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Clinical utility and implications of asparaginase antibodies in acute lymphoblastic leukemia

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Abstract

Hypersensitivity to asparaginase is common but the differential diagnosis can be challenging, and the diagnostic utility of antibody tests is unclear. We studied allergic reactions and serum antibodies to *E. coli*-asparaginase (Elspar) in 410 children treated on St. Jude Total XV protocol for acute lymphoblastic leukemia. Of 169 patients (41.2%) with clinical allergy, 147 (87.0%) were positive for anti-Elspar antibody. Of 241 patients without allergy, 89 (36.9%) had detectable antibody. Allergies ($P = 0.0002$) and antibodies ($P = 6.6 \times 10^{-6}$) were higher among patients treated on the low-risk than among those on the standard/high-risk arm. Among those positive for antibody, the antibody titers were higher in those who developed allergy than in those who did not ($P < 1 \times 10^{-15}$). Antibody measures at week 7 of continuation therapy had a sensitivity of 87%–88% and a specificity of 68%–69% for predicting or confirming clinical reactions. Antibodies inversely associated with serum asparaginase activity ($P = 7.0 \times 10^{-6}$). High antibodies associated with a lower risk of osteonecrosis (odds ratio = 0.83; 95% confidence interval, 0.78–0.89; $P = 0.007$). Antibodies were related to clinical allergy and to low systemic exposure to asparaginase, leading to lower risk of some adverse effects of therapy.

Keywords

ALL; asparaginase; antibody; allergy; pharmacodynamics; osteonecrosis

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Introduction

Asparaginase is a critical treatment component for acute lymphoblastic leukemia (ALL);^{1–5} however, its use is complicated by the development of clinical hypersensitivity.^{6–11} Allergic reactions typically require discontinuation of the offending formulation (e.g., native *E. coli* asparaginase, Elspar) and substitution with other formulations (e.g., Erwinase or the pegylated form of *E. coli* asparaginase, Oncaspar); however, differentiating allergy to asparaginase from other acute reactions can sometimes be challenging. Serum asparaginase antibody has been associated with clinical allergy, but few studies have focused on its utility as a diagnostic test.¹⁰

Some previous studies have indicated that serum antibodies, even in the absence of clinical allergy, may inhibit serum asparaginase activity¹² and attenuate its anticancer effect,¹³ although there are conflicting data.^{14–18} Many studies lack properly timed control samples from patients whose asparaginase therapy is identical to that of patients who do develop antibodies.

Here, we prospectively measured IgG antibodies to asparaginase at predetermined time points among 410 pediatric patients treated on a front-line trial of ALL, St. Jude Total XV protocol, and evaluated the predictive utility of antibody measures for allergy, and their association with asparaginase activity and adverse effects.

Methods

Patients

Between 2000 and 2007, 498 patients with newly diagnosed childhood ALL were enrolled in St. Jude Children's Research Hospital front-line Total XV protocol: 239 treated on the low-risk (LR) arm and 259 on the standard/high risk (SHR) arm. All patients received asparaginase treatment, and 410 (197 LR and 213 SHR) had serum samples evaluable for anti-asparaginase antibodies (Supplemental Table S1). The informed consent, IRB approval, risk arm assignment, and detailed treatment regimens have been described previously.¹⁹ Race/ethnicity groups were assigned using germline genomic variation from Affymetrix mapping arrays to assess ancestry, as described.²⁰

Asparaginase regimen and sample collection

During remission induction, Elspar was administered intramuscularly at a dose of 10000 U/m² thrice weekly, for a total of 6 doses (on days 6, 8, 10, 12, 14, and 16) or 9 doses (additionally on days 19, 21, 23) in patients with high levels (i.e., 1% or more) of leukemic cells in bone marrow on day 19 of remission induction.

Patients on the LR arm received 9 doses of 10000 U/m² Elspar during reinduction I (weeks 7–9 from start of continuation treatment), and 9 doses during reinduction II (weeks 17–19) (Supplemental Figure S1). Patients on the SHR arm received Elspar at 25000 U/m² weekly for 19 doses in continuation treatment (weeks 1–19). For SHR patients with Philadelphia chromosome-positive ALL or induction failure, an additional dose of 25000 U/m² Elspar was given in the reintensification phase (after consolidation or after reinduction I based on minimal residual disease (MRD) status). Patients who exhibited clinical allergy to Elspar were subsequently given Erwinia asparaginase (Erwinase) or polyethylene glycol-conjugated Elspar (Oncaspar), based on their availability (Erwinase was used preferentially when both were available), which was influenced by manufacturer-related drug shortages. Erwinase was given at 20000 U/m² thrice weekly during remission induction for both LR and SHR patients, 20000 U/m² thrice weekly during reinduction for LR patients, and 25000 U/m³ twice weekly in weeks 1–19 of continuation therapy for the SHR patients. Oncaspar

was given at 2500 U/m² weekly according to treatment phase. All forms of asparaginase were given intramuscularly. If clinical allergy was confirmed for all three forms, asparaginase was discontinued.

Blood was collected into tubes without anticoagulant for asparaginase measures on days 5, 19, and 34 of remission induction, day 1 of reinduction I, and day 1 of reinduction II. Samples were collected before the asparaginase injection if given on the same day of sampling. Serum was frozen at -80°C until analysis.

Phenotyping of clinical allergy to asparaginase

The allergic reactions to asparaginase were characterized by local (pain, swelling, erythema) and/or systemic manifestations (fever, urticaria, or edema) and graded using the National Cancer Institute Common Toxicity Criteria (NCICTC version 3.0); only patients who developed grade 2 or higher reactions received drug substitution.²¹

Anti-asparaginase antibodies

Serum was available for assay of antibodies in 410 patients on Total XV (Supplemental Table S1). A total of 2010 serum samples (Supplemental Table S2) were measured by ELISA for total IgG antibodies to three forms of asparaginase (Elspar, Erwinase, and Oncaspar); IgE and other classes of antibodies were not measured. The method was based on a modification of a previously reported assay.²² Anti-asparaginase antibodies were analyzed as a continuous variable (optical density (OD) readings at 1:400 dilution after normalization across instruments) and also classified as positive or negative, using thresholds as defined in detail in the Supplement. Those with positive antibodies in the absence of clinical allergy were considered to have silent hypersensitivity to asparaginase.

To estimate the long-term exposure to antibodies, the area under the antibody-concentration-versus-time curve (AUC) was estimated using the method of trapezoids in the 360 patients who had at least four out of five scheduled antibody tests over the first 35 weeks of therapy. Analyses were performed using the sum of the induction AUC (antibody AUC between day 5 and day 34 of remission induction) and continuation AUC (antibody AUC between week 7 and week 17 of continuation) to Elspar (Supplemental Figure S1 and Supplemental methods). Patients were considered to have detectable antibody if the AUC was greater than 2.12 (OD value × days), which corresponds to 2.58 standard deviations above the mean value of negative cases (i.e., the antibody AUCs of the patients negative for anti-Elspar antibody consistently during therapy).

Sensitivity, specificity and predictive values

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated using the numbers of patients with true positive (TP), false positive (FP), true negative (TN) and false negative (FN) antibody results. For this purpose, patients who had clinical reactions attributed to Elspar over a given time period (e.g., weeks 7–9) were classified as TP or FN, depending on whether they were positive for anti-Elspar at an adjacent time point (e.g., week 7); the patients who never had a clinical reaction were classified as TN or FP, depending on whether they were ever positive for anti-Elspar during therapy. Sensitivity was defined as the percentage of reacting patients who were positive for anti-Elspar (calculated as $TP / [TP + FN]$); specificity was defined as the percentage of non-reacting patients who were negative for anti-Elspar (calculated as $TN / [TN + FP]$); PPV was defined as the likelihood that a patient who was positive for antibody had a clinical reaction (calculated as $TP / [TP + FP]$); and NPV was defined as the likelihood that a patient who was negative for antibody never had a clinical reaction (calculated as $TN / [TN + FN]$).

Determining serum asparaginase activity

Serum asparaginase activity was determined in a subset of samples from patients in the SHR arm: 77 week-7 samples and 69 week-17 samples which were collected 6–8 days after the last Elspar administration of 25000 U/m²; 9 week-7 samples and 10 week-17 samples which were collected 3–4 days after the last Erwinase administration of 25000 U/m²; and 14 week-7 samples and 16 week-17 samples which were collected 6–8 days after the last Oncaspar administration of 2500 U/m². We used a kinetic spectrophotometric assay similar to a previously described assay²³ based on an enzymatically coupled oxidation of reduced nicotinamide adenine dinucleotide (NADH). Details are described in the Supplement.

Asparaginase Adverse Effects

Other than allergy, the adverse effects possibly related to asparaginase include pancreatitis, thrombosis, and osteonecrosis, which exposure were uniformly assessed in all patients. Patients were prospectively screened by magnetic resonance imaging for osteonecrosis of the hips and knees, which was graded as 0 (absent), 1 (asymptomatic), 2 (symptomatic), 3 (severe), and 4 (disabling) based on the NCICTC Version 3.0, as previously reported.²⁴ All cases of grade 2–4 thrombosis and pancreatitis before week 20 of continuation therapy were considered possible adverse effects of asparaginase.

Statistical analysis

Comparisons of continuous variables between groups were conducted with Wilcoxon rank sum test. Fisher's exact test was used to determine the difference in frequencies of categorical variables (e.g., allergy or antibody positivity). Nonlinear and linear regression analyses were used to describe the change of antibody OD readings with time or with the frequency of clinical reaction. The prognostic capacity of antibody tests at different threshold levels were evaluated in terms of the receiver-operating characteristic (ROC) curve (see Supplement for details). Logistic regression was applied for multivariate analysis of risk factors of osteonecrosis; and the cumulative incidence of osteonecrosis was compared between groups by log-rank test. Pancreatitis and thrombosis were compared between groups using time-dependent analysis,²⁵ a Cox proportional-hazards regression model where antibody status was treated as a time-dependent covariate. Statistical analyses were conducted using R 2.11.1 (R Development Core Team, <http://www.r-project.org>) and SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Asparaginase allergy

Among 410 patients, 169 (41%) had clinical allergy to Elspar, of whom 72 were first rechallenged with Erwinase and 94 with Oncaspar, and 3 were switched to other drugs. Thirty-three patients experienced a second episode of allergy (9 to Erwinase and 24 to Oncaspar), and 2 patients developed allergy to all three preparations. Patients in the LR arm were more likely than those in the SHR arm to experience hypersensitivity to Elspar, with cumulative incidences of clinical allergy being 51% versus 32% ($P = 0.0002$), a finding consistent with that of our prior report.²¹ Antibody against Elspar was also more common in patients in the LR arm than the SHR arm: 69% versus 47%, $P = 6.6 \times 10^{-6}$ (Figure 1). The majority of allergic reactions occurred upon first re-exposure to Elspar following a 10- to 16-week hiatus after the induction phase: 78% (54 of 69) of patients in the SHR arm who had clinical allergy reacted during continuation weeks 1–6, and 96% (96 of 100) of patients in the LR arm who had clinical allergy reacted at reinduction I (Supplemental Table S3).

We also investigated whether other clinical features such as age, gender, race, or ALL lineage were associated with the risk of developing antibodies or an allergic reaction to

Elspar (Supplemental Table S4–5). Age and gender were not associated with allergy or antibody status; age was not related to allergy ($P = 0.68$) or antibody status ($P = 0.86$) even when the analysis was restricted to those with B-lineage ALL (adjusting for treatment arm and race). Allergies differed by race: reactions occurred in 45% of white, 31% of black, and 33% of other (Hispanic and Asian) patients ($P = 0.037$), and the frequencies of positive antibodies during therapy were 62%, 55%, and 40% in those patient groups ($P = 0.005$), respectively. All patients with T-cell ALL were enrolled in the SHR arm, and they had significantly fewer clinical reactions (19% vs. 39%, $P = 0.003$) and lower antibodies (35% vs. 52%, $P = 0.02$) to Elspar than did those with B-lineage ALL in the SHR arm.

Antibodies and allergy

Anti-Elspar antibodies were more frequent among the patients with clinical allergy to Elspar: 87.0% (147 of 169 reacting patients) compared to only 36.9% (89 of 241) among non-reacting patients ($P < 1 \times 10^{-15}$). Among the 236 patients who were considered positive for anti-Elspar antibody, those who had reactions had higher levels of antibody (based on OD readings) at the end of induction ($P = 0.0002$) and during the continuation phase (week 7, $P = 8 \times 10^{-9}$; and week 17, $P < 1 \times 10^{-15}$), and higher cumulative antibody titer based on anti-Elspar antibody AUC (27.8 ± 17.9 vs. 10.8 ± 13.3 , $P < 1 \times 10^{-15}$) than those who did not have reactions (i.e., the group who would be classified as “silent hypersensitivity”; Figure 2). However, patients with more severe clinical toxicity (grade 3–4 reactions) against Elspar did not have higher cumulative anti-Elspar antibody AUC (22.8 ± 23.0 vs. 28.6 ± 16.9 , $P = 0.11$) than those with mild toxicity (grade 2 reactions). For Oncaspar, similarly, we observed no difference in anti-Oncaspar antibody AUC in those with grade 3–4 vs. grade 2 reactions (5.7 ± 7.8 vs. 5.4 ± 4.8 , $P = 0.83$). For Erwinase, all allergic reactions were grade 2, and thus no comparisons are possible.

This study’s sampling strategy was designed to have evaluable antibody samples at comparable times relative to asparaginase dosing for all patients (those who developed an allergy and those who did not). Because allergic reactions may have occurred at times during which no samples were obtained, maximal values may not have been captured if antibody levels are maximal at the time of reaction. However, serial samples that happened to be obtained before, during, and after reactions indicated that antibody levels were estimated to be maximal at about 50 days after an allergic reaction (Supplemental Figure S2).

We investigated the utility of serum anti-Elspar antibody positivity to predict future (if sampling just before) or to confirm (if sampling just after) clinical allergic reactions. The sensitivity, specificity, and predictive values are shown in Table 1. The greatest utility was evident for the week 7 serum samples, which happened to be obtained closest to the time period during which most reacting patients had their allergic reaction (continuation weeks 7–9 for those in the LR arm and weeks 1–6 for those in the SHR arm). The week 7 anti-Elspar antibody test had a sensitivity of 87–88% and a specificity of 68–69% for predicting clinical reactions to Elspar at weeks 7–9 for LR patients and for confirming clinical reactions to Elspar at weeks 1–6 for SHR patients. The prognostic utility of the antibody tests at day 34, week 7 and week 17, as evaluated by the AUC of the ROC curves, ranged from 0.70 to 0.91 (Supplemental Figure S3). Antibody test obtained at day 34 of induction for future clinical reactions showed a lower prognostic capacity compared with those at week 7 and week 17 (Supplemental Figure S3).

Interestingly, we observed a strong positive linear relationship between the anti-Elspar level (natural log of OD reading) at week 7 and the probability of patients reacting to Elspar at weeks 7–9 on the LR arm (R-square = 0.96, $P = 6 \times 10^{-8}$) or weeks 1–6 on the SHR arm (R-square = 0.83, $P = 4 \times 10^{-5}$; Supplemental Figure S4), consistent with our finding that anti-

Elspar level was higher in patients with allergy than those with silent hypersensitivity (Figure 2).

The frequency of secondary allergy to Oncaspar was higher than to Erwinase (24/94 or 26% vs. 9/72 or 13%, $P = 0.05$). For secondary treatment with Erwinase and Oncaspar, there was less association between clinical reactions to these agents and corresponding antibodies. We measured anti-Erwinase and anti-Oncaspar antibodies in 63 of the 72 patients who went on to be treated with Erwinase and 85 of the 94 patients who went on to receive Oncaspar. Among the 63 patients receiving Erwinase, 9 had an allergy to it, of whom only 4 had detectable anti-Erwinase antibody. Among the 85 patients receiving Oncaspar, 23 had a clinical reaction, of whom 20 had detectable anti-Oncaspar antibody. In addition, silent hypersensitivity occurred among 8 of 63 (13%) patients treated with Erwinase and among 47 of 85 (55%) treated with Oncaspar (Supplemental Figure S5).

Antibodies and serum asparaginase activity

In addition to testing the clinical utility of asparaginase antibodies as a tool to predict or confirm clinical allergy, antibodies have been hypothesized to be important as indicators of lower systemic exposure to asparaginase. Indeed, we found that *in vivo* serum asparaginase activity after Elspar was lower in the samples positive for antibodies than in those negative for antibodies ($P = 7 \times 10^{-6}$, Figure 3A), and was inversely related to the level of antibody (based on OD readings, $P = 7 \times 10^{-11}$, Figure 3B).

To determine whether antibody was also inversely related to asparaginase activity after secondary treatment with Erwinase and Oncaspar, we measured *in vivo* serum asparaginase activity in 19 patients on Erwinase and 30 patients on Oncaspar at the time of the anti-Elspar antibody measurement. Asparaginase activity did not differ by anti-Elspar antibody positivity in patients who received second line treatment with Erwinase ($P = 0.29$), but it was lower (median 0.03 IU/mL, range 0–2.73 IU/mL) in those who were positive for anti-Elspar antibody and received second line treatment with Oncaspar compared to those who were antibody negative (median 2.04 IU/mL, range 1.07–2.77 IU/mL; $P = 0.005$).

Antibodies and adverse effects

As we have shown that asparaginase can potentiate exposure to dexamethasone,²⁶ we investigated whether cumulative asparaginase antibody titer affected the risk of symptomatic (grade 2 to 4) osteonecrosis. After adjustment for age and treatment arm, risk factors for the osteonecrosis, higher cumulative antibody titer (based on antibody AUC) was associated with lower risk of symptomatic osteonecrosis (odds ratio for $\log_2$ antibody AUC = 0.83; 95% CI, 0.78–0.89; $P = 0.007$), consistent with antibodies causing less asparaginase exposure.

We also compared the cumulative risk of symptomatic osteonecrosis between patients negative for anti-Elspar antibody (i.e., anti-Elspar antibody AUC ≤ 2.12) and those with detectable antibody (i.e., anti-Elspar antibody AUC > 2.12 ; Figure 4). There was a lower cumulative incidence of symptomatic osteonecrosis in those with a higher anti-Elspar antibody AUC who were older than 10 years of age (in the LR arm, $P = 0.048$; and the SHR arm, $P = 0.037$), and in younger patients (in the LR arm, $P = 0.047$; but not on the SHR arm, $P = 1.0$) (Figure 4).

Seven patients had pancreatitis before the end of reinduction II, and five of them were positive for antibody. Twenty-eight patients had thrombotic events before the end of reinduction II, and seven of them were positive for antibody. After adjusting for age and treatment arm, there was no apparent association between antibody positivity and pancreatitis ($P = 0.22$) or thrombosis ($P = 0.20$).

Discussion

Although the relationship between antibodies and allergic reactions to asparaginase has been observed in many studies,^{8, 10, 11, 23} this is the first report focusing on the diagnostic utility of asparaginase antibody measures to predict or to confirm clinical allergy to asparaginase. Particularly in children, differentiating allergy from other diagnoses can be challenging. In the context of an asparaginase-intensive front-line clinical trial and by dichotomizing samples as positive versus negative, the most informative sample (at week 7 of continuation phase) had reasonably high (88%) sensitivity to predict reactions in the next 3 weeks but less favorable specificity (68%) to confirm past reactions (see Table 1). In this regard, there was a relatively frequent occurrence of silent hypersensitivity during therapy (22%, 90 of 410 patients on the protocol). Based on this test performance, for every 100 patients with a reaction during weeks 1–9, 88 would be expected to have positive and 12 would have negative antibody tests during this time period (at week 7); for every 100 patients without an allergy, 32 would have positive and 68 would have negative antibody tests at that time (week 7). The diagnostic value of antibody test at this time point is similar to those of some widely used clinical laboratory tests, such as the diagnosis of venous thromboembolism with d-dimer (sensitivity 80–95% and specificity 40–70%);²⁷ the prediction of rheumatoid arthritis with rheumatoid factor (sensitivity 40–80%, specificity 70–90%);²⁸ and the diagnosis of systemic lupus erythematosus with antinuclear antibody (sensitivity 93%, specificity 50–80%).²⁹

We were unable to identify a more informative time point that yielded better antibody test performance. The early antibody test at the end of induction lacked the sensitivity to predict future clinical allergy. The later antibody tests (i.e., week 17) did not confirm as well for patients in the LR arm; for those in the SHR arm, the week 17 result had slightly reduced sensitivity (72.7%) and increased specificity (88.2%), but clinically, it would not be possible to use such a result for making therapeutic decisions. Because antibody levels tend to increase over time with successive asparaginase exposures, even in the absence of a reaction, comparisons between reacting and non-reacting patients should control for placebo-in-therapy. In our study, serum samples were timed uniformly relative to therapy but were not available at the exact time of the reaction in most cases (and control samples, of course, would not have been available in non-reacting patients at exactly comparable times); thus, we were not able to assess the specificity of the serum anti-Elspar antibody test at the time of clinical reaction to Elspar. However, even if we limited analysis to those reacting patients who had serum obtained 5–30 days before or after the allergy ($n = 132$ patients), the estimated true positive rate was not higher than 80% (data not shown).

We also investigated whether the predictive utility of the antibody measures was improved by leaving the measure as a continuous measure (OD of anti-Elspar antibody titers) instead of dichotomizing the results as positive versus negative. We observed a linear correlation between the antibody OD at week 7 and the probability of patients having a clinical reaction in the subsequent 3-week asparaginase course, and a correlation between week 7 antibody OD and the proportion who had clinical reactions in the previous 6-week asparaginase course (Supplemental Figure S4). For the LR patients with anti-Elspar OD > 0.39 at week 7, 90% of them are predicted to have an event in the subsequent course; for the SHR patients with anti-Elspar OD > 1.06 at week 7, 80% of them would have had an event in the previous course. However, falsely positive readings (high OD readings in patients with no known reaction) remained a limitation, and thus we were not able to define an alternative universal threshold for distinguishing positive from negative antibody status that resulted in better overall balanced test performance (based on the ROC curve of the antibody tests, see Supplemental Figure S3).

In addition to the possible utility of an anti-asparaginase antibody test to facilitate diagnosis of allergy, antibodies may be an indicator of reduced serum asparaginase exposure. In samples obtained at a uniform time (6–8 days) post-dose, we found that serum asparaginase activity was indeed inversely related to antibody level (Figure 3), consistent with reports from other groups.^{12, 13, 23, 30} Whether this attenuation of serum asparaginase activity is due to a direct neutralizing effect of antibodies, or because the presence of antibodies is an indicator of other immune-based mechanisms that enhance drug clearance (e.g., via the reticuloendothelial system) is unknown; the latter may explain why a few samples had low asparaginase activity despite being low in asparaginase antibodies (Figure 3).

We explored whether the presence of antibodies, with its likely attendant reduced exposure to asparaginase over time, would reduce other adverse effects of asparaginase therapy. Even though dexamethasone is the major cause of osteonecrosis in children with ALL,^{31–35} we have shown that asparaginase can increase the risk of osteonecrosis,³⁵ which we hypothesize could be because asparaginase inhibits protein synthesis, decreases dexamethasone clearance,²⁶ alters lipid metabolism,^{36, 37} and induces coagulopathy.^{38, 39} Because osteonecrosis is a long-term complication, we used antibody AUC as a measurement of long-term exposure to antibodies against Elspar, the front-line asparaginase preparation. We observed that, among younger patients on the LR arm and all older patients on either LR or SHR arm, those with high antibody AUC were at lower risk to develop symptomatic osteonecrosis than those with low antibody AUC (Figure 4). The difference was not apparent among the younger patients on the SHR arm, possibly because younger patients are less prone to osteonecrosis, and those on the SHR arm also had lower antibodies, perhaps too low to have any effect on the development of osteonecrosis (Figure 4D, right panel). In multivariate analysis, after adjusting for age and treatment arm, antibody AUC was significantly associated with grade 2–4 osteonecrosis. This is the first report that anti-asparaginase antibodies were inversely related to the risk of osteonecrosis, but is consistent with our hypothesis that asparaginase potentiates glucocorticoid effects.³⁵ We recently reported that asparaginase antibodies were associated with a higher risk of central nervous system relapse,⁴⁰ but did not find that asparaginase antibodies were related to pancreatitis or thrombosis, perhaps indicating that these latter two adverse events are less “dose-related” than others.

In the Total XV study, with Elspar as the front-line asparaginase preparation, the frequency of clinical hypersensitivity to Elspar was 41% (169/410) for all patients who started with Elspar, and the incidence of IgG antibodies to Elspar was 58% (236/410), similar to the average from other reported series.^{11–14} The hypersensitivity appeared to differ by treatment arm and immunophenotype, consistent with the result from our previous ALL trial, Total XIII.^{11, 14} Patients on the LR arm exhibited more hypersensitivity to asparaginase than did those on the SHR arm, possibly because the LR arm included less immunosuppressive chemotherapy and included longer periods of asparaginase “holiday” followed by re-challenge, a practice noted to be associated with allergy by others.⁹

There was a very high frequency of “silent hypersensitivity” observed in patients who subsequently received Oncaspar (55% of patients with no allergy had positive antibodies against Oncaspar). However, 24% of patients who never received Oncaspar had positive antibodies at week 7 and 17, which could be due to cross reactivity between sera positive to both Elspar and Oncaspar in patients who are exposed initially to Elspar (Oncaspar is pegylated Elspar).⁴¹ Consistent with that idea, only 13% of patients who received Erwinase had silent hypersensitivity, compared to 6% of patients who never received Erwinase. Allergy was also more common ($P = 0.05$) in those who received Oncaspar rather than Erwinase as their second-line agent, and serum asparaginase activity was lower in those with (compared to those without) anti-Elspar antibodies who received Oncaspar ($P = 0.005$), but

not in those who received Erwinase ($P = 0.29$), consistent with recent reports.³⁰ Together, these data suggest that Erwinase may be preferred over Oncaspar in those who receive primary treatment with Elspar and experience allergy, although it is possible that such a decision depends upon the dose of Oncaspar versus Erwinase and the absolute level of anti-Elspar antibodies.³⁰

Interestingly, patients with T-cell ALL were less likely to develop asparaginase allergy and had lower antibody levels at post-asparaginase time points than those with B-lineage ALL. The mechanisms underlying this difference are unclear, but suggest an intriguing added value for asparaginase in T-cell ALL.

In summary, we comprehensively analyzed anti-Elspar antibody in an asparaginase-intensive front-line clinical trial. In this context, in which Elspar is given as the primary form of asparaginase, serum anti-Elspar antibodies have good utility as tools to help make the diagnosis of clinical hypersensitivity. Our data show that higher antibody levels are associated with greater clinical pharmacologic effects including a higher risk of clinical allergy, and a greater attenuation of serum asparaginase enzyme activity with associated lower risk of osteonecrosis. Thus, measures of serum antibodies to asparaginase can be useful in patients with ALL.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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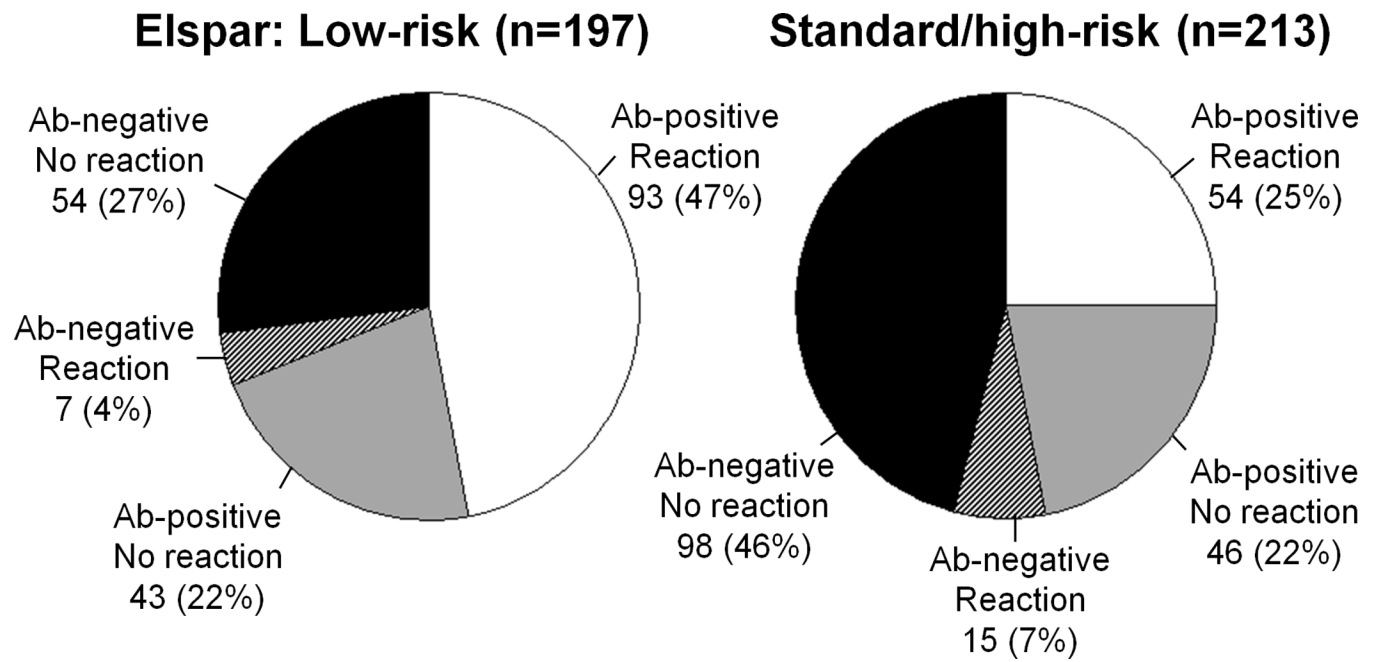


Figure 1. Frequency of hypersensitivity to Elspar

The frequency of allergic reactions and anti-Elspar antibody (Ab) positivity in patients enrolled on the low-risk (LR) and standard/high-risk (SHR) treatment arms. Compared with those on the SHR arm, patients on the LR arm had more reactions ($P < 0.00001$) and anti-Elspar antibody ($P = 0.0002$).

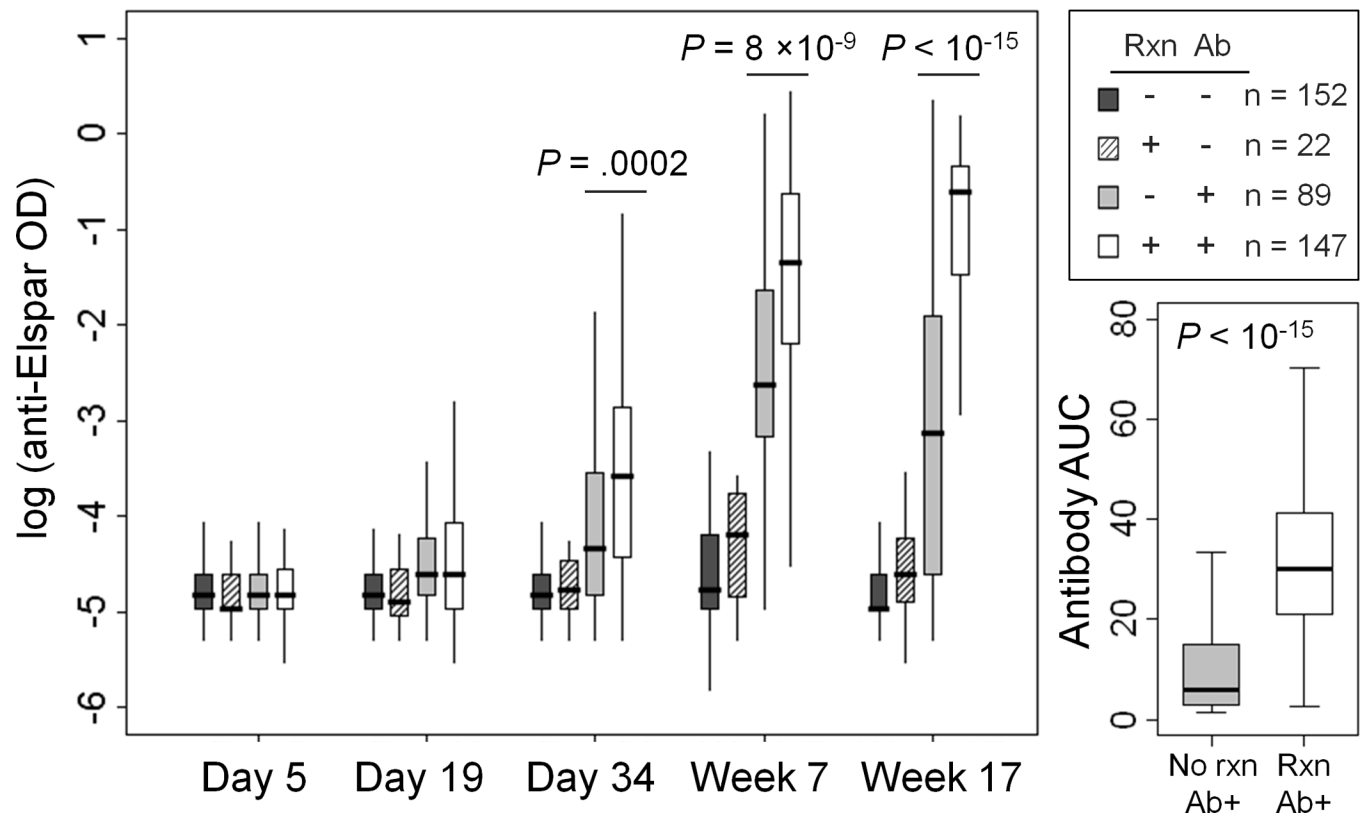


Figure 2. Antibody levels in patients with and without clinical reactions

Left: Anti-Elspar optical density (OD) measures (natural log) for 410 patients at days 5, 19, and 34 of remission induction and weeks 7 and 17 of continuation therapy. At each time point, patients are grouped based on whether they ever (or never) experienced a clinical allergic reaction (Rxn), and whether they were ever (or never) positive for anti-Elspar antibody (Ab) at anytime during therapy. Among the patients positive for anti-Elspar, those who had reactions (white bars) had significantly higher anti-Elspar levels than did those who had silent hypersensitivity (light grey) at day 34, week 7, and week 17. Among the patients negative for anti-Elspar antibodies (as defined by the threshold in the Supplement), those who had allergic reactions (hatched) exhibited slightly elevated anti-Elspar levels compared with those without any allergic reactions (dark grey) at weeks 7 ($P = 0.05$) and 17 ($P = 0.03$). Right: cumulative anti-Elspar antibody AUC over the first 35 weeks of therapy for antibody-positive patients who had allergic reactions versus those who had silent hypersensitivity. Box plots: median, quartiles, non-outlier range.

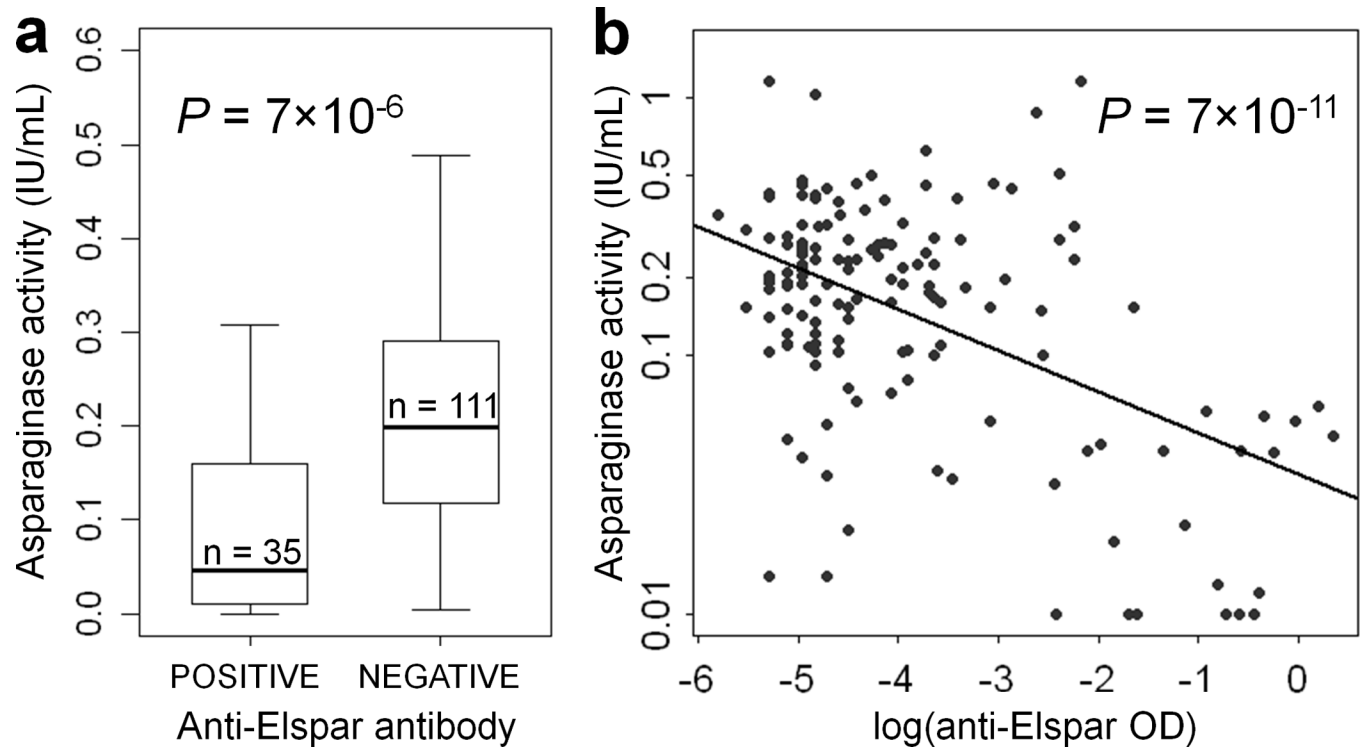


Figure 3. Correlation between serum asparaginase activity and antibody level

Serum asparaginase activity (IU/mL) measured 6–8 days after the last Elspar dose of 25000 U/m². (A) Asparaginase activity was significantly lower in the samples positive than in those negative for anti-Elspar antibodies. Box plots: median, quartiles, non-outlier range. (B) Asparaginase activity inversely correlated with anti-Elspar OD reading of serum samples. Individual data points and the regression line are shown for all cases (n = 146).

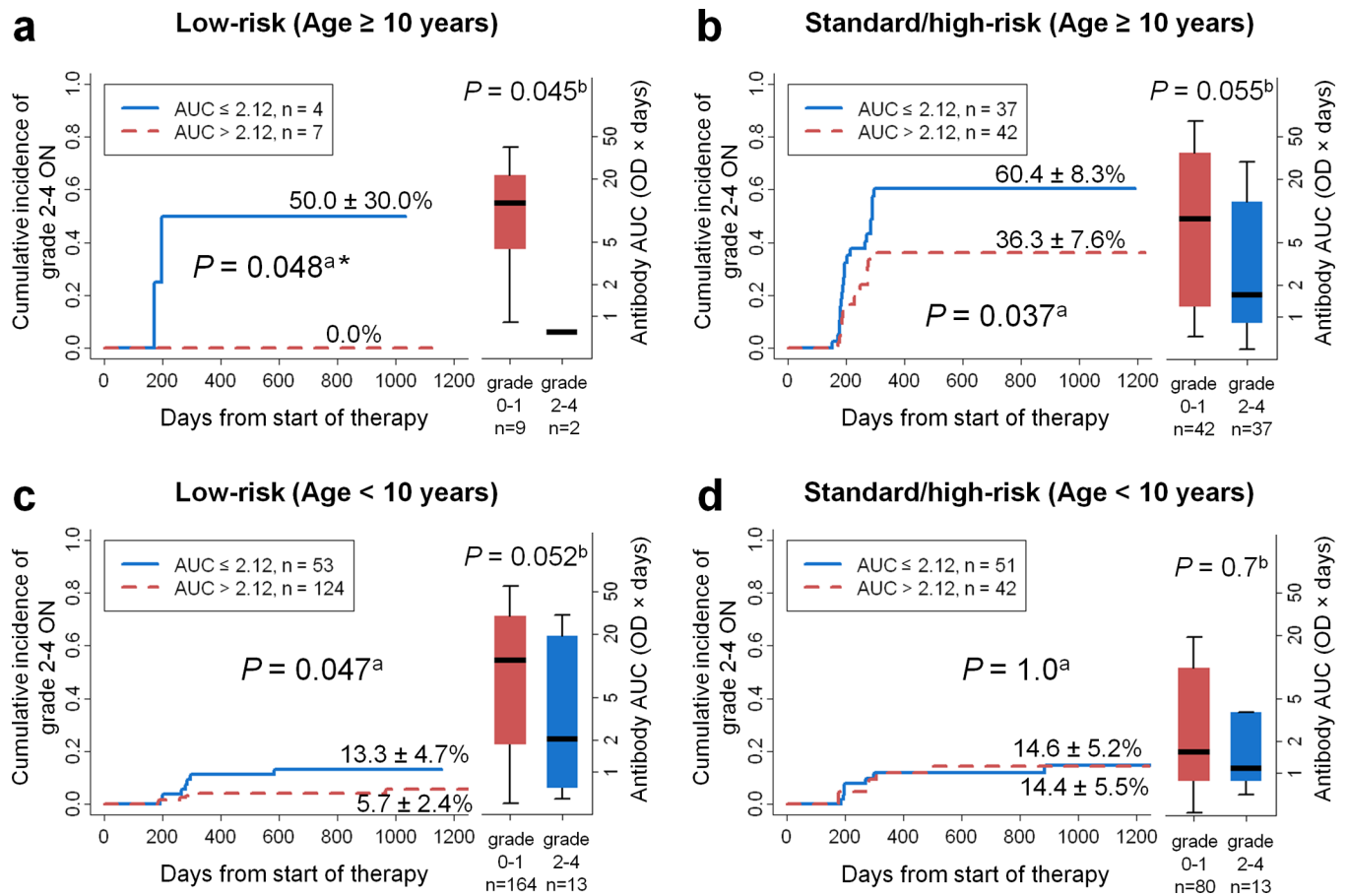


Figure 4. Cumulative incidence of osteonecrosis based on anti-asparaginase antibody AUC
 Patients were stratified by low-risk (LR) versus standard/high-risk (SHR) treatment arm and age at diagnosis: (A) patients in the LR arm with age greater than 10 years; (B) patients in the SHR arm with age greater than 10 years; (C) patients in the LR arm with age under 10 years; (D) patients in the SHR arm with age under 10 years. Left: Cumulative incidence of symptomatic (grade 2 to 4) osteonecrosis (ON) in patients with anti-Elspar antibody AUC (OD $\times$ days) lower than 2.12 (blue) versus greater than 2.12 (red). Right: box plot of anti-Elspar antibody AUC in patients with (blue) versus without symptomatic ON (red). a = P value based on log rank test and b = P value based on Wilcoxon rank sum test. *Fisher's exact test ($P = 0.11$) was also performed because the sample size is small.

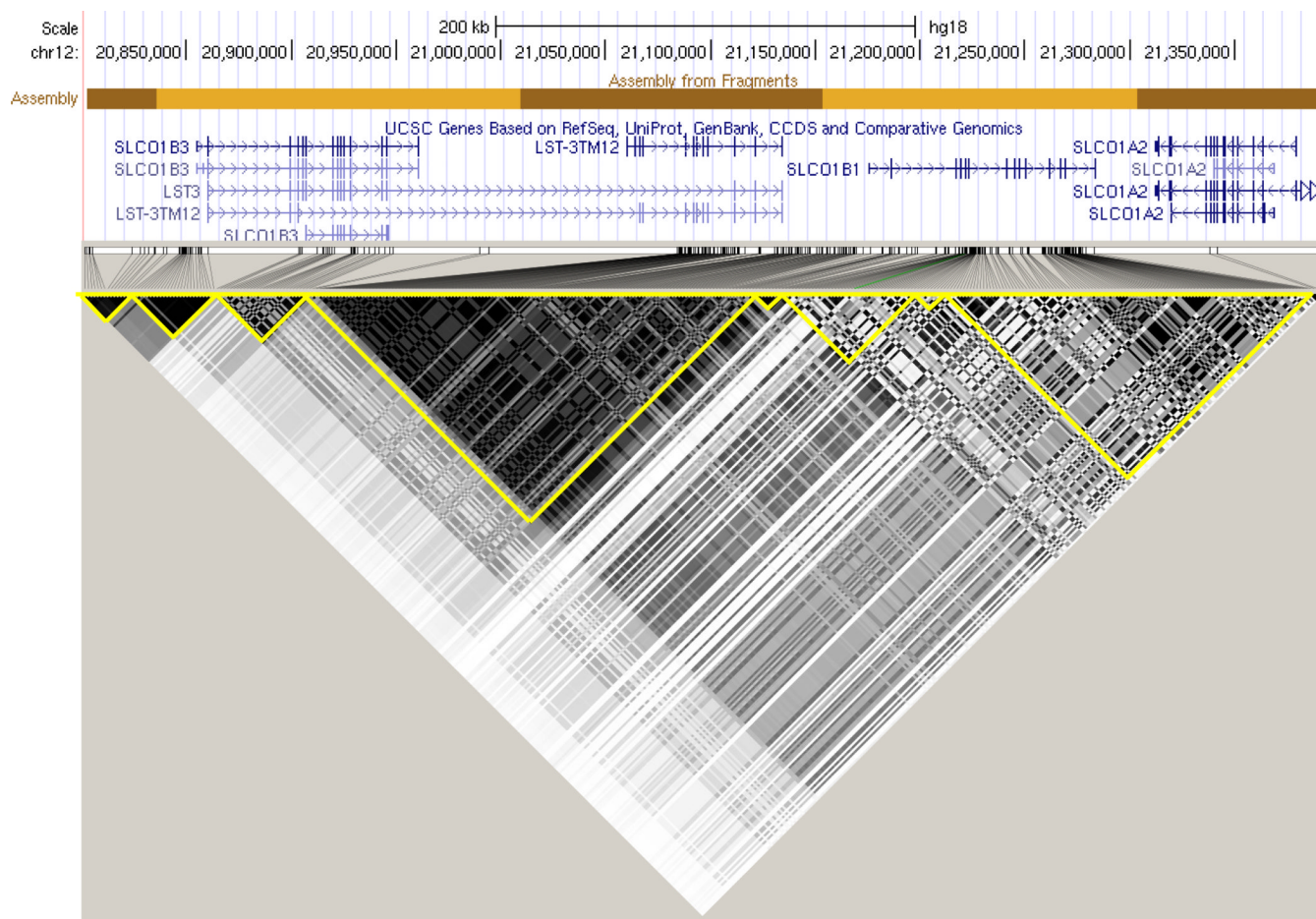


Figure 5.

Sensitivity, specificity, and predictive values of three anti-Elspar antibody tests for predicting or confirming clinical reactions to Elspar

Table 1

Risk arm	n	Time of Ab test	Time of Rxn	Time between test and Rxn, weeks	Sensitivity %	Specificity %	PPV %	NPV %
Prediction of future reactions								
LR	185	Day 34	Wks 7–9	17–20	42.8	92.5	84.8	62.6
SHR	190	Day 34	Wks 1–6	10–17	26.1	93.1	54.5	79.8
LR	192	Wk 7	Wks 7–9	0–3	87.4	68.0	72.8	84.6
Confirmation of past reactions								
SHR	184	Wk 7	Wks 1–6	0–6	87.8	68.9	50.6	93.9
LR	189	Wk 17	Wks 7–9	7–10	89.4	65.3	71.8	86.1
SHR	174	Wk 17	Wks 1–16	0–16	72.7	88.2	74.1	87.5

SHR, standard/high-risk; LR, low-risk; PPV, positive predictive value; NPV, negative predictive value; Ab, anti-Elspar antibody; Rxn, clinical allergic reaction; Day 34, day 34 of remission induction therapy (34 days from diagnosis of ALL); Wk 7, week 7 of continuation therapy (23 weeks from diagnosis of ALL); and Wk 17, week 17 of continuation therapy (33 weeks from diagnosis of ALL).