

Hypersensitivity reactions to asparaginase therapy in acute lymphoblastic leukemia: immunology and clinical consequences

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Asparaginase is commonly used in combination therapy of acute lymphoblastic leukemia. However, as an immunogenic protein, hypersensitivity reactions (HSRs) during asparaginase therapy are frequent, indicating the development of anti-asparaginase antibodies. These can be associated with diminished clinical effectiveness, including poorer survival. Therapeutic drug monitoring of serum asparaginase activity to confirm complete asparagine depletion is therefore crucial during asparaginase therapy. Switching to alternative types of asparaginase is recommended for patients experiencing HSRs or silent inactivation; those with HSRs or silent inactivation on *Escherichia coli*-derived asparaginases should switch to another preparation. However, prior global shortages of *Erwinia* asparaginase highlight the importance of alternative non-*E. coli*-derived asparaginase, including recombinant *Erwinia* asparaginase.

Plain language summary: Asparaginase is commonly used as a part of a multidrug regimen for acute lymphoblastic leukemia treatment. As foreign proteins, asparaginases have the potential to induce immune responses known as hypersensitivity reactions (HSRs), which can range from a mild rash to a severe allergic reaction. Here, we provide an overview of HSRs and their prevalence in asparaginase-based therapies, and clinical approaches to reduce HSRs. We also review the current understanding of cellular and molecular mechanisms of HSRs, consequences of HSRs and current recommendations for the management of immune reactions to asparaginase. Prior global shortages of *Erwinia* asparaginase due to manufacturing and supply issues have limited access of asparaginase treatment to patients. In this context, newer therapies have recently been developed.

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Asparagine is a nonessential amino acid required for protein synthesis and normal cell growth [1]. Although healthy cells are able to synthesize asparagine from aspartic acid via the enzyme asparagine synthetase, leukemia cells require extracellular asparagine for continued growth, as they do not possess the necessary intracellular capacity to synthesize sufficient quantities [1,2].

Following these observations, therapies containing L-asparaginase were developed for the treatment of hematologic malignancies, including acute lymphoblastic leukemia (ALL) [2]. The proposed rationale for these agents was that reduction of the available extracellular asparagine pool would lead to death of malignant cells [3]. Indeed, studies have shown that the hydrolysis of asparagine by L-asparaginase produces aspartic acid and ammonia, resulting in the depletion of serum asparagine [1], ultimately leading to DNA breaks, cell cycle arrest and apoptosis (Figure 1) [4,5]. L-asparaginase can also hydrolyze glutamine to produce glutamic acid and ammonia, albeit to a lesser extent (the K_m for glutamine is ~ 100 times higher than that of asparagine) [1].

There are several types of therapeutic asparaginases available that have the same mechanism of action (MOA); these include native preparations derived from *Escherichia coli* and *Erwinia chrysanthemi*, chemically modified

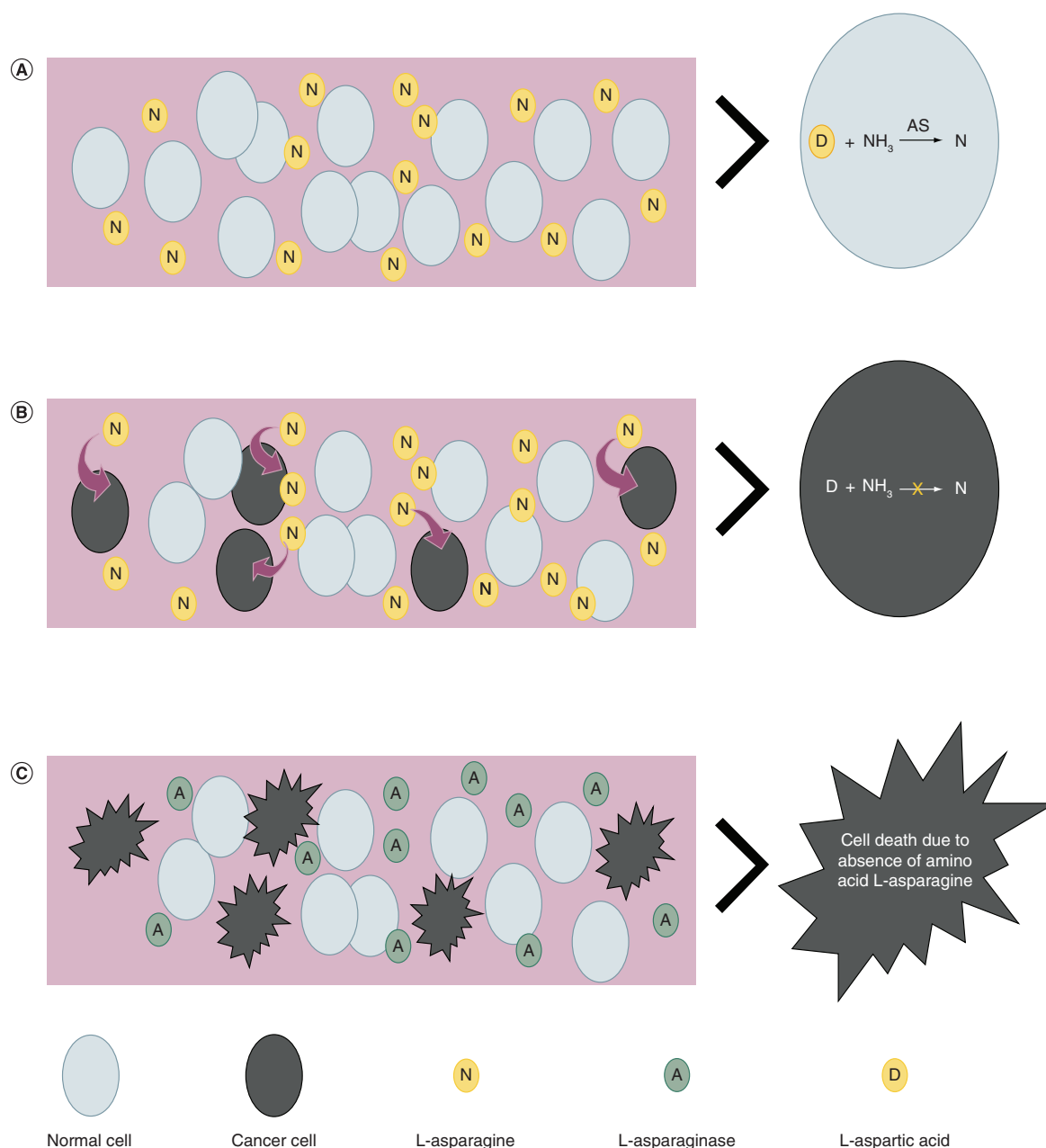


Figure 1. Asparaginase toxicity to tumor cells. (A) Normal cells synthesize amino acid L-asparagine (N) with the help of enzyme asparagine synthetase (AS). **(B)** Cancer cells are unable to synthesize amino acid L-asparagine (N) due to the absence of asparagine synthetase (AS), and they depend on uptake of extracellular L-asparagine for their survival. **(C)** L-asparaginase (A) enzyme decreases concentration of extracellular L-asparagine (N). Tumor cells selectively die due to absence of protein synthesis, and normal cells survive due to asparagine synthetase (AS). Adapted with permission from [5].

preparations of *E. coli* asparaginase conjugated with polyethylene glycol (PEG-asparaginase) [6], recombinant preparations of *E. coli*-derived asparaginases [7] and a recombinant *Erwinia* asparaginase derived from a novel *Pseudomonas fluorescens* expression platform [8].

As these treatments utilize enzymes foreign to humans, which have the potential to induce immune responses, it is vital to have multiple therapeutic options for asparaginase-based therapy with distinct immunogenic profiles [9]. However, as asparaginases are administered as a component of a multiagent regimen for the treatment of ALL, it can be difficult to determine which adverse effects (AEs) are related to an individual therapeutic agent [1].

More than one-half of all patients treated with an asparaginase-containing treatment regimen develop mild-to-moderate loss of appetite, nausea, vomiting or a slight increase in body temperature [1]. More severe AEs (SAEs) have also been reported. An increased risk of thromboembolic events in patients receiving asparaginase therapies has been observed, with an average overall incidence of approximately 3–5% in children with ALL. However, these incidences are more frequently observed in adolescent patients relative to younger children (2–7% in patients aged <9 years vs 15–20% in older patients) [9–11]. It has been suggested that the increased risk of thromboembolism could be a result of impaired coagulation due to a deficiency of clotting inhibitors and increased prothrombotic factors, stemming from a depletion of asparagine [1,12].

Asparaginase-related pancreatitis has been reported in 5–10% of pediatric ALL patients, also with higher incidence observed in adolescents (15 vs 5%) [9,10]. These events range in clinical severity from mild amylase/lipase elevations to life-threatening complications [9,13]. Nearly all patients receiving an asparaginase-containing treatment regimen report abnormalities of liver function and hepatic transaminase levels; however, most resolve without intervention within 2–3 weeks of the end of treatment, and liver failure is rarely seen [9]. Hyperglycemia is commonly reported during the induction phase of asparaginase-containing treatment (when administered in conjunction with corticosteroids) and generally resolves within 2–4 weeks; however, diabetic ketoacidosis has been reported in 1–3% of ALL patients, although this rarely requires long-term insulin treatment [9].

One of the more common AEs of asparaginase therapy are hypersensitivity reactions (HSRs), which are immunogenic reactions, ranging from localized erythema and urticaria to systemic anaphylaxis [1,9,14]. Clinical HSRs are generally reported in 10–30% of patients receiving native *E. coli*-derived asparaginase, 3–24% of patients receiving PEG-asparaginase and 3–37% of patients receiving *Erwinia* asparaginase and are one of the most frequent reasons for discontinuation of asparaginase therapy [15]. HSRs may occur following the first dose of asparaginase, but the risk increases with repeated administrations, particularly during consolidation and reinduction treatment phases [15–17]. However, despite these relatively high rates of HSRs, only 20–25% of patients experience additional HSRs after switching to an asparaginase with a different immunogenic profile, allowing more than 90% of patients to complete the prescribed asparaginase regimen [1].

HSRs are indicative of the development of anti-asparaginase antibodies that can reduce the clinical effectiveness of the asparaginase-containing regimen, and treatment guidelines do not recommend the continued administration of the same formulation of asparaginase after an HSR has occurred [18]. Although measures such as steroid premedication and desensitization may decrease symptoms, they may not prevent the inactivation of asparaginase activity via anti-asparaginase antibodies, which can negatively impact treatment outcomes [18].

Therapeutic drug monitoring (TDM) through the measurement of serum asparaginase activity (SAA) is therefore a critical element to the current clinical guidelines for asparaginase-based regimens, especially in cases where premedication is used, as premedication can mask clinical symptoms indicating asparaginase inactivation [18]. A trough SAA of ≥ 0.1 IU/ml has generally been accepted as the level required for complete serum asparagine depletion, as has been confirmed and utilized in numerous clinical trials [18].

Immunology of HSRs

Prevalence of HSRs can depend on several patient- and treatment-related variables. Certain genetic alterations have been linked to a predisposition toward asparaginase hypersensitivity, including carriage of the major histocompatibility complex II *HLA-DRB1**07:01 allele [19]. Notably, studies have shown conflicting results regarding the influence of age on the risk of asparaginase hypersensitivity [10,20,21]. However, relatively lower rates of asparaginase hypersensitivity in adults may be associated with the greater use of antihistamine and corticosteroid premedication [14].

It has been suggested that patients treated with high-intensity chemotherapy regimens and asparaginase therapy administered continuously with few breaks in treatment experience a lower risk of asparaginase HSRs [14]. Type of asparaginase therapy is also a key factor: in a multivariate analysis of data from more than 3300 patients, use of native *E. coli* asparaginase was associated with a significantly higher frequency of HSRs relative to use of PEG-asparaginase [21]. However, exposure to an immunologically similar asparaginase therapy can increase the risk of a subsequent HSR after switching products, as studies have shown cross-reactivity when switching from native *E. coli* asparaginase to PEG-asparaginase [6,22].

Upon initial exposure to large foreign protein-based molecules such as asparaginase, the primary immune response leads to the development of low-affinity anti-asparaginase immunoglobulin M (IgM) antibodies by circulating B cells (Figure 2) [1,18,23]. Simultaneously, antigen-presenting cells (APCs) detect and engulf peptide fragments of

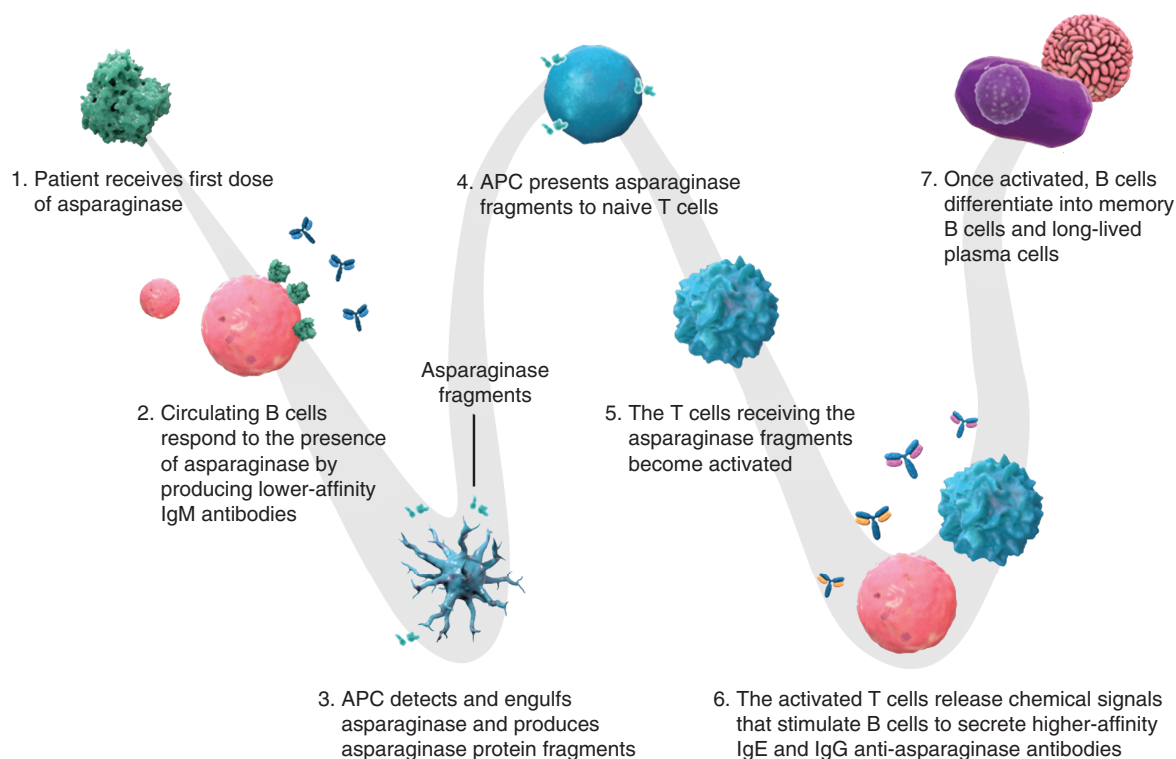


Figure 2. Formation of immune memory[†] – the primary immune response.

[†]Not every patient receiving asparaginase will experience an immune reaction.

APC: Antigen-presenting cell; IgE: Immunoglobulin E; IgG: Immunoglobulin G; IgM: Immunoglobulin M.

the drug before presentation to naive T helper (Th) cells via cell surface major histocompatibility complex class I and II molecules [23]. The Th cells become activated via this signal and by costimulatory molecules on the surface of the APCs. Subsequently, interactions between the activated antigen-specific Th cells and B cells initiate a class switch from IgM production by the B cells to higher-affinity immunoglobulin E (IgE) and immunoglobulin G (IgG) antidrug antibodies [23]. A specific subpopulation of the B cells matures into long-living plasma cells, which largely reside in the bone marrow [23].

Re-exposure to an immunogenic drug activates memory B cells and long-living plasma cells to secrete antidrug antibodies more rapidly than during the primary immune response [23]. Antidrug IgE antibodies bind to cells expressing the FcεRI receptor, including mast cells and basophils, resulting in the release of histamine upon antigen exposure [24,25]. Cross-linking of IgE by drug antigens thereby causes clinical symptoms, including skin reactions, respiratory effects and potentially decreased blood pressure and cardiovascular collapse in severe cases [26]. IgG antibodies can create immune complexes with the antigen, binding to the Fcγ receptors on neutrophils, macrophages, basophils and mast cells, leading to the release of platelet-activating factor and inducing phagocytosis [24,27], which might explain the rapid clearance of asparaginase and significant reduction in asparaginase activity on development of anti-asparaginase antibodies (Figure 3) [24–28]. Studies have shown that patients with HSRs have elevated IgG levels, specifically IgG₁, IgG₃ and IgG₄, which have been correlated with asparaginase inactivation [1].

In a study of 80 children and adolescents with ALL who had received two consecutive courses of asparaginase therapy, at the end of the induction treatment phase, IgG anti-asparaginase antibodies were found in 28% of patients, IgM anti-asparaginase antibodies in 25% of patients and IgE anti-asparaginase antibodies in 18% of patients, with only 5% of patients exhibiting all three classes of antibodies [28]. During the reinduction treatment phase, both IgG and IgM antibodies were more common, while the percentage of patients with IgE antibodies remained the same [28]. IgG and IgM anti-asparaginase antibodies were significantly associated with HSRs/allergy, whereas IgE antibodies were not. Similarly, during the reinduction phase, mean asparaginase activity was reduced in patients with IgG and IgM antibodies to below 100 IU/l, but remained above this level for patients with IgE antibodies (Figure 4) [28].

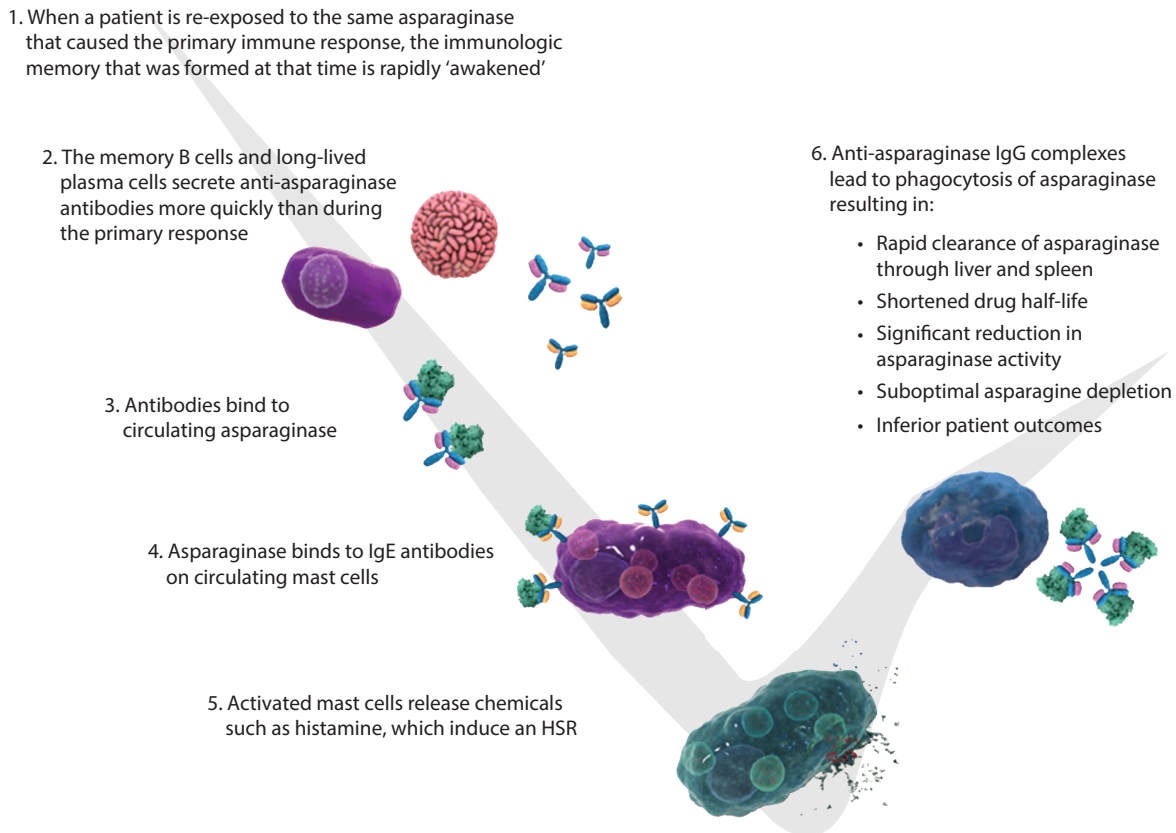


Figure 3. Secondary immune response following re-exposure to asparaginase.
HSR: Hypersensitivity reaction; IgE: Immunoglobulin E; IgG: Immunoglobulin G.

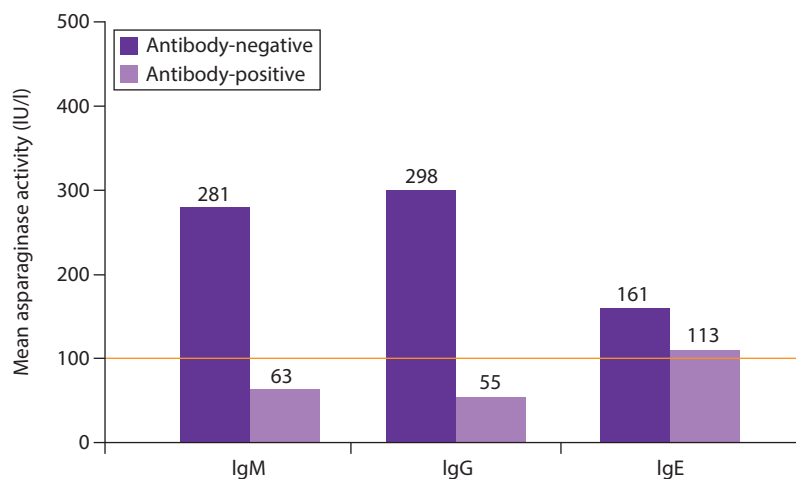


Figure 4. Asparaginase activity following the development of anti-asparaginase antibodies during reinduction therapy. p-value for the difference between antibody-positive and antibody-negative patients (IgG, $p < 10^{-5}$; IgM, $p < 10^{-5}$). The recommended level of asparaginase reflecting effective depletion of asparagine (≥ 100 IU/l) is denoted by the orange line.

IgE: Immunoglobulin E; IgG: Immunoglobulin G; IgM: Immunoglobulin M.
Adapted with permission from [28].

An investigation of the relationship between specific classes of anti-asparaginase antibodies and clinical outcomes has also been reported [17]. In 47 children (aged 3–17 years) with newly diagnosed ALL treated in Poland, ten developed IgG anti-asparaginase antibodies and 17 developed IgM antibodies [17]. Of these, seven demonstrated low enzyme activity (<0.1 IU/ml). Event-free survival (EFS) and overall survival (OS) were significantly lower for patients with IgG and IgM anti-asparaginase antibodies during induction therapy relative to those who did not develop these antibodies [17].

Silent inactivation

Antibody inactivation of asparaginase can occur without presenting clinical manifestations of HSRs, and this is termed ‘silent inactivation’ or ‘inadequate activity of asparaginase’ [18]. Studies have reported varying rates of silent inactivation in clinical trials of patients with ALL, ranging between eight and 44% for *E. coli*-derived asparaginase [14].

Silent inactivation can be identified via measuring trough SAA and is defined as SAA <0.1 IU/ml in two independent samples from a patient, in the absence of clinical symptoms of HSRs. As described by van der Sluis *et al.*, patients receiving pegaspargase, which is administered every 2 weeks, SAA levels <0.1 IU/ml on Day 7 and/or below the lower limit of quantitation (LLQ) on Day 14 are consistent with silent inactivation. Patients receiving native *E. coli*-derived asparaginase who display SAA levels below the LLQ 72 h postdose (in a two administrations/week schedule) or 7 days postdose (in a weekly administration schedule) are consistent with silent inactivation. Patients receiving *Erwinia*-derived asparaginase who show an SAA level below the LLQ 48 h postdose are consistent with silent inactivation [18]. Guidelines recommend that all patients undergoing therapy with asparaginase should undergo TDM for silent inactivation, at least when first starting treatment, and again following a gap in therapy [18]. Patients who display silent inactivation are recommended to switch asparaginase therapy, as silent inactivation will likely prevent a patient from experiencing the full clinical benefit of asparaginase treatment [18].

Infusion reactions

Infusion reactions, unlike HSRs, are not antibody mediated. However, the exact mechanism of action by which chemotherapeutic drugs elicit infusion reactions is less clear, although it has been suggested that the direct release of cytokines or histamine or direct activation of the complement system are involved [29]. Infusion reactions with asparaginase therapy may also result from sharp increases in serum ammonia levels, following the rapid hydrolysis of asparagine [14,30]. Symptoms of hyperammonemia can include nausea, vomiting, headache, dizziness, lethargy and rash, which are typically mild and short-lived [14]. The clinical manifestations of HSRs and infusion reactions therefore overlap considerably; however, the timing of symptom onset can be a critical distinguishing factor as HSRs generally require subsequent exposure to promote antibody development, while infusion reactions can occur following the initial exposure to asparaginase [14]. Measuring SAA can also be helpful here (with sub-therapeutic levels indicating antibody-mediated inactivation), as direct measurement of serum ammonia levels can be challenging due to residual asparaginase activity during sample processing, even with cooling samples on ice, and varying ammonia levels throughout the asparaginase treatment course [31–33].

As infusion reactions are not antibody mediated, they typically resolve with supportive care once the infusion is halted. Patients who experience infusion reactions can often be rechallenged with the same asparaginase therapy (typically at a slower infusion rate and with appropriate prophylaxis) and are often able to successfully complete further treatment [14,29].

Potential consequences of HSRs

Numerous studies have shown that following the development of anti-asparaginase antibodies, a loss of asparaginase activity has been observed.

One study evaluated the relationship between development of anti-asparaginase antibodies and HSRs during treatment with asparaginase in children with newly diagnosed ALL [34]. Of 89 patients who received PEG-asparaginase therapy, 20 experienced HSRs and a further seven demonstrated silent inactivation. Patients who developed allergic reactions demonstrated undetectable SAA, while those without allergy or silent activation had a mean trough SAA of 899 IU/l [34]. During the treatment intensification phase, the levels of anti-asparaginase antibodies were significantly higher in children who had experienced HSRs or silent inactivation compared with those who had not [34]. In a smaller study of 16 ALL patients treated with *E. coli*-derived asparaginase, in the

reinduction phase, 11 patients experienced HSRs, and of these, nine had developed anti-asparaginase antibodies, six of whom also had antibodies against PEG-asparaginase [6]. Of the five remaining children without HSRs, four were positive for anti-asparaginase antibodies [6].

It has also been shown that the half-life of both native *E. coli*-derived asparaginase and PEG-asparaginase are substantially decreased in patients following an HSR [35]. In a study of ten patients who developed HSRs following treatment with either native *E. coli* asparaginase or PEG-asparaginase, at Day 1 after treatment initiation, SAA levels were <0.6 IU/ml [35]. Furthermore, the mean serum asparaginase half-lives were undetectable or 1.8 days for native *E. coli* and PEG-asparaginase formulations, respectively, which were significantly shorter than those seen in patients without HSRs, indicating a more rapid clearance [35].

More importantly, it is well established that immunological reactions and their impact on asparaginase pharmacodynamics and pharmacokinetics are associated with poorer clinical outcomes in patients with ALL. In a study of 85 adults with ALL, 22 did not achieve complete asparagine depletion during treatment with PEG-asparaginase [36]. In the latter group, median disease-free survival (DFS; hazard ratio [HR], 2.21; $p = 0.01$) and OS (HR, 2.37; $p = 0.001$) were significantly shorter compared with those patients who achieved persistent total asparagine depletion [36]. Recently, a study from the Children's Oncology Group in over 3000 patients with high-risk ALL compared outcomes between patients who received all planned PEG-asparaginase doses to patients who substituted remaining asparaginase doses with *Erwinia* asparaginase and patients who missed asparaginase doses. Of the 3081 patients enrolled in COG AALL0232, DFS was similar in a multivariable analysis between patients who received *Erwinia* asparaginase and patients who received all PEG-asparaginase doses (HR, 1.1; $p = 0.52$). However, patients who missed asparaginase doses had inferior DFS compared with patients who received all planned PEG-asparaginase doses (HR, 1.5; $p = 0.002$) [37].

Another study showed that, in patients with ALL whose asparaginase dosing regimen was individualized according to SAA monitoring, 5-year EFS was significantly improved versus those who received a fixed dose of asparaginase (90 vs 82%; $p = 0.04$) [38]. However, 5-year EFS was similar for patients who developed anti-asparaginase antibodies without clinical HSRs and those who did not [38]. Notably, routine use of dexamethasone during induction therapy also improved 5-year EFS [38]. In a previous study, HSRs and development of anti-asparaginase antibodies were shown to be independently associated with increased dexamethasone clearance [39]. Consequently, higher dexamethasone clearance was significantly associated with the risk of relapse. Dexamethasone clearance and positive anti-asparaginase antibody status were also significantly associated with central nervous system relapse [39].

Management of immune reactions to asparaginase-based therapy

A representative treatment algorithm based on published algorithms from Bleyer *et al.* and Burke *et al.*, highlighting clinical signs and recommendations to address non-antibody-based reactions, HSRs, silent inactivation and accelerated asparaginase clearance following a reaction to an asparaginase-based therapy, is shown in Figure 5 [14,40]. This algorithm is for illustrative purposes, as management of individual patients can vary.

Consensus guidelines recommend switching asparaginase therapies for patients who experience antibody-mediated reactions, either HSRs or silent inactivation. The standardized format for determining the severity of an adverse event is of National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) grading; however, grading of allergic reactions can be challenging due to the limited terminology to link a clinical manifestation to a causative agent and a lack of granularity imposed by five possible grades [41]. In response to HSRs of NCI-CTCAE grade ≥ 2 , it is recommended to switch to another asparaginase formulation (without definite need to check SAA levels); following occurrence of NCI-CTCAE grade 1 HSRs, SAA monitoring is also recommended to identify asparaginase inactivation [18]. When switching asparaginase therapies, patients experiencing HSRs or silent inactivation to an *E. coli*-derived asparaginase should switch to a non-*E. coli*-derived asparaginase [18]. When switching to an *Erwinia*-derived asparaginase, continued SAA monitoring is recommended, as inter-patient pharmacokinetic variability (primarily clearance) can be high [18].

Premedication with either antihistamines and/or corticosteroids, which stabilizes mast cells and prevents release of certain proinflammatory mediators [42,43], can be employed to mitigate some of the adverse clinical effects of asparaginase administration, including infusion reactions [44]. There have been conflicting reports on the clinical utility of premedication in reducing HSRs in patients with ALL. In a study involving adults and children with ALL receiving PEG-asparaginase, premedication with acetaminophen, diphenhydramine and methylprednisolone reduced the number of patients experiencing grade 3–4 allergic reactions relative to the previous protocol of no premedication [45]. However, in a separate study, the use of acetaminophen, diphenhydramine and corticosteroid

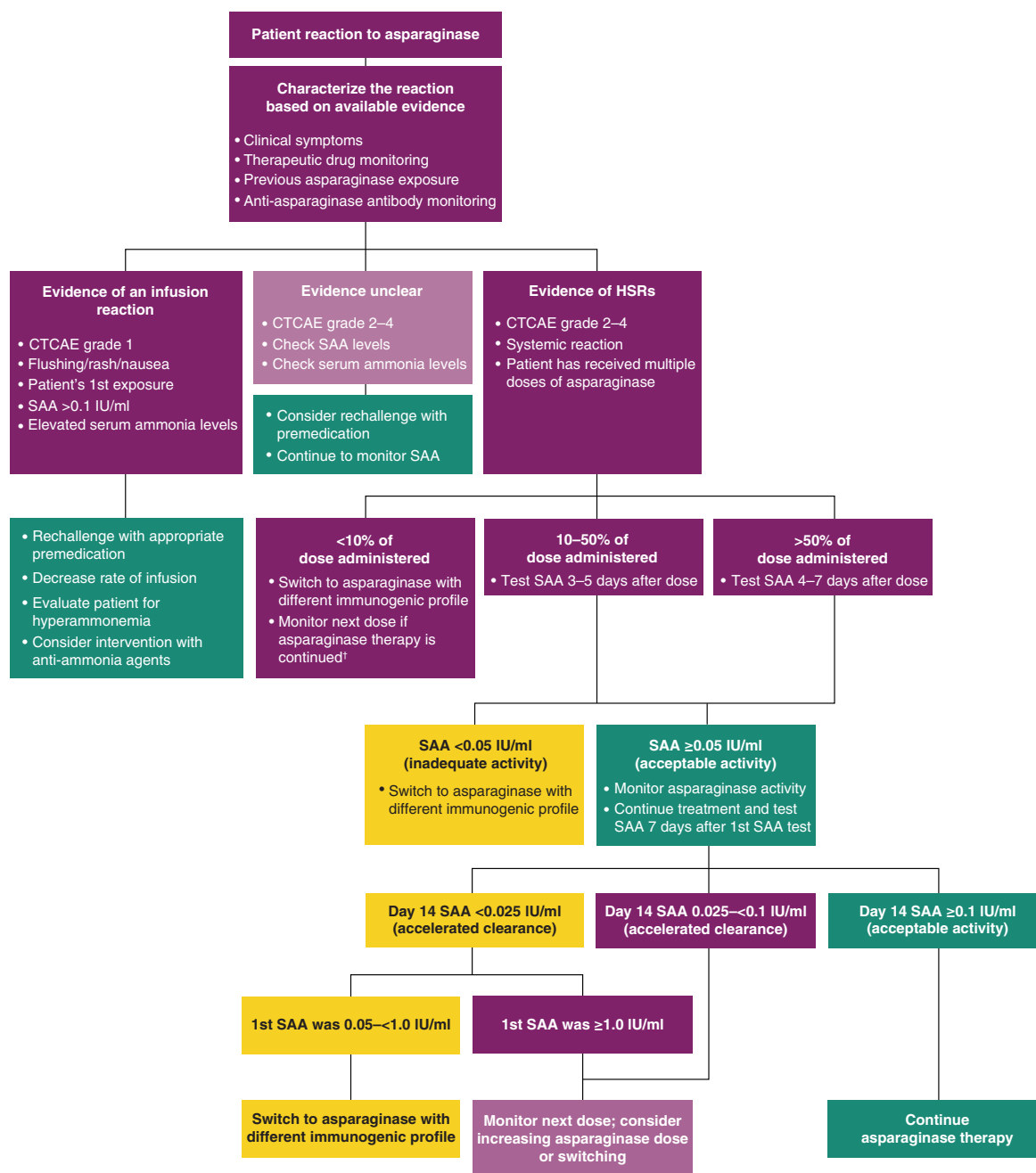


Figure 5. Treatment algorithm following reaction to asparaginase-based therapy. †In most cases, the asparaginase preparation should be discontinued.

CTCAE: Common Terminology Criteria for Adverse Event; HSR: Hypersensitivity reaction; SAA: Serum asparaginase activity.

Adapted with permission from [14,40].

premedication did not reduce the number of reactions in patients receiving treatment with PEG-asparaginase relative to no premedication [46]. Additionally, antihistamines and corticosteroids do not prevent the antibody-mediated inactivation of asparaginase activity [18]. As these agents can mask clinically important signals of immune system activation, premedication should not be utilized in the absence of checking SAA levels (Figure 6) [18,42,43,47].

Several studies have reported results following universal premedication strategies in conjunction with TDM that various institutions have adopted. Cooper *et al.* conducted a retrospective chart review (November 2011 to April 2018) of 177 patients, of which 109 patients were treated with PEG-asparaginase prior to the implementation

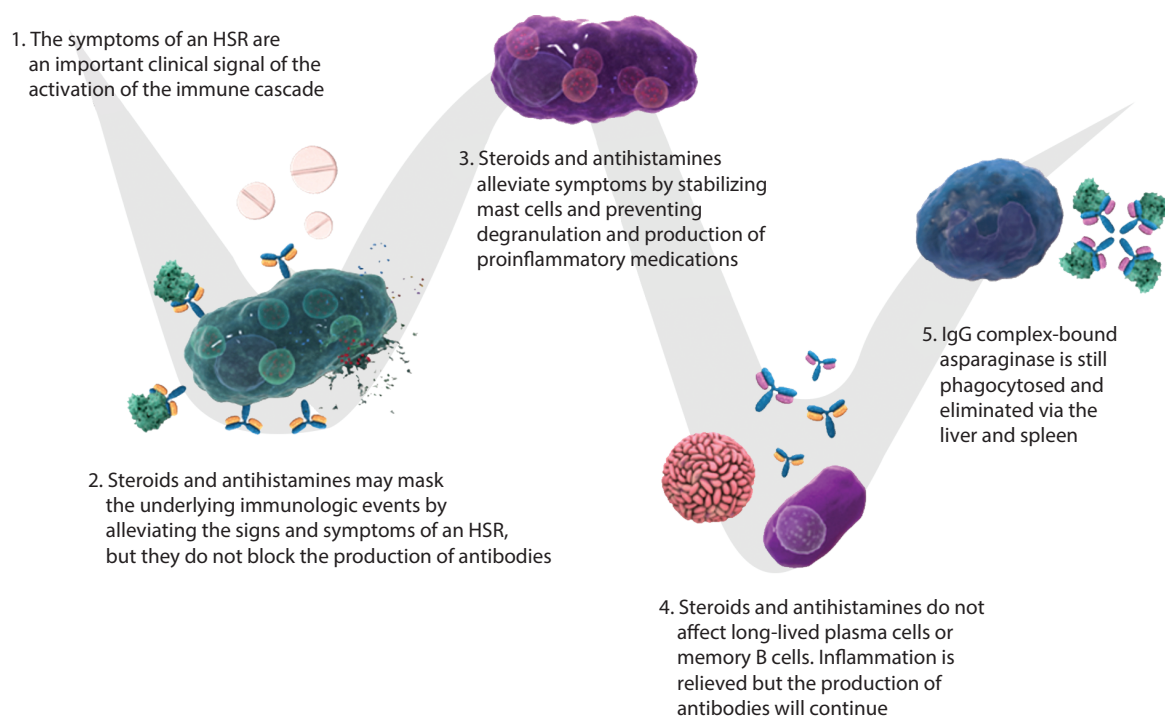


Figure 6. Premedication with steroids and antihistamines stabilizes mast cells but does not prevent the production of antibodies.

HSR: Hypersensitivity reaction; IgG: Immunoglobulin G.

of universal premedication, 55 patients received premedication, and 13 patients received PEG-asparaginase both with and without premedication [48]. The patients received a regimen of diphenhydramine and either famotidine or ranitidine; patients who were rechallenged after a previous acute AE with PEG-asparaginase also received hydrocortisone. TDM was performed 7 days (± 3 days) following intravenous PEG-asparaginase administration. Prior to implementation of universal premedication, 21 of 122 (17.2%) patients had clinically significant reactions to PEG-asparaginase prompting a switch to *Erwinia*-derived asparaginase versus five of 68 (7.4%) patients postimplementation, representing a 57.3% reduction in switching ($p = 0.028$). Further, the rate of AEs dropped from 17.2 to 5.9%, representing a 65.8% reduction ($p = 0.017$) [48]. A similar study by Marini *et al.* of 297 patients (163 preimplementation and 134 postimplementation) found a significant reduction in switching to *Erwinia*-derived asparaginase (21% preimplementation vs 7% postimplementation; $p = 0.0035$) following implementation of universal premedication with a regimen of acetaminophen, diphenhydramine, hydrocortisone and ondansetron in conjunction with TDM [49]. However, several studies have shown that universal premedication offers limited utility in the reduction of HSRs. A retrospective study found that HSRs occurred in 12 of 65 (18.5%) patients preimplementation of universal premedication versus 16 of 74 (21.6%) postimplementation ($p = 0.64$) with a premedication regimen of diphenhydramine and hydrocortisone [50]. Additionally, a similar study by Hughes *et al.* found that a slower rate of PEG-asparaginase infusion after implementation of a premedication regimen of ranitidine and diphenhydramine had no effect on the probability of HSRs ($p = 0.56$) [51]. Further studies are needed to determine the clinical benefit of universal premedication in conjunction with TDM.

Due to historical global shortages of *Erwinia* asparaginase [52], treatment switching was not always a viable option. In such cases, real-time monitoring of SAA has been employed in conjunction with rapid desensitization protocols to ensure that the therapeutic levels of asparaginase activity are maintained [14]. Although the mechanism of rapid desensitization is incompletely understood, it has been hypothesized that this approach may render mast cells and basophils unresponsive to low levels of antigen, as well as inducing internalization of antigen cross-linked IgE Fc ϵ RI receptors [26]. Desensitization has shown mixed results, with some patients still experiencing subsequent HSRs and some patients unable to maintain adequate asparaginase activity [52–54]. However, with the recent US

FDA approval of *Erwinia chrysanthemi* (recombinant)-rywn (Rylaze™) [55], the prior global shortages of *Erwinia* asparaginase that gave rise to the necessity of rapid desensitization protocols should be alleviated.

In a small study of eight patients (aged 2–19 years) with previous HSRs to PEG-asparaginase, PEG-asparaginase was diluted to three different concentrations (0.05, 0.5 and 10 IU/ml) [54]. Four separate infusions were administered at each dose level, together with a progressive increase in infusion rate (and therefore higher dose of PEG-asparaginase), resulting in a total of 12 steps [54]. All patients received the same dose of PEG-asparaginase during the first 11 steps and, for the final step, the dose was calculated based on the patient's body surface area [54]. All patients received premedication with antihistamines, hydrocortisone and montelukast, which continued for at least 24 h. Following intensive monitoring after the first desensitization dose, each further dose was administered if the patients tolerated the previous dose and maintained asparaginase SAA >0.1 IU/ml for at least 14 days [54]. Asparaginase activity was intensively monitored at infusion completion and at 24–96 h, 7 days and 14 days after each PEG-asparaginase dose [54]. A total of seven patients were able to complete the desensitization protocol, with the only AE of rash, although the remaining one patient was unable to continue due to a progressive rash. Six patients demonstrated SAA >0.1 IU/ml for at least 2 weeks [54]. Four patients without anti-asparaginase or anti-PEG antibodies at baseline were successfully desensitized; however, two of four patients positive for antibodies to PEG and PEG-asparaginase had reactions and were unable to maintain adequate asparaginase activity [54].

In a separate study of ten patients (aged 3–18 years) with ALL or lymphoblastic lymphomas with a history of severe PEG-asparaginase HSRs, a 13-step desensitization protocol was created [52]. Serial dilutions of PEG-asparaginase were made (three blocks of 0.29 IU/ml, 2.9 IU/ml and 29 IU/ml, with a 13th dose of 29 IU/ml), and these were administered with infusion rate increases every 15 min. The total PEG-asparaginase dose was 2500 IU/m² [52]. All patients received premedication with antihistamines, corticosteroids and montelukast, and were admitted to the pediatric intensive care unit for the desensitization [52]. Eight of the ten patients did not experience any HSRs during the desensitization protocol and received all planned doses of PEG-asparaginase [52]. The remaining two patients experienced mild HSRs and received epinephrine and additional antihistamines and were able to receive the remaining dose of PEG-asparaginase with additional infusion steps [52]. Seven patients exhibited sustained asparaginase activity for at least 14 days [52]. Given the encouraging results in patients who developed a prior HSR to PEG-asparaginase, desensitization should be considered on a case-by-case basis. Patients undergoing desensitization should continue to be monitored closely, as desensitization has not been successful in all patients and adverse reactions may still occur.

Alternatively, the historical shortages of *Erwinia* asparaginase may be addressed by a newly formulated recombinant *Erwinia* asparaginase preparation that was recently approved by the FDA in adult and pediatric patients aged 1 month or older who have developed HSRs to *E. coli*-derived asparaginase [55]. The amino acid sequence in recombinant *Erwinia* asparaginase is the same as in *Erwinia* asparaginase, with similar *in vitro* activity, and is not anticipated to show immunologic cross-reactivity with the *E. coli*-derived asparaginase [56]. In a dose-ranging phase 1 study in healthy volunteers, total depletion of asparagine was demonstrated with recombinant *Erwinia* asparaginase at 25 mg/m², intramuscularly. Further, adequate sustained SAA ≥ 0.1 IU/ml at 72 h and no HSRs were observed, and dosing responses in terms of asparaginase activity and pharmacokinetics were similar to *Erwinia* asparaginase at 25,000 IU/m², intramuscularly, within the phase I study [8]. Modeling and simulations, based on preliminary results from a phase II/III study, have shown that 94% of patients are expected to maintain nadir serum asparaginase levels ≥ 0.1 U/ml at 48 h following intramuscular administration of 25 mg/m² of recombinant *Erwinia* asparaginase [55].

Two cases are presented below to illustrate potential management strategies in patients experiencing immune reactions to asparaginase therapy.

Case study 1

Here, we present a case in which a patient displayed allergic symptoms while receiving asparaginase during their initial induction therapy phase for B-cell ALL. A 12-year-old-boy diagnosed with B-cell ALL was treated in accordance with the high-risk arm of the COG AALL1131 protocol [57]. This protocol provides a single dose of PEG-asparaginase at a dose of 2500 U/m² on day 4 of induction therapy. During the intravenous administration of the PEG-asparaginase, 50 min into the 1-h infusion, the patient developed symptoms of headache, elevated blood pressure, nausea and a macular erythematous rash over his arms and chest that was non-pruritic. The infusion was stopped, and SAA and ammonia levels were sent for analysis. Given this was the patient's first exposure to PEG-asparaginase and the timing of the reaction, an infusion reaction was suspected. The serum ammonia level

shortly returned at a value of 235 $\mu\text{mol/l}$ (normal range: 11–35 $\mu\text{mol/l}$) consistent with hyperammonemia and an infusion reaction and the cause of his apparent allergic symptoms. The SAA level returned 2 days later at a level of 0.57 IU/ml consistent with good asparaginase activity. The patient continued to receive PEG-asparaginase, without evidence of an HSR, throughout the remainder of his ALL therapy as assigned according to the AALL1131 protocol.

Case study 2

Here, we present a case study in which a patient displayed allergic symptoms during the intense reinduction phase of asparaginase treatment. A 15-and-a-half-year-old girl diagnosed with B-cell ALL was treated in accordance with the standard arm of the AIEOP/BFM 2017 treatment protocol. This protocol provides for a total of three administrations of PEG-asparaginase at a dose of 2500 IU/ m^2 , two during remission induction and one in the intensive reinduction phase. In accordance with the guidelines adopted in Poland, the patient underwent therapeutic monitoring of asparaginase activity, with assessment on days 8 and 15 following the PEG-asparaginase administration. During the induction phase, the patient received the prescribed doses of PEG-asparaginase without significant complications. SAA levels were as follows: day 19: 0.77 IU/ml; day 26: 0.31 IU/ml; day 33: 0.83 IU/ml; and day 40: 0.57 IU/ml. In the intensive reinduction phase, immediately after starting the infusion with PEG-asparaginase, the patient began to complain of abdominal pain and headache, as well as skin rash with urticarial eruptions. The infusion was interrupted, and steroids and antihistamines were administered, after which the symptoms subsided in about 20 min. After 2 days, *Erwinia* asparaginase was administered at a dose of 20,000 IU/ m^2 , anticipating seven consecutive doses. SAA levels before administration of the next asparaginase dose were as follows: 0.15 IU/ml, 0.18 IU/ml, 0.26 IU/ml, 0.16 IU/ml, 0.18 IU/ml, 0.14 IU/ml, 0.14 IU/ml, and 0.1 IU/ml and, after two consecutive days, 0.03 IU/ml. The switch from PEG-asparaginase to *Erwinia* asparaginase after the occurrence of HSR symptoms allowed the patient to complete the treatment successfully.

Conclusion

HSRs, together with silent inactivation, are often associated with negation of asparaginase activity due to anti-asparaginase antibody development [34]. This can result in a lack of asparagine depletion and increased clearance of asparaginase, which negatively impacts clinical outcomes, including survival [17,35,36]. The clinical manifestations of HSRs can overlap considerably with infusion reactions, both of which are common in patients with ALL undergoing asparaginase therapy as part of a multiagent treatment regimen [15]. The timing of AEs can usually act as a differentiator between HSRs and infusion reactions: infusion reactions typically occur immediately during the first dosing of asparaginase, while HSRs generally occur from second administration onward due to the mechanisms of antibody-mediated immune responses [14]. To ameliorate the clinical manifestations of HSRs, premedication has been employed [48,49]; however, premedication with antihistamines and/or steroids only suppresses the symptoms arising from an antibody-mediated reaction and do not prevent the antibody-mediated neutralization of asparaginase. Therefore, TDM is important in monitoring SAA to ensure therapeutic asparaginase activity, especially in cases with silent activation as there are no clinical manifestations to indicate development of anti-asparaginase antibodies [18].

Ideally, if HSRs occur with the first asparaginase formulation, switching to another product is recommended, although cross-reactivity can occur in some patients [18]. Premedication and desensitization have been employed to allow for further dosing of the same asparaginase formulation to delay switching to an *Erwinia*-derived asparaginase with conflicting reports on the extent of clinical benefit [52,53]. However, premedication and desensitization do not address antibody-mediated immune reactions and thus premedication should always be accompanied by TDM [18]. Due to the historical shortages of *Erwinia* asparaginase, switching has not always been possible; however, with the recent FDA approval of recombinant *Erwinia* asparaginase, these challenges should be alleviated [52,53,55].

Future perspective

Routine TDM for patients with ALL receiving premedication and other hematologic malignancies starting and continuing asparaginase therapy will hopefully allow for timely identification of the effects of anti-asparaginase antibody formation. Given the previous shortage of *Erwinia* asparaginase, the newly FDA-approved product, asparaginase *Erwinia chrysanthemi* (recombinant)-rywn (Rylaze™) will hopefully alleviate concerns of not having an asparaginase alternative in the setting of HSRs.

Executive summary

- Treatment with asparaginase is a key component of acute lymphoblastic leukemia (ALL) treatment.
- Hypersensitivity reactions (HSRs) are a frequent complication of asparaginase therapy and can range from localized erythema and urticaria to systemic anaphylaxis.

Immunology of HSRs

- Initial exposure to asparaginase induces the development of low-affinity immunoglobulin M (IgM) antibodies by circulating B cells, with simultaneous activation of T helper (Th) cells.
- Th cells and B cells drive a class switch from IgM to higher-affinity immunoglobulin E (IgE) and immunoglobulin G (IgG) antibodies.
- Re-exposure to asparaginase activates memory B cells and plasma cells to produce antibodies; IgE antibodies may be associated with HSR symptoms, while IgG antibodies are associated with significant reductions in asparaginase therapy.

Silent inactivation

- Silent inactivation (inadequate asparaginase activity) can occur in the absence of HSRs and can only be detected during therapeutic drug monitoring of serum asparaginase activity (SAA).
- Asparaginase inactivation is defined as SAA <0.1 IU/ml.

Infusion reactions

- Although some symptoms of HSRs and infusion reactions are similar, infusion reactions can occur following initial exposure to asparaginase, while HSRs typically occur following subsequent exposure to asparaginase.
- As infusion reactions are not antibody mediated, they typically resolve following cessation of the infusion, and treatment may be restarted with prophylaxis and slowing of the infusion rate.

Potential consequences of HSRs

- Development of anti-asparaginase antibodies has been associated with loss of asparaginase activity.
- Some studies have shown that the half-lives of both native *Escherichia coli* asparaginase and polyethylene glycol (PEG) – asparaginase are reduced in patients experiencing HSRs as a consequence of increased clearance.
- Incomplete asparaginase depletion may be associated with decreased survival in patients with ALL.

Management of immune reactions to asparaginase-based therapy

- Patients experiencing HSRs or silent inactivation on *E. coli*-derived asparaginases should be switched to a non-*E. coli*-derived asparaginase to maintain SAA levels ≥ 0.1 IU/ml.
- The historical global shortage of *Erwinia* asparaginase required development of new therapies. The newly FDA-approved product, asparaginase *Erwinia chrysanthemi* (recombinant)-rywn (Rylaze™) should hopefully improve availability of an asparaginase alternative in the setting of HSRs.
- Continued monitoring of SAA is advised after switching.
- Premedication can prevent some symptoms of HSRs but does not prevent silent inactivation.
- Desensitization is a last resort approach, as it is not always effective and may be associated with adverse reactions.

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