In summary, in the TXVI study with PEG-ASP as the primary formulation, the majority of patients (81.5%) with allergy had antibodies to PEG-ASP, similar to the percent of allergic patients who had anti–L-ASP in the TXV study. With PEG-ASP, the primary antigen was PEG, not L-ASP. Patients who received fewer ITs had a higher risk of reaction. Some patients who have an apparent allergic reaction to PEG-ASP can be successfully rechallenged. Predictors of successful rechallenge include a lack of anti–PEG-ASP antibodies and lack of angioedema. Measurement of anti–PEG-ASP, especially anti-PEG, could be of use in managing patients who are treated with PEG-ASP and other PEGylated therapeutics.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST AND DATA AVAILABILITY STATEMENT

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Antibodies Predict Pegaspargase Allergic Reactions and Failure of Rechallenge

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