

Hypersensitivity or Development of Antibodies to Asparaginase Does Not Impact Treatment Outcome of Childhood Acute Lymphoblastic Leukemia

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Purpose: Development of antibodies and hypersensitivity to asparaginase are common and may attenuate asparaginase effect. Our aim was to determine the relationship between antiasparaginase antibodies or hypersensitivity reactions and event-free survival (EFS).

Patients and Methods: One hundred fifty-four children with acute lymphoblastic leukemia received *Escherichia coli* asparaginase 10,000 IU/m² intramuscularly three times weekly for nine doses during multiagent induction and reinduction phases and for seven monthly doses during continuation treatment. *Erwinia* asparaginase was used in case of clinical hypersensitivity to *E coli* but not for subclinical development of antibodies. Plasma antiasparaginase antibody concentrations were measured on day 29 of induction in 152 patients.

Results: Antibodies were detectable in 54 patients (35.5%), of whom 30 (55.6%) exhibited hypersensitivity to asparaginase. Of the 98 patients who had no

detectable antibodies, 18 (18.4%) had allergic reactions. Patients with antibodies were more likely to have a reaction than those without antibodies ($P < .001$). Among the 50 patients who experienced allergic reactions (including two for whom antibodies were not measured), 36 (72.0%) were subsequently given *Erwinia* asparaginase; seven (19.4%) reacted to this preparation. EFS did not differ among patients who did and did not have antibodies ($P = .54$), with 4-year EFS (± 1 SE) of $83\% \pm 6\%$ and $76\% \pm 5\%$, respectively. Similarly, EFS did not differ among patients who did and did not develop allergic reactions ($P = .68$), with 4-year estimates of $82\% \pm 6\%$ and $78\% \pm 5\%$, respectively.

Conclusion: In this setting, in which most patients with allergy were switched to another preparation, there was no adverse prognostic impact of clinical or subclinical allergy to asparaginase.

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ASPARAGINASE IS an effective antileukemic agent that exploits a metabolic difference between normal cells and malignant cells. Asparaginase catalyzes the hydrolysis of asparagine to aspartic acid and ammonia in the extracellular space. Although most normal cells can synthesize asparagine, asparagine synthetase activity or inducibility is diminished in some malignant lymphoblasts such that this amino acid is not produced in sufficient quantities in leukemic cells.^{1,2} As a result of asparagine depletion, synthesis of protein and nucleic acids is diminished, which decreases leukemic cell proliferation.

Because asparaginase is isolated from bacterial sources (*Escherichia coli* or *Erwinia chrysanthemi*), development of allergies is a major limitation to its clinical use. Available pharmaceutical preparations of asparaginase vary in their pharmacokinetic and immunogenic properties.³⁻⁵ *E coli* is the source of the most commonly used asparaginase preparation in the United States and is also widely used throughout the world. In the United States, *Erwinia* asparaginase is used only in patients who have an allergy to the *E coli* preparation.

The reported frequency of antiasparaginase antibodies is highly variable; antibodies have been reported in as many as 79% of adults⁶ and as many as 70% of children⁷ after intravenous or intramuscular administration of *E coli* asparaginase. Antibody concentrations increase with repeated

asparaginase administration and are higher in patients who exhibit overt allergic reactions.⁸ Development of antibodies may attenuate the antileukemic effect of asparaginase by shortening its half-life, preventing or delaying absorption after intramuscular injection, or interfering with enzyme activity.^{3,6,9}

Clinical hypersensitivity reactions to *E coli* asparaginase are also common, and their reported frequency is highly variable (ranging from 0% to 45%).^{2,6,7,10-16} Factors that may affect the incidence of allergic reactions include different manufacturers, dosage, ongoing asparaginase ad-

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ministration, readministration after a hiatus, and concurrent chemotherapy.^{7,10,11,17-19} These reactions frequently require discontinuation of *E coli* asparaginase and subsequent substitution with another asparaginase preparation.

The relationship between the development of antiasparaginase antibodies and allergic reactions and outcome in childhood acute lymphoblastic leukemia (ALL) has not been clearly defined. Recently, asparaginase allergy was found not to be prognostic among adults with ALL,²⁰ although antibodies were not measured. In the current study, children were treated on a single protocol and had anti-asparaginase antibody concentrations measured at a fixed time point relative to asparaginase dosing. Our objective was to determine whether the development of antiasparaginase plasma antibodies or clinical hypersensitivity reactions after intramuscular administration of *E coli* asparaginase had a significant impact on the event-free survival (EFS) of a group of uniformly treated children with newly diagnosed ALL.

PATIENTS AND METHODS

Patient Eligibility and Treatment Schedules

All 154 children with newly diagnosed ALL enrolled on St. Jude Children's Research Hospital front-line protocol Total XIII-HR were evaluated. The criteria for eligibility, diagnosis, risk-group classification, and treatment have been reported elsewhere.^{21,22} Patients received *E coli* asparaginase (Elspar; Merck & Co, Inc, West Point, PA) 10,000 IU/m² intramuscularly three times weekly for a total of nine doses during both induction and reinduction phases, as well as seven monthly doses during the first 7 months of continuation therapy. Two months after the last patient was accrued, the protocol was amended such that asparaginase doses 7, 8, and 9 during reinduction and all doses from maintenance were eliminated to reduce the risk of secondary acute myeloid leukemia (AML).²¹ In patients who developed clinical asparaginase allergy, patients were either premedicated with diphenhydramine, with or without glucocorticoids, or switched to a different preparation (*Erwinia* asparaginase or polyethylene glycol-conjugated [PEG] asparaginase), or both. The multiagent treatment regimen for Total XIII-HR is listed in Table 1. All allergic reactions were graded according to the standard National Cancer Institute common toxicity criteria. All treatment protocols were approved by the institutional review board, and informed consent was obtained from patients or their parents or guardians according to institutional guidelines.

Sample Collection

For measurement of antiasparaginase antibodies, blood samples from a peripheral vein were collected in heparinized tubes on day 29 of induction therapy (ie, 10 days after nine doses of asparaginase) in 152 patients; two of 154 patients did not have plasma available for study for logistic reasons. Samples were centrifuged at 3,000 rpm for 5 minutes, and the plasma was stored at -70°C until analysis. Plasma or CSF suitable for asparaginase measures was not available.

Determination of Antiasparaginase Antibodies

Antiasparaginase antibody concentrations were measured by enzyme-linked immunosorbent assay as previously described.⁸ Briefly, *E*

Table 1. Treatment Regimen for Total XIII-HR Protocol

Remission induction/ reinduction	
Weeks 0-4	Methotrexate, either 1 g/m ² IV $\times$ 24 hours or 30 mg/m ² PO every 6 hours $\times$ 6, followed in 96 hours by prednisone 40 mg/m ² PO daily for 29 days, vincristine 1.5 mg/m ² weekly for 4 weeks; daunomycin 25 mg/m ² IV days 1 and 8, and asparaginase 10,000 IU/m ² IM 3 times per week $\times$ 9 doses.
Weeks 5-6	Etoposide 300 mg/m ² IV + cytarabine 300 mg/m ² IV days 22, 25, and 29.
Weeks 7-8	Methotrexate 2 g/m ² IV days 44 and 51; mercaptopurine 75 mg/m ² PO daily $\times$ 14.
Continuation (120 weeks)	
Week 1	Etoposide 300 mg/m ² IV + cyclophosphamide 300 mg/m ² IV.
Week 2	Mercaptopurine 75 mg/m ² PO daily + methotrexate 40 mg/m ² IV/IM.
Week 3	Methotrexate 40 mg/m ² IV/IM + cytarabine 300 mg/m ² IV.
Week 4	Prednisone 40 mg/m ² PO daily + vincristine 1.5 mg/m ² IV + asparaginase 10,000 IU/m ² IM.
Week 5	Etoposide 300 mg/m ² IV + cyclophosphamide 300 mg/m ² IV.
Week 6	Mercaptopurine 75 mg/m ² PO daily + methotrexate 2 g/m ² IV.
Week 7	Etoposide 300 mg/m ² IV + cytarabine 300 mg/m ² IV.
Week 8	Prednisone 40 mg/m ² PO daily + vincristine 1.5 mg/m ² IV + asparaginase 10,000 IU/m ² IM.

NOTE. Weeks 1 through 8 of continuation were repeated for a total of 120 weeks of therapy. At weeks 32 through 38, reinduction therapy was given, which was identical to remission induction therapy, except that etoposide/cytarabine was given on day 22 only, and only one high-dose methotrexate dose was given. High-dose methotrexate was replaced by methotrexate 40 mg/m² after 1 year of continuation therapy, and asparaginase was omitted after reinduction. Thus asparaginase was administered for a total of nine doses (on days 2, 4, 6, 8, 10, 12, 15, 17, and 19) during induction therapy and on the same days of reinduction (weeks 32 through 34). Asparaginase was also administered every 4 weeks during continuation therapy (weeks 4, 8, 12, 16, 20, 24, and 28).

Abbreviations: IV, intravenously; PO, orally; IM, intramuscularly.

coli asparaginase 5 μ g/mL (Elspar 10,000 IU, lot no. 0496D) was used as the antigen, and bovine serum albumin 0.1% (radioimmunoassay grade; Sigma, St. Louis, MO) was used as the blocking solution. Wells with multiple serial dilutions were developed with horseradish peroxidase-conjugated goat antihuman immunoglobulin (Ig) G (Sigma). After incubation with substrate solution, wells were read on an enzyme-linked immunosorbent assay reader set to read optical density (OD) at a wavelength of 490 nm minus the OD at 650 nm (Molecular Devices Corp, Sunnyvale, CA). Data at the 1:3200 plasma dilution are reported as OD readings. Samples giving an OD of $\geq$ 0.016 (mean OD of 20 untreated normal human volunteers + 2 SDs) at 1:3200 plasma dilution were considered to be positive for antibodies.

Statistical Methods

Fisher's exact test and the exact χ^2 test were used to determine whether or not categorical variables of interest differed in their distributions between two groups. The Wilcoxon rank sum test was used to compare continuous variables.

EFS was measured from the date on which the patient was enrolled onto the study to the first failure of any kind (ie, relapse, secondary malignancy, or death) or the date of last follow-up. Patients who did not achieve a complete remission by day 43 were assigned an EFS value of zero. Distributions of EFS were estimated by the method of Kaplan and Meier,²³ and associated SEs were calculated according to the method of Peto et al.²⁴ Distributions of EFS were compared using the Mantel-Haenszel test stratified for immunophenotype, age at diagnosis, and WBC count at diagnosis.

Logistic regression was used to test for relationships between explanatory variables of interest and whether patients experienced hypersensitivity. A Cox proportional hazards model, stratified for immunophenotype, age at diagnosis, and leukocyte count at diagnosis, was used to determine the impact of antiasparaginase plasma antibody concentration on EFS.

No adjustments were made for multiple testing. All *P* values reported are two-sided, and all statistical analyses were conducted using SAS Release (Version 6.12, SAS Institute, Inc, Cary, NC).

RESULTS

Antiasparaginase Antibodies and Allergic Reactions

Of the 154 patients, 151 achieved a complete remission. Antiasparaginase antibody concentrations were measured in 149 of the 151 patients who achieved remission and in the three who did not. Of the 152 children who had antibodies measured, 54 (35.5%) had detectable antibodies; all three children who did not achieve remission did not have detectable antibodies. Patient characteristics are listed in Table 2. Patients with B-lineage immunophenotype were more likely to develop antibodies than patients with T-lineage ALL (Table 2; *P* = .028). Patients who developed antibodies were more likely to experience a hypersensitivity reaction to asparaginase than those who did not (30 of 54 v 18 of 98; *P* < .001) (Fig 1). Among those who did develop antiasparaginase antibodies, the odds of experiencing a hypersensitivity reaction to asparaginase increased by approximately 5.6 times for each 0.01 OD increase in antibody concentration (*P* = .019). The proportion of children receiving the maximal number of asparaginase doses was not different in those who did versus those who did not exhibit antibodies (*P* = .31). The two patients who were not tested for antiasparaginase antibody experienced a hypersensitivity reaction.

Fifty (32.5%) of the 154 patients had one or more hypersensitivity reactions to *E coli* asparaginase, with initial reactions developing after three to 22 doses (median, 12 doses). The majority of hypersensitivity reactions to asparaginase occurred after the time point (day 29) at which

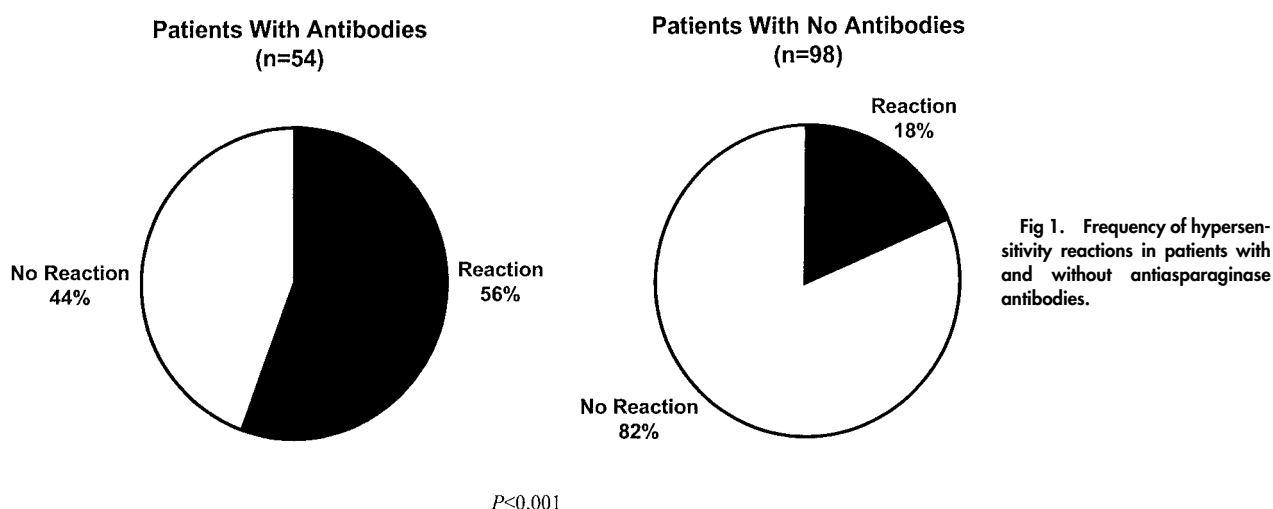
Table 2. Results of Tests for Associations Between Presenting Features and the Presence/Absence of Measurable Antiasparaginase Antibodies

Factor	No. of Patients Tested	Patients With Antibodies at Day 29 (%)	<i>P</i>
Age at diagnosis			
< 1 year	5	0	
≥ 1 and < 10 years	104	36	.23*
≥ 10 years	43	40	
Sex			
Male	88	34	.73
Female	64	37	
Race			
White	127	38	.25
Nonwhite	25	24	
WBCs at diagnosis			
≤ 50 × 10 ⁹ /L	109	40	
> 50 and ≤ 100 × 10 ⁹ /L	19	16	.083*
> 100 × 10 ⁹ /L	24	29	
Immunophenotype			
B-lineage	130	39	.028
T-lineage	22	14	
DNA index			
≥ 1.16 and ≤ 1.6	22	41	.63
< 1.16 or > 1.6	130	35	
Philadelphia chromosome			
Negative	145	36	.99
Positive	6	33	
MLL gene			
Germline	123	35	.16
Rearranged	10	10	
TEL gene			
Germline	100	31	.19
Rearranged	29	45	

NOTE. Statistical analyses were used to determine whether the distribution of presenting features differed in patients who did and did not develop antiasparaginase antibodies.

*Computed using the exact χ^2 test. All other *P* values were calculated using the Fisher's exact test.

antibody concentrations were measured. Six patients had nine reactions during induction; four of these patients had detectable antibodies at day 29, with two of the levels being among the highest antibody concentrations measured. In addition, there were 54 reactions during continuation and 26 reactions during reinduction therapy. Timing of initial (first) reactions were as follows: one patient each reacted to the third through seventh doses (one patient reacted to the fourth dose, but it was not given until continuation because of coagulopathy during induction), two reacted to the eighth dose, four reacted to the 10th dose, nine each reacted to the 11th and 12th doses, six reacted to the 13th dose, seven reacted to the 14th dose, three reacted to the 15th dose, and one each reacted to the 16th, 17th, 19th, 20th, and 22nd doses. After a reaction to asparaginase, patients either



continued on the same preparation with premedication or switched to another asparaginase product (Fig 2).

Characteristics of the hypersensitivity reactions to asparaginase are listed in Table 3. Fifty patients experienced a total of 89 reactions to asparaginase (79 reactions to the *E coli* preparation and 10 to the *Erwinia* preparation). There was an approximately equal number of patients who presented with localized reactions alone and with generalized reactions alone; however, most reactions manifested as a plethora of symptoms rather than as single symptoms. One patient who exhibited a clinical allergic reaction to both *E coli* and *Erwinia* asparaginase received PEG asparaginase, a conjugate of the native *E coli* asparaginase covalently

linked to polyethylene glycol, and reacted to this preparation with generalized erythema and angioedema.

EFS

The 4-year EFS $\pm$ 1 SE for patients with and without antibodies was $83\% \pm 6\%$ and $76\% \pm 5\%$, respectively (Fig 3). After stratifying for immunophenotype, age at diagnosis, and diagnostic WBC count, there was no evidence to suggest that the EFS of patients with anti-asparaginase plasma antibodies was significantly different from that of those without antibodies ($P = .54$). Moreover, there was no evidence to suggest that anti-asparaginase plasma

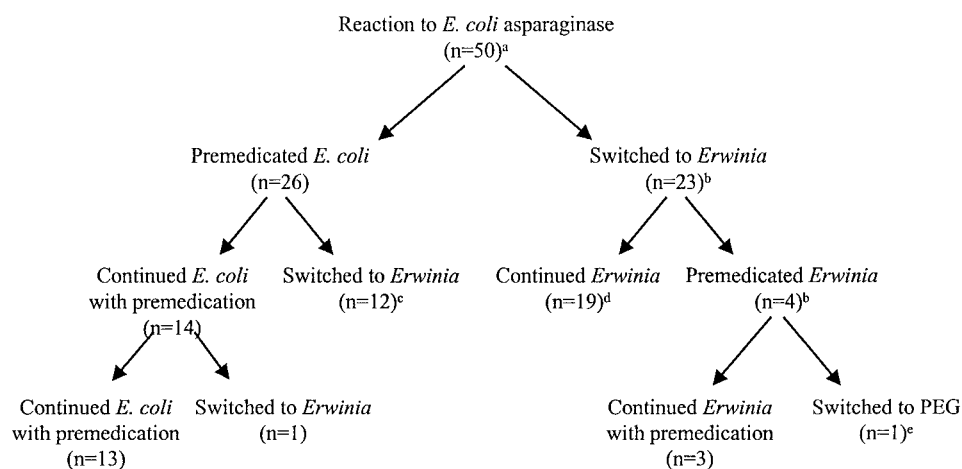


Fig 2. Schematic of asparaginase therapy in patients exhibiting clinical hypersensitivity reactions ($n = 50$). One hundred fifty-three patients were able to tolerate some form of asparaginase (with or without premedication); one patient reacted to all three asparaginase preparations. ^a One patient was diagnosed with hepatitis B 9 days after a reaction to *E coli* and did not receive any additional asparaginase doses. ^b One patient received occasional premedication with *Erwinia*. ^c One patient received premedication with each *Erwinia* dose. ^d One patient received three *E coli* doses and nine *Erwinia* doses. ^e The patient exhibited a clinical allergic reaction to the final scheduled dose of PEG asparaginase.

Table 3. Clinical Characteristics of Reactions to Asparaginase

	<i>E coli</i> Asparaginase		<i>Erwinia</i> Asparaginase	
	With Other Manifestations	Alone	With Other Manifestations	Alone
Localized reaction				
Pain	21	6	2	2
Edema	18	3	2	1
Urticaria	2	1	0	0
Erythema	5	8	1	0
Macular rash	2	0	0	0
Pruritis	1	0	0	0
Generalized reaction				
Pain	4	1	0	0
Erythema	13	7	1	0
Urticaria	8	5	2	0
Macular	3	2	1	2
Pruritis	4	0	0	0
Respiratory distress	7	2	0	0
Fever	5	0	0	0
Angioedema	12	1	1	0
Loss of consciousness	1	0	0	0
Anaphylaxis	0	1	0	0
Localized reaction	34		5	
Generalized reaction	35		5	
Localized + generalized reaction	10*		0	

*Each reaction consisted of both localized and generalized signs/symptoms.

antibody concentrations, evaluated as a continuous variable, had independent prognostic significance ($P = .154$).

The 4-year EFS ± 1 SE for patients who reacted to asparaginase and those who did not was $82\% \pm 6\%$ and $78\% \pm 5\%$, respectively (Fig 4). After stratifying for immunophenotype, age at diagnosis, and diagnostic WBC count, there was no evidence to suggest that the EFS of patients who had hypersensitivity reactions to asparaginase was significantly different from that of those who did not ($P = .68$).

DISCUSSION

We observed no relationship between the development of anti-asparaginase antibodies or hypersensitivity reactions and outcome in children with newly diagnosed ALL. Antibodies were measured at a fixed time point relative to asparaginase administration: day 29 of remission induction therapy after nine doses of intramuscular *E coli* asparaginase. Variables that may influence antibody levels and/or allergic reactions, such as previous exposure, time of treatment, and concurrent chemotherapy,^{4,7,10,11,18,19} were not confounding factors, because all patients were on the same treatment protocol. In this setting, in which asparagi-

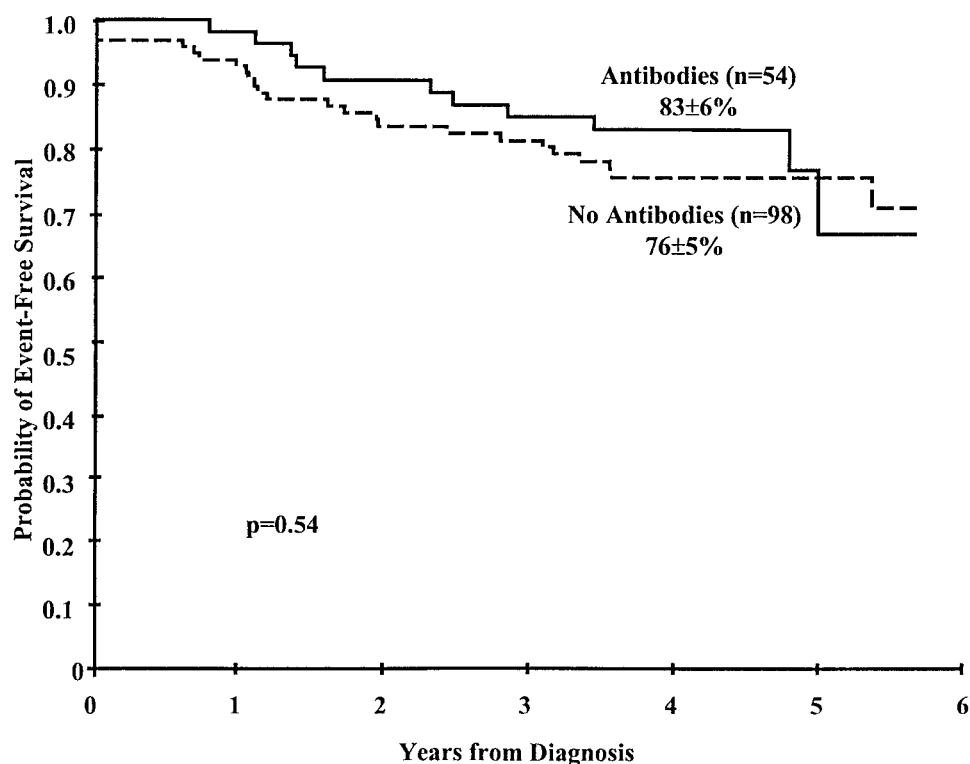


Fig 3. EFS according to the presence or absence of antibodies to asparaginase. There was no evidence to suggest that the EFS of patients with antibodies was significantly different from that of those without antibodies ($P = .54$).

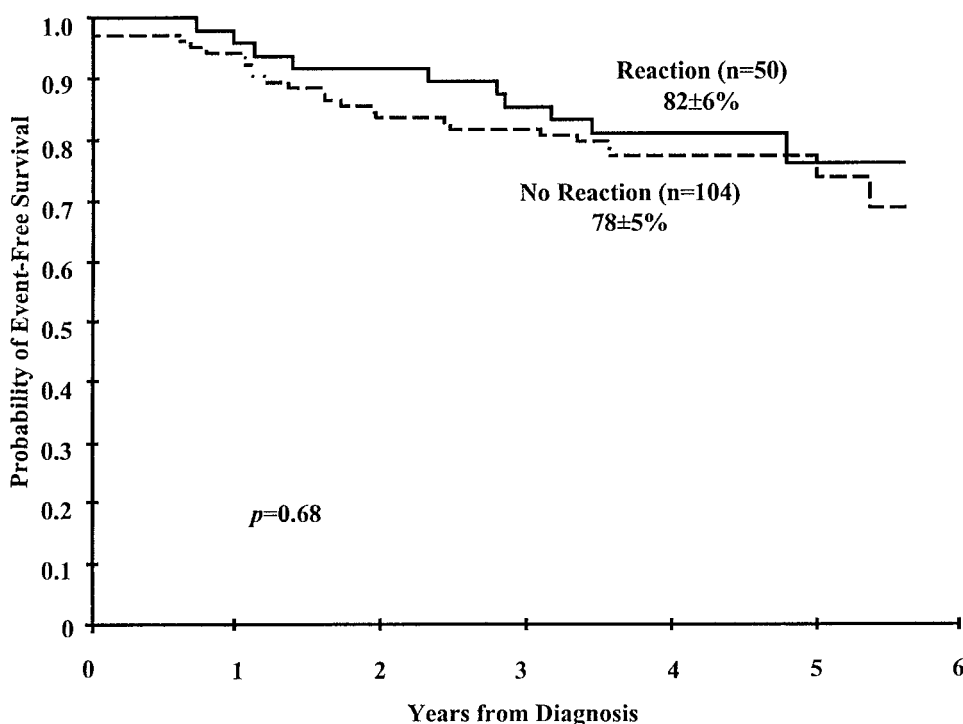


Fig 4. EFS according to the presence or absence of hypersensitivity reactions to asparaginase. There was no evidence to suggest that the EFS of patients who experienced hypersensitivity reactions was significantly different from that of those who did not ($P = .68$).

nase preparations were changed after reactions occurred, development of anti-asparaginase antibodies at the end of remission induction or hypersensitivity reactions did not compromise EFS.

Thirty-six percent of 152 patients, including 63% of the patients who exhibited clinical hypersensitivity, had measurable antibody levels. It has been suggested that anti-asparaginase antibodies may have a deleterious effect on systemic exposure or effect of asparaginase, as development of antibodies has been associated with attenuated serum asparaginase activity in six of seven children and 15 of 16 adults.⁶ We did not measure asparaginase activity or asparaginase concentrations in this study. However, it may be that the schedule of asparaginase was intense enough—either a high enough dose or a great enough frequency—such that attenuation of asparaginase by antibodies was not important with respect to outcome in these patients.

Conflicting data exist regarding the relationship between anti-asparaginase antibodies and clinical outcome, and studies have typically involved small numbers of patients and heterogeneous populations. In a study of children and adults with ALL and AML, the frequency of remission among antibody producers was 68% (13 of 19 patients) and among nonproducers was 27% (three of 11 patients).⁶ Antibody formation was hypothesized to be a surrogate for an active

immune response against leukemic cells and thus a good prognostic factor. In contrast, Cheung et al¹⁰ found that six children who did not make appreciable amounts of antibody were in continuous marrow remission after 26 months, whereas four of seven patients who had higher levels of antibody experienced relapse and died. These investigators hypothesized that asparaginase inactivation by antibodies had occurred and was deleterious in their patients. In our large cohort of patients who received uniform therapy for the same disease, we found no significant differences in EFS between patients who developed antibodies at the end of remission induction and those who did not.

It has been proposed that hypersensitivity to asparaginase may attenuate its pharmacologic effect. Asselin et al³ were unable to detect asparaginase activity 1 week after a reaction to *E coli* asparaginase in 80% of patients who continued to receive the same preparation, and the enzyme half-life was significantly shorter in patients who were switched to PEG asparaginase (mean $\pm$ SD, 1.82 ± 0.26 days) than in those who never had a reaction (5.73 ± 3.24 days; $P < .01$). Furthermore, Gentili et al²⁵ reported that no patient who reacted to *Erwinia* asparaginase experienced plasma asparaginase depletion. Despite prior reports of the effect of hypersensitivity reactions on asparaginase pharmacokinetics and pharmacodynamics, we found no significant

differences in EFS between patients who reacted to asparaginase and those who did not. Adverse consequences of hypersensitivity in our study may have been minimized, as patients who had a hypersensitivity reaction to *E coli* were quickly switched to the *Erwinia* preparation and did not receive fewer total doses of asparaginase. Consistent with our finding, in a study of adults with newly diagnosed ALL, even patients who had hypersensitivity reactions requiring discontinuation of asparaginase did not have a shorter remission duration than did patients who received all planned doses.²⁰

Thirty-three percent of the 154 children included in this study had a hypersensitivity reaction to asparaginase, which is consistent with the reported frequency in children (0% to 45%).^{5,6,10-12,14,17,18,26-28} The majority of the reactions occurred during the continuation phase (54 reactions v nine during induction and 26 during reinduction therapies), which may be related to the monthly administration schedule, as the risk of hypersensitivity reactions may increase with readministration after a period of no asparaginase therapy for at least a month.¹⁷ Also, the reduced incidence of allergic reactions during induction and reinduction may be caused by immunosuppression associated with concomitant chemotherapy,^{10,11,17-19} particularly glucocorticoids. Fourteen percent of patients who reacted to *E coli* asparaginase eventually reacted to *Erwinia* asparaginase, which is similar to results from previous reports.^{5,17,18,29,30} Patients generally reacted sooner to *Erwinia* asparaginase than they did to the *E coli* preparation, as initial reactions to *E coli* asparaginase occurred after a median of 12 doses (range, three to 22 doses), and subsequent reactions after *Erwinia* asparaginase occurred after a median of four doses (range, one to 11 doses). The rapid hypersensitivity may have been related to previous reactions to *E coli* asparaginase and the total number of asparaginase doses received, which are both risk factors for reactions to *Erwinia* asparaginase.¹⁷

Patients who developed antiasparaginase antibodies were more likely to experience a hypersensitivity reaction than those who did not. Furthermore, the risk of developing a hypersensitivity reaction among patients who developed antiasparaginase antibodies was related to the antibody concentration. In this subset of patients, 50% of patients who experienced an allergic reaction had an antibody concentration greater than an OD of 0.135 at 1:3200 dilution, whereas there were no patients who did not experience allergic reactions whose levels exceeded this value (data not shown). Although antiasparaginase antibodies were measured only on day 29 of induction and the majority of the hypersensitivity reactions occurred later,

antibody analysis may be of clinical value, especially if patients are to receive multiple or additional courses of asparaginase treatment, because elevated antibody levels may indicate an increased risk of future hypersensitivity. We have also previously shown that antibody levels increase with the number of asparaginase doses, but the antiasparaginase antibody concentration at the end of induction and before any hypersensitivity reaction was higher in patients who experienced a subsequent reaction than in those who never experienced a reaction.⁸

Although drug hypersensitivity has most commonly been related to IgE, antibodies to asparaginase have been characterized as IgG.¹⁰ Cheung et al¹⁰ detected IgG antibodies in eight patients who experienced a hypersensitivity reaction (mean, 42 U/mL; range, 21 to 70 U/mL) after multiple doses of intramuscular or intravenous *E coli* asparaginase. In contrast, the 18 patients who did not have a reaction had a mean IgG antibody level of 6 U/mL (range, 2 to 12 U/mL). The eight patients with hypersensitivity reactions did not have a significantly different IgE level as compared with normal, unimmunized, nonallergic volunteers (mean $\pm$ SD, 77 $\pm$ 65 cpm v 65 $\pm$ 63 cpm, respectively). Thus in our study, we measured IgG antiasparaginase antibodies and not IgE antibodies.

The use of asparaginase immediately before etoposide administration has been implicated in an increased rate of secondary AML; this is possibly the result of decreased synthesis of a protein that is required to repair DNA damage or reverse recombination induced by etoposide.^{21,31,32} In our study, there were nine patients whose first adverse event was secondary AML; five of the nine patients had detectable antibodies (median OD, 0.026; range, 0.00 to 0.133), and three developed allergic reactions. One additional patient initially experienced an isolated CNS relapse but was diagnosed with secondary AML approximately 1 year later. This patient did not develop detectable antibodies or experience hypersensitivity.

In general, *E coli* asparaginase was well tolerated in these pediatric patients, and most patients received all of the planned therapy. Patients who had antiasparaginase antibodies or patients who had hypersensitivity reactions to *E coli* asparaginase did not have a significantly different EFS than the remaining children treated on this ALL protocol. It is possible that *E coli* asparaginase is inactivated in patients who develop antiasparaginase antibodies or become hypersensitive, but any adverse prognostic significance of such inactivation may have been attenuated in our patients because we substituted another asparaginase preparation. In addition, patients on this protocol received several addi-

tional antileukemic agents during continuation treatment (etoposide, cyclophosphamide, methotrexate, mercaptopurine, prednisone, vincristine, and cytarabine); thus any attenuation of the asparaginase effect may have been obscured by the intensity of other active antileukemic agents.

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