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CLINICAL AND DEMOGRAPHIC FACTORS ASSOCIATED WITH PEG-ASPARAGINASE-RELATED TOXICITIES IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

PRIYADARSHANI DHARIA

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by

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PUBLIC HEALTH

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By

Priyadarshani Dharia, MD, MPH, PhD
2019

DEDICATION

To Pallavi and Prabhakar Dharia

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TOXICITIES IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

by

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TOXICITIES IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Introduction: Polyethylene glycol (PEG)-asparaginase is one of the first-line drugs in pediatric ALL treatment. Although a complete and prolonged treatment with PEG-asparaginase has been instrumental in improving the survival of ALL patients, it is associated with four common toxicities: hypersensitivity, hyperbilirubinemia, pancreatitis, and venous thromboembolism (VTE). PEG-asparaginase-related toxicities may require modifications and delays in treatment, which often leads to poor treatment outcomes. Previous studies of PEG-asparaginase-related toxicities have mainly focused on individual toxicities. Further, these studies were conducted in study populations which were predominantly self-reported non-Hispanic whites. Therefore, there is a gap in the knowledge regarding the cumulative burden of the toxicities in a multi-ethnic population and the role of genetic ancestry in the development of these toxicities. This study focused on the clinical and demographic factors influencing the incidence of individual and cumulative burden of PEG-asparaginase-related toxicities. **Methods:** Data from 548 newly diagnosed patients with ALL were analyzed to examine the association of clinical factors and

self-reported race/ethnicity with individual and cumulative burden of PEG-asparaginase-related toxicities. Using a novel approach of k-mode cluster analysis, we identified groups of patients with distinct toxicity profiles. From 170 patients who had genotype data available, we analyzed the role of Native American ancestry in the development of PEG-asparaginase related toxicities using multivariable logistic regression. **Results:** Older (>10 years) patients and those with higher BMI (overweight/obese) were at an increased risk of individual PEG-asparaginase-related hyperbilirubinemia, pancreatitis, and VTE; and were at a higher risk of co-occurring toxicities. Hypersensitivity was more likely in patients treated with high/very high-risk treatment protocols compared to low/standard risk treatment protocols. Although we did not find a significant association with self-reported or genetically defined race/ethnicity and PEG-asparaginase-related toxicities, the proportion of Native American genetic ancestry increased with an increasing number of toxicities. **Conclusion:** The incidence and cumulative burden of PEG-asparaginase-related toxicities are associated with age at diagnosis, BMI, and ALL risk group. More research with a larger more diverse population is necessary to improve the risk-benefit ratio of treatment with PEG-asparaginase.

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BACKGROUND

Literature Review

Pediatric acute lymphoblastic leukemia (ALL) accounts for approximately 25% of all childhood cancers diagnosed annually in the United States (U.S.) (Siegel et al., 2017). Over the past several decades, improvements in the treatment of pediatric ALL have resulted in five-year survival rates exceeding 90%; however, not all groups have benefitted equally from these improvements and the rate of treatment failure and relapse remains high (10-20%) (Silverman, L. B. et al., 2010; Stephen P. Hunger et al., 2012). Specifically, racial and ethnic differences in both the incidence of ALL and the response to therapy persist across decades (Abrahao et al., 2015; Bhatia et al., 2002). Recent investigations have reported racial and ethnic disparities in the incidence of treatment-related toxicity (Kahn et al., 2018; Taylor et al., 2018), which may lead to treatment modifications and potentially contribute to disparities in outcomes (Avramis & Panosyan, 2005; Hunger, S. P. & Mullighan, 2015; Narta et al., 2007). Therefore, there exists a critical need to identify which patients are at greatest risk of adverse response to modern ALL chemotherapy. However, few studies have evaluated the predictors and patterns of toxicity to asparaginase, one of the cornerstones of pediatric ALL therapy. The objective of this study is to characterize the incidence and patterns of asparaginase-related toxicities in a diverse, contemporary population of pediatric ALL patients.

Acute Lymphoblastic Leukemia Epidemiology

ALL is the most common cancer diagnosed in children <15 years of age in the U.S. (Siegel et al., 2017). With approximately 3,500 incident cases every year, ALL accounts for 26% of all childhood cancers diagnosed in the U.S. (Ward, DeSantis, Robbins, Kohler, & Jemal, 2014). In the U.S., the incidence of pediatric ALL between 2010 and 2014 was 3.4 cases per 100,000 persons, with a peak incidence observed between 2 to 5 years of age (7.5 per 100,000 persons during 2010-2014) and a steady decline into adolescence and young adulthood (Siegel et al., 2017). Males are more likely to develop ALL compared to females (38.0 compared to 29.7 per 1 million) (Md Jobayer Hossain, Li Xie, & Suzanne M. McCahan, 2014; Siegel et al., 2017).

Clinical Classification

Classification of ALL depends on cytological and morphological characteristics. The World Health Organization updated the classification of ALL in 2016, as shown in Table 1 (Arber et al., 2016).

Table 1: WHO Classification of Acute Lymphoblastic Leukemia

B-lymphoblastic leukemia/lymphoma, NOS
B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
B-lymphoblastic leukemia/lymphoma with t (9;22) (q34.1; q11.2); BCR-ABL1
B-lymphoblastic leukemia/lymphoma with t(v;11q23.3); KMT2A rearranged
B-lymphoblastic leukemia/lymphoma with t (12;21) (p13.2; q22.1); ETV6-RUNX1
B-lymphoblastic leukemia/lymphoma with hyperdiploidy
B-lymphoblastic leukemia/lymphoma with hypodiploidy
B-lymphoblastic leukemia/lymphoma with t (5;14) (q31.1; q32.3) IL3-IGH
B-lymphoblastic leukemia/lymphoma with t (1;19) (q23; p13.3); TCF3-PBX1
Provisional entity: B-lymphoblastic leukemia/lymphoma, BCR-ABL1-like
Provisional entity: B-lymphoblastic leukemia/lymphoma with iAMP21
T-lymphoblastic leukemia/lymphoma
Provisional entity: Early T-cell precursor lymphoblastic leukemia
Provisional entity: Natural killer (NK) cell lymphoblastic leukemia/lymphoma

Risk Stratification

The choice of chemotherapeutic drugs and treatment duration depends on the risk-based stratification of the child. Risk-stratification is determined by factors affecting the prognosis, possibility of treatment-failure, and clinical and biochemical features of the patient. Children with high-/very high-risk ALL are treated more aggressively and for a longer duration of time compared to children with the low-/standard-risk disease (Cooper, S. L. & Brown, 2015). The risk group of a child with ALL is determined by the oncologist. Presence of the following features is associated with being in the high-risk group:

- Children <1 year old and >10 years old at the time of diagnosis

- High white blood cell count at the time of diagnosis ($>50,000/\mu\text{L}$)
- Testicular involvement
- Central nervous system involvement
- Chromosomal alterations such as Down syndrome (trisomy 21)
- Male gender

Treatment

Individual drugs, dosage, and duration of administration of drugs differ with the patient's type of ALL and risk-based group. However, all treatment protocols have the Berlin-Frankfurt-Munster backbone with three phases: remission-induction, post-induction, and maintenance. The Berlin-Frankfurt-Munster protocol was introduced in 1975 in Germany and based the treatment for pediatric ALL with eight drugs used in the aforementioned three phases (Biondi, 2016). Children's Oncology Group (COG) is a National Cancer Institute supported clinical trials group, which is dedicated to pediatric cancer. There are currently more than 100 active clinical trials across the U.S. and Canada. COG develops clinical trials for the frontline treatment of many childhood cancers including ALL, by a collaboration of physicians, scientists, and researchers. Each protocol has specific inclusion/exclusion criteria, for instance, trial protocol number AALL0932 included children with newly diagnosed standard-risk B-lymphoblastic leukemia who were 1 to 9 years old (O'Leary, Krailo, Anderson, & Reaman, 2008). These clinical trial protocols based on the Berlin-Frankfurt-Munster backbone are established with the aim to improve and individualize ALL treatment, which considers the age of diagnosis, immunophenotype of ALL, and involvement of central nervous system or testicular involvement, for best possible outcomes (Hunger, Stephen P. et al., 2012). AALL0932 is used for

patients with low-risk leukemia, AALL0631 is used for standard and intermediate risk, AALL1131 protocol is used to treat patients who are at high risk and very high risk. Although the specific duration of treatment and the doses of each drug differ with protocol, the general outline of treatment is divided into the three phases mentioned earlier - remission-induction, consolidation, and maintenance.

Remission-Induction Phase:

The first phase of treatment lasts for four to six weeks after the initial diagnosis. The goal of this phase of the treatment is to achieve a complete remission with no minimum residual disease (MRD). MRD diagnosis is based on flow cytometry detection of aberrant immunophenotypes and polymerase chain reaction (PCR) analysis of T-cell receptor gene rearrangements (Bene & Eveillard, 2018). When leukemic cells are undetectable by day 29 of treatment, it is defined as complete remission without MRD. Low-risk MRD is defined as having <0.001% detectable leukemic cells at day 29 of treatment, and high-risk MRD is defined as having >0.001% detectable leukemic cells. Approximately, 95% of all children with ALL have complete remission but those with positive MRD either suffer from induction failure or succumb to therapy-related mortality. The absence of complete remission by 6 weeks of induction chemotherapy is known as induction failure (Pui, Ching-Hon & Campana, 2017).

The remission-induction phase includes glucocorticoids (prednisone, prednisolone, or dexamethasone), vincristine, and asparaginase. Depending on the risk group, some protocols may also administer an anthracycline (daunorubicin, doxorubicin, or epirubicin) in this phase (Chang et al., 2008; Johnston, 2016; Pui, Ching-Hon & Evans, 2006). The weight, age, risk group, and other factors determine the individual dose and duration of the treatment. Vincristine,

asparaginase, and anthracyclines act by selectively disrupting the cell cycle of tumor cells resulting in apoptosis or cell death. Glucocorticoids' anti-tumor activity is a result of glucose uptake and metabolism block in ALL cells; they also have an immunosuppressive property which helps in reducing the hypersensitivity reactions due to asparaginase (Dyczynski et al., 2018).

Polyethylene glycol asparaginase (PEG-asparaginase) is initiated on day 4 of the remission-induction phase in most treatment protocols. It is administered intravenously with a dose of 2500 international units/m²/dose. The number of doses may be higher in protocols for children with high- or very high-risk disease.

Post-induction Phase:

The post-induction phase is divided into consolidation and intensification phases. The complete phase lasts from six to nine months. The goal of this phase is to eliminate submicroscopic diseased cells after achieving MRD in the remission-induction phase. Similar to remission-induction phase the individual dose and duration of this phase is governed by clinical factors of the patient, subjecting children with high-risk ALL to a longer and more intensive treatment compared to their standard-risk counterparts (Chang et al., 2008; Johnston, 2016; Pui, Ching-Hon & Evans, 2006). The decision of delayed intensification or re-induction is considered in high-risk children.

In accordance with the Berlin-Frankfurt-Munster protocol, the consolidation is characterized by high-dose methotrexate with or without leucovorin rescue and mercaptopurine. Other drugs included in this phase are asparaginase, cytarabine, and cyclophosphamide (Pui, Ching-Hon & Evans, 2006). Cytarabine and mercaptopurine are anti-metabolite analogs which replace

purines and pyrimidines, and cyclophosphamide acts as an alkylating agent disrupting normal DNA function to result in cell death (Lennard, 1992; Wiley, Jones, Sawyer, & Paterson, 1982). Methotrexate competitively inhibits the production of metabolites necessary for DNA synthesis and eliminates the tumor cells (Howard, McCormick, Pui, Buddington, & Harvey, 2016). PEG-asparaginase in this phase of treatment is given on day 15 in most treatment protocols. It may be repeated between days 43-64 of the post-induction phase in treatment protocols for children with high-risk or very high-risk ALL.

Maintenance Phase:

This phase, lasting for approximately two to three years, usually starts only after complete remission is achieved and there is no evidence of submicroscopic malignant cells. As the purpose of this phase of treatment is to prevent the recurrence of the disease and slow the progression if recurrence occurs, along with continuous chemotherapy for two years, children may require bone marrow transplantation and intrathecal methotrexate injections to prevent CNS infiltration of leukemia cells (Pui, Ching-Hon & Evans, 2006).

Drugs used in the maintenance phase include methotrexate, vincristine, mercaptopurine, and glucocorticoids but their doses are usually lowered compared to the remission-induction and consolidation phase. The maintenance phase may be truncated in children with low-risk ALL.

Table 2: Pediatric Acute Lymphoblastic Leukemia treatment phases

PHASE	Remission-induction	Post-induction	Maintenance
GOAL	Achieve complete remission	Eliminate submicroscopic leukemic cells	Prevent recurrence and slow progression if ALL recurs
DRUGS	Glucocorticoids Asparaginase Vincristine Doxorubicin	Methotrexate Mercaptopurine Cytarabine Cyclophosphamide Asparaginase	Methotrexate Mercaptopurine Vincristine Glucocorticoids
DURATION	2-4 weeks	4-6 weeks	2-3 years

Pediatric ALL Outcomes & Disparities

Although overall survival rates for pediatric ALL exceed 90% with current treatment regimens, there is considerable inter-patient variability in treatment responses (Hunger, S. P. & Mullighan, 2015). Factors associated with pediatric ALL survival include age at diagnosis, gender, and race and ethnicity (Barrington-Trimis et al., 2017; Giddings, Whitehead, Metayer, & Miller, 2016). Compared to those diagnosed in infancy, children between the age of 1 to 5 years of age at diagnosis are 85% more likely to survive. The hazard ratio for death in males was 1.29 times higher compared to females (Md Jobayer Hossain et al., 2014). The survival rates vary significantly by race and ethnicity, with the lowest survival in non-Hispanic blacks and highest in non-Hispanic whites (Abrahao et al., 2015; Bhatia et al., 2002).

Evidence from the literature suggests racial and ethnic disparities are seen in both pediatric ALL incidence and survival. The age-adjusted incidence rate per 1,000,000 person-years was highest for Hispanic whites (24.9) and lowest for the non-Hispanic black population (10.4). Non-

Hispanic whites and Asian/Pacific Islanders had intermediate incidence (16.6 and 14.8 respectively) (Dores, Devesa, Curtis, Linet, & Morton, 2012). A population-based analysis of SEER data from 1992-2001 showed similar results where the incidence rate for Hispanic children was 1.3-times the rate observed for non-Hispanic white children and 2.6-times that of non-Hispanic black children (Matasar, Ritchie, Consedine, Magai, & Neugut, 2006). Another study of SEER data from 2001-2007 confirmed that non-Hispanic black children have significantly lower incidence rates compared to non-Hispanic white children; with age-adjusted incident rates per 100,000 of 1.1 and 1.7, respectively (Dores et al., 2012).

Racial and ethnic differences are also well documented for treatment outcomes, with inferior treatment outcomes observed among Hispanics and non-Hispanic blacks compared to non-Hispanic whites of the same age group. A large-scale population-based retrospective study of 8,447 children treated on Children's Cancer Group treatment protocols between 1981 and 1994 showed that the 5-year survival among non-Hispanic white children was 75.1% compared to 65.9% and 61.5% in Hispanic and non-Hispanic black children, respectively (Bhatia et al., 2002). Another retrospective study from 1988 to 2012 including 9,295 children from the California Cancer Registry found that the 5-year survival in children diagnosed with ALL is lowest for non-Hispanic black children (74.4%), followed by Hispanic children (79.0%), and highest in non-Hispanic white children (85.0%) (Abrahao et al., 2015). Although the difference in 5-year survival has decreased over recent decades, it is still consistently lower among Hispanic children compared to non-Hispanic white children.

The causes of racial and ethnic differences in the incidence and survival of children with ALL remain uncertain but can be partly explained by non-genetic factors such as access to health care and treatment adherence as evident in a single-institution study at St. Jude Children's Research Hospital (Lim, J. Y., Bhatia, Robison, & Yang, 2014). Evidence also suggests that genetic factors such as levels of ploidy and ancestry are associated with relapse and survival (Aldrich et al., 2006; Cheng et al., 2011). To determine the role of ethnicity in incidence and survival of ALL, prior studies have used self-reported race and ethnicity as well as genetic ancestry (Bhatia et al., 2002; Hunninghake, Weiss, & Celedon, 2006; Lim, J. Y. et al., 2014).

Self-reported race and ethnicity are defined by virtue of their biological (e.g., physical appearance) and non-biological (e.g., language and traditions) features leading to heterogeneous and imprecise groups with respect to genetic background (Cooper, R. S., Kaufman, & Ward, 2003). Hispanics are one of the most broadly defined race/ethnic groups and include individuals with varying proportions of European, African, and Native American genetic ancestry. For instance, Hispanic ethnicity comprises people of Mexican, Cuban, Puerto Rican or Dominican origin (Humes, Jones, & Ramirez, 2011). Mexican Hispanic persons will have different proportions of European, African and Native American genetic ancestry compared to Puerto Rican Hispanic persons. Since culture, religion, and racial traits play a role in self-reported ethnicity, there is a level of self-identification and perception of society that might differ from the genetic ancestry (Cooper, R. S. et al., 2003).

Genetic ancestry, on the other hand, is defined based on variation in genomic structure among different populations (Mersha & Abebe, 2015). Genetic ancestry is most commonly estimated

based on an assessment of variation in ‘ancestry informative markers’ (AIMs). AIMs are preselected diploid and unlinked autosomal markers inherited from both the parents. Proportions of Native American, African, and European ancestry can be established for each individual, and then ancestry can be inferred by statistically comparing the AIMs to that of reference populations such as 1000 Genome Project phase 3 samples (Ding et al., 2011). These genetic ancestry proportions vary with each individual. For example, two self-identified African American people can have different proportions of European and African genetic ancestry. Within self-identified ethnicity, there is genetic variability; this is particularly true for Hispanics who have differing proportions of Native American genetic ancestry. The ‘Hispanic’ or ‘Latino’ populations have broad ranges of variation in admixture (variable proportion of Native American, European, and African ancestral origin). On average, Hispanic populations from Mexico have 35-64% Native American genetic ancestry and 3 to 5% African genetic ancestry; in contrast, those from Puerto Rico have 12-15% Native American genetic ancestry and 18-25% African genetic ancestry (Hunninghake et al., 2006).

Self-reported race and ethnicity allow us to assess the association of biological and non-biological factors with drug-related toxicity. Genetic ancestry, on the other hand, enables us to evaluate the population-specific biological variability associated with drug-related toxicities. Therefore, to comprehensively discuss the implications of racial and ethnic disparities in childhood ALL, it is essential to consider both self-reported race/ethnicity and genetic ancestry.

Racial and ethnic disparities in treatment outcomes may also occur as a result of increased susceptibility to drug-associated toxicities within some racial and ethnic groups, leading to

treatment interruption and less efficacious treatment. (Taylor et al., 2018) Pediatric ALL therapy constitutes treatment phases consisting of unique combinations of drugs including asparaginase, vincristine, cyclophosphamide, cytarabine and mercaptopurine, glucocorticoids, methotrexate and doxorubicin (Inaba, Greaves, & Mullighan, 2013). Drug-associated toxicities are a common occurrence during treatment in children with ALL, and variation in incidence and severity of these toxicities is associated with various clinical, demographic and genetic factors. For example, glucocorticoid-associated osteonecrosis occurs in 1% in children less than 9 years of age, compared to almost 10% in children 10-15 years of age (Mattano, Sather, Trigg, & Nachman, 2000). Some recent studies have reported differences in drug-associated toxicities by race/ethnicity. For example, Taylor et al. discovered that, compared to non-Hispanic whites, Hispanic ALL patients are at increased risk of methotrexate neurotoxicity, and episodes of neurotoxicity were associated with patients receiving fewer courses of chemotherapy (Taylor et al., 2018). Similarly, Moriyama and colleagues identified genetic variants in the *NUDT15* gene that were associated with thiopurine-associated myelosuppression in pediatric ALL patients (Moriyama et al., 2017). Notably, the frequency of these risk variants is highest in individuals of Hispanic or Asian descent, suggesting susceptibility to specific drug toxicities varies across genetic ancestry. Drug-associated toxicities may lead to discontinuation and interruption of treatment. Treatment interruption, particularly during the remission-induction phase, may affect treatment efficacy and increase the probability of relapse. Between 10% to 20% of children suffer relapsed ALL, which is the leading cause of cancer death in children (Silverman et al., 2010; Stephen P. Hunger et al., 2012). Hence, efforts are now directed towards

decreasing the incidence of drug-associated toxicities and minimizing treatment delays and failures with the aim of improved long-term survival and quality of life.

Asparaginase Chemotherapy

Asparaginase, a drug derived from an *E. coli* bacterium is one of the first-line drugs for the treatment of pediatric ALL (Clavell, L. A. et al., 1986; Panetta et al., 2009). PEG-asparaginase, a preparation variant of asparaginase, has pharmacological benefits over native *E. coli* L-asparaginase such as longer half-life and lower immunogenicity (Avramis et al., 2002; Panetta et al., 2009). Evidence has shown that several drug-related toxicities (e.g., hypersensitivity, pancreatitis, hyperbilirubinemia, and pancreatitis) are linked with PEG-asparaginase, and vary with respect to clinical and demographic factors (Hunger, S. P. & Mullighan, 2015; Pui, C. H., Robison, & Look, 2008). However, differences in toxicity rates across ethnic groups have not been fully assessed.

Introduction of asparaginase derived from *E. coli* into ALL treatment protocols starting in 1972 saw improvement in the pediatric ALL survival rates from approximately 30% to 60% (Hunger, S. P. & Mullighan, 2015). As such, asparaginase has been the cornerstone of pediatric ALL treatment since its introduction. However, like any other chemotherapeutic agent, asparaginase is associated with specific drug toxicities (hypersensitivity, pancreatitis, hyperbilirubinemia, and coagulation issues) (Pui, C. H. et al., 2008). These four most common asparaginase-associated toxicities appear to share a similar mechanism of toxicity and may occur simultaneously (Avramis & Panosyan, 2005; Parmentier et al., 2015; Reinert et al., 2006). Moreover, the relationship between variation in severity and incidence of asparaginase-associated toxicities and ethnicity in pediatric ALL patients has not been well-studied.

Therefore, the overall goal of this study is to evaluate the association of race and ethnicity (self-reported and genetically defined) and PEG-asparaginase-related toxicities in recently diagnosed pediatric ALL patients.

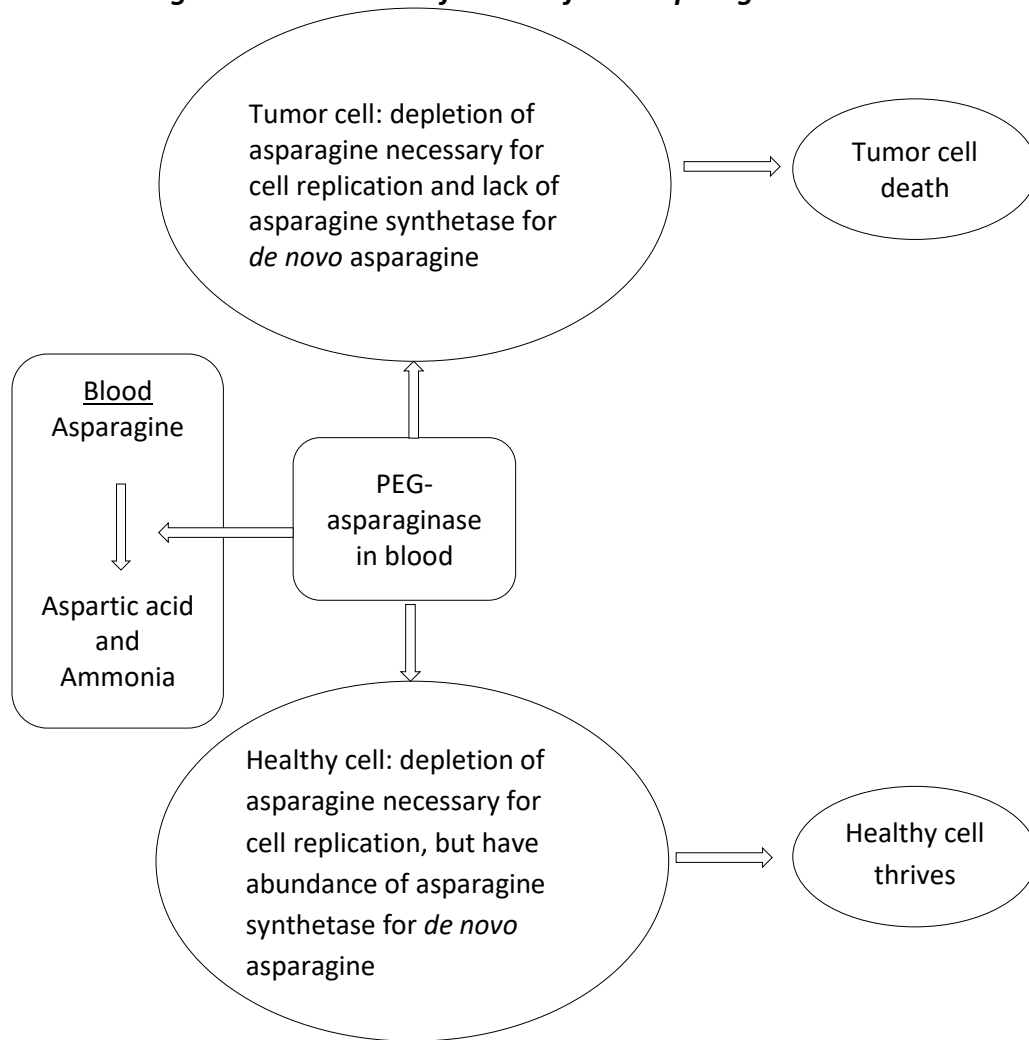
Mechanism of action of PEG-asparaginase

Asparaginase is derived either from *Escherichia coli* (*E. coli*) or *Erwinia chrysanthemi* (*Erwinia*).

In December 2012, the manufacturer withdrew asparaginase derived from native *E. coli* (Elspar®) and replaced it with pegylated *E. coli* known as PEG-asparaginase (Oncaspar®) as the pegylated form of asparaginase has a longer half-life and less immunogenicity compared to native asparaginase (Place et al., 2015). Another formulation is *Erwinia* asparaginase (Erwinaze®), which was approved by the FDA in 2011. PEG-asparaginase and *Erwinia*-asparaginase are approved for intramuscular (IM) and intravenous (IV) administration. These three preparations have different half-lives which reflect the required frequency of doses.

As seen in Figure 2, asparaginase catalyzes the hydrolysis of asparagine to aspartic acid and ammonia. Unlike normal cells, leukemia cells lack asparagine synthetase and are incapable of synthesizing asparagine *de novo*, making the uptake of extracellular asparagine imperative for the growth of leukemia cells. Asparaginase preparations deplete the extracellular asparagine, thereby arresting the cell cycle of leukemia cells in the G1 phase leading to breakage of DNA strands and impeding leukemia cell proliferation. Asparaginase also helps in the conversion of glutamine to glutamic acid, which decreases the levels of glutamine in the circulation. As glutamine is the nitrogen donor for DNA and RNA synthesis by leukemia cells, depletion of glutamine further increases the apoptosis of leukemic cells (Narta, Kanwar, & Azmi, 2007).

Figure 1: Mechanism of action of PEG-asparaginase

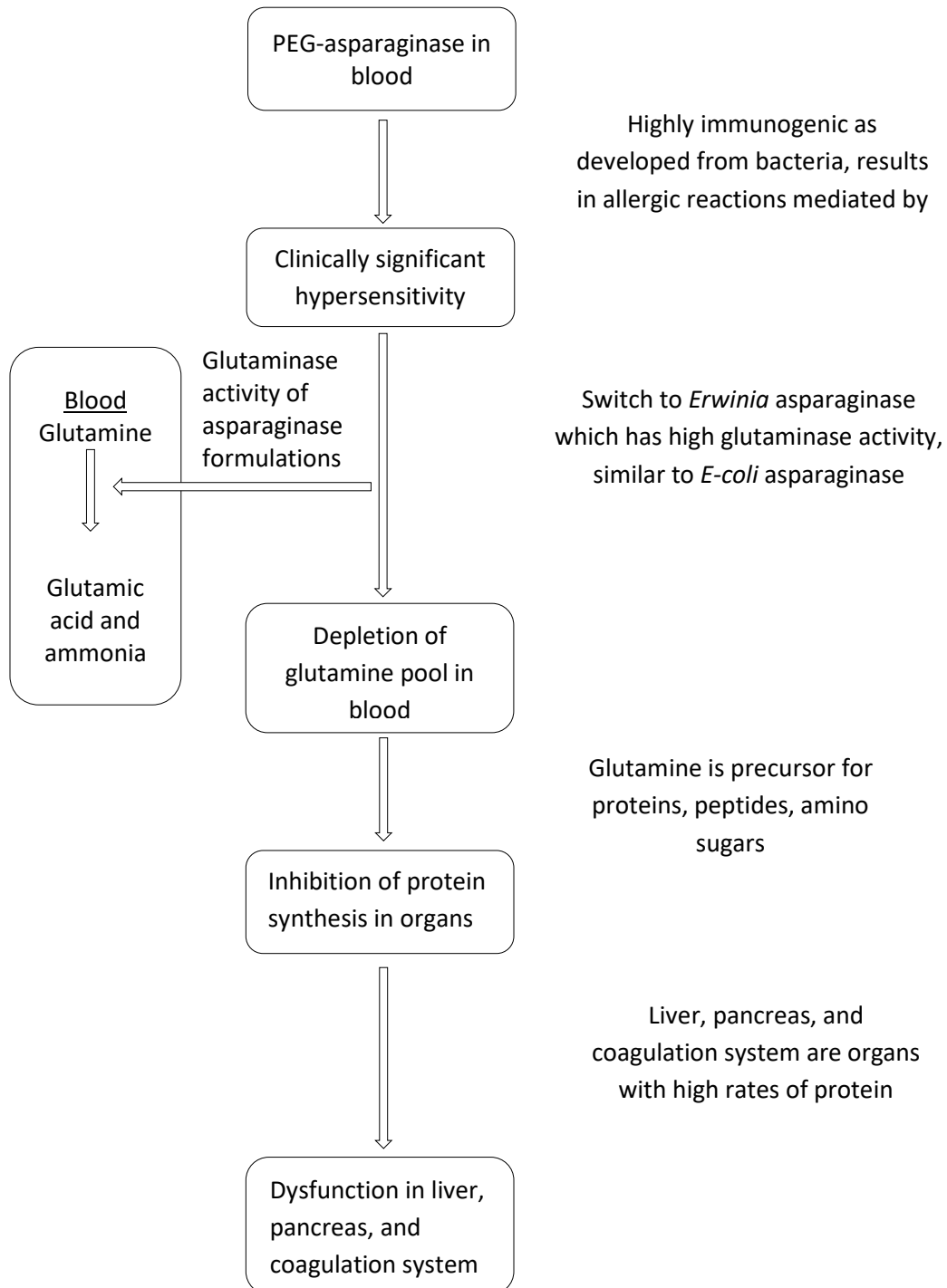


PEG-asparaginase-related toxicities

The most commonly reported PEG asparaginase-associated toxicities are hypersensitivity, hyperbilirubinemia, pancreatitis, and coagulation disorders. PEG-asparaginase is developed from *E. coli*, which as a foreign substance elicits an allergic or hypersensitivity reaction. The gold standard is to switch to asparaginase developed from *Erwinia* when hypersensitivity is clinically relevant. All asparaginase formulations have some degree of glutaminase activity, and studies

have shown that *Erwinia* asparaginase has higher glutaminase activity compared to PEG-asparaginase. Similar to asparaginase, glutaminase catalyzes the hydrolysis of glutamine to glutamic acid and ammonia depleting glutamine pools in the blood (Figure 3). As glutamine is an essential component of asparagine synthetase and is a precursor for protein peptides and amino sugars, depletion of glutamine pools depletes asparagine synthetase enzyme thus hindering the asparagine neo-synthesis in normal cells and inhibits protein synthesis. The toxicities arising from inhibition of protein synthesis mostly affects organs with high rates of protein synthesis – liver, pancreas, and coagulation system, increasing the probability these four toxicities occurring simultaneously (Avramis & Panosyan, 2005; Parmentier et al., 2015; Reinert et al., 2006). The relationship between mechanisms of action of different toxicities reiterates the plausibility that these four toxicities occur simultaneously. The investigation of the predicting factors for these toxicities will aid in improving the course of treatment.

Figure 2: Mechanism of action of PEG-asparaginase-related toxicities



Details of the individual toxicities are provided below.

Hypersensitivity

Hypersensitivity due to asparaginase is immunological sensitization to foreign bacterial proteins of *E. coli* or *Erwinia chrysanthemi*. Immunological sensitization is the production of antibodies in response to the bacterial proteins resulting in IgE, IgG, and IgM antibodies cascades, degranulation of mast cells and releasing immune mediators such as histamines, prostaglandins, and leukotrienes (Shinnick, Browning, & Koontz, 2013). This may be clinically manifested as mild cases of urticaria to life-threatening anaphylaxis. Hypersensitivity of grade 3 and above is defined as prolonged recurrence of transient flushing or rash and fever ≥ 100.4 degrees F, which are not rapidly responsive to symptomatic treatment. In a few cases, anaphylaxis can be observed which is characterized by symptomatic bronchospasm with or without urticaria, angioedema, and hypotension (US Department of Health and Human Services, National Institutes of Health, National Cancer Institute, 2017; Yun, Adam, Yerly, & Pichler, 2012).

The incidence of hypersensitivity differs by asparaginase formulation, highest seen with native *E. coli* followed by PEG-asparaginase, and the lowest incidence with *Erwinia* asparaginase.

Overall, 3-78% of patients treated with native *E. coli* asparaginase experience some degree of hypersensitivity; this wide range can be contributed by variation in definition and various clinical manifestations (Vrooman et al., 2010). The introduction of PEG-asparaginase reduced the incidence of clinically significant asparaginase-associated hypersensitivity to approximately 30% (Asselin, 1999). Clinically significant hypersensitivity corresponds to grade 3 and above

hypersensitivity requiring treatment modification to *Erwinia* (Raetz & Salzer, 2010; Silverman et al., 2001; Vrooman et al., 2010). PEG-asparaginase may also undergo 'silent inactivation' or develop 'subclinical hypersensitivity' making the drug harmful and therapeutically ineffective in 26-71% of children (Tong et al., 2014). Silent inactivation is due to the presence of anti-asparaginase antibodies in the blood, which can be counteracted by maintaining asparaginase activity through regular therapeutic drug monitoring and often requires changes in dose (Tong et al., 2014).

Despite the high incidence of hypersensitivity and silent inactivation of the drug, PEG-asparaginase is one of the first-line drugs in many treatment protocols due to its long half-life (5.7 days) (Kurre, Helen A. et al., 2002). In the presence of clinically significant PEG-asparaginase-related hypersensitivity, it is replaced by *Erwinia* asparaginase (Vrooman et al., 2010). Although *Erwinia* asparaginase has no cross-reactivity with PEG-asparaginase and has low immunogenicity, it has low adherence due to a short half-life of 0.6 days. As a result, *Erwinia* needs to be administered ~3 times a week to maintain therapeutic asparagine levels in the blood (Vrooman et al., 2010).

Pancreatitis

Pancreatitis is another well-established asparaginase-associated toxicity. It is seen in 0.7% - 18% of children treated with PEG-asparaginase (Denton, Rawlins, Oberley, Bhojwani, & Orgel, 2018; Kearney et al., 2009). The wide range of reported incidence can be accounted for by the inconsistencies in the definition of asparaginase-related pancreatitis. Children older than 10 years of age are more likely to experience asparaginase-related pancreatitis compared to younger children (Denton et al., 2018; Moghrabi et al., 2007).

Pancreatitis is diagnosed by clinical assessment and blood tests for high levels of pancreatic enzymes including lipase and amylase. Radiological imaging used to confirm the diagnosis of pancreatitis shows findings of pancreatic edema, necrosis or pseudocyst (Raja, Schmiegelow, & Frandsen, 2012; Raja et al., 2014). The Common Terminology Criteria for Adverse Events (CTCAE) v.5.0 defines pancreatitis as a disorder associated with inflammation of the pancreas. Symptomatic pancreatitis results in treatment interruption until it is resolved. Symptomatic pancreatitis is seen in Grade 3 and above with increased levels of enzymes (amylase and lipase) and symptoms including severe pain and vomiting requiring radiologic or surgical intervention. (US Department of Health and Human Services, National Institutes of Health, National Cancer Institute, 2017). Grade 3-4 toxicity is defined by a serum lipase $>2.0-5.0 \times \text{UNL}$ (Upper Normal Limits) and serum amylase $>5.0 \text{ UNL}$ (US Department of Health and Human Services, National Institutes of Health, National Cancer Institute, 2017)

Treatment with asparaginase is re-initiated in children with pancreatitis once it has resolved. However, large prospective trials are necessary to assess the rate of leukemia relapse and long-term effects such as chronic pancreatitis or insulin-dependent diabetes mellitus. Complications include surgical interventions for pseudocysts, necrotic pancreatitis or hemorrhagic pancreatitis (Denton et al., 2018; Knoderer, Robarge, & Flockhart, 2007; Raja et al., 2014).

Hyperbilirubinemia

The incidence rate for PEG-asparaginase-related hyperbilirubinemia is approximately 4-5% (Avramis et al., 2002; Dinndorf, Patricia Anne, Gootenberg, Cohen, Keegan, & Pazdur, 2007; Kurre, Helen A. et al., 2002). Onset typically occurs between 2 to 3 weeks after treatment initiation. Clinically significant hyperbilirubinemia may require treatment delay, thus affecting

treatment efficacy. Clinically, hyperbilirubinemia grade 3 and above is manifested as pain experienced in the right upper quadrant of the abdomen, anorexia, and signs of jaundice (Dinndorf, P. A., Gootenberg, Cohen, Keegan, & Pazdur, 2007; Kurre, H. A. et al., 2002) CTACE v.5.0 defines hyperbilirubinemia in terms of lab values; as grade 3 is an elevation of serum bilirubin > 3.0 to 10.0 *UNL, and grade 4 is elevation of these enzymes >10.0 *UNL (US Department of Health and Human Services, National Institutes of Health, National Cancer Institute, 2017).

Similar to pancreatitis, hyperbilirubinemia is diagnosed by clinical assessment and laboratory values of bilirubin (conjugated and unconjugated). PEG-asparaginase-related hyperbilirubinemia is usually self-limiting in children but may require treatment interruption in some cases (US Department of Health and Human Services, National Institutes of Health, National Cancer Institute, 2017).

Venous thromboembolic events (VTE)

Considering the key role of the liver in the coagulation system, children experiencing hyperbilirubinemia are more likely to have some coagulation disorders (Tripodi et al., 2009). Also, the hemostasis equilibrium changes in patients with ALL by increasing coagulation factors VIII, IX, von Willebrand, and proteins, while simultaneously decreasing some coagulation factor-like factors XIII A, XIII S, and protein C, ultimately resulting in the hypercoagulable state (Mitchell et al., 1994). Asparaginase treatment further increases the risk of the pre-thrombotic state by decreasing anti-thrombin, protein C, and protein S. A majority of cases are diagnosed during induction phase within an average of 3.5 months of the start of the induction phase, whereas, 10% of cases are seen in consolidation. (Levy-Mendelovich, Barg, & Kenet, 2018)

VTE is seen in approximately 5% of children treated with asparaginase. Of this 5%, approximately 50% of VTE cases is seen in the central nervous sinus, 27.5% in the upper limbs, 1% in the pulmonary system, and 1% in the right atrium. Central lines placed in the jugular vein for medication delivery further increase the risk of VTE (Caruso et al., 2006).

Low molecular weight heparin (LMWH) is a first-line anticoagulant drug for VTE. Asparaginase is withheld in clinically significant cases until symptoms are resolved and is typically restarted with the administration of LMWH (Truelove, Fielding, & Hunt, 2013). CTCAE v.5.0 does not have an explicit definition for thrombosis or venous thromboembolism, hence a usual definition consists of those children showing signs of VTE and who are administered Lovenox or warfarin (Sanofi-aventis, 1993).

Public Health Significance

Pediatric ALL accounts for approximately 25% of all childhood cancers diagnosed annually in the U.S. (Siegel et al., 2017). Although survival for pediatric ALL has improved considerably, with current 5-year survival exceeding 90%, the rate of treatment failure and relapse remains high (10-20%) (Silverman et al., 2010; Stephen P. Hunger et al., 2012). While asparaginase has been the cornerstone of ALL treatment since its introduction, PEG-asparaginase-related toxicities may lead to treatment delays and treatment modifications, potentially impacting treatment efficacy (Avramis & Panosyan, 2005; Hunger, S. P. & Mullighan, 2015; Narta et al., 2007). These drug toxicities significantly affect the ability to attain MRD, thus influence relapse and survival rates (Bene & Eveillard, 2018; Denton et al., 2018). Despite the serious consequences of treatment-related toxicity, it remains unclear why some racial and ethnic groups experience greater toxicities than others (Lim, Joshua Yew Suang, Bhatia, Robison, & Yang, 2014; Moriyama

et al., 2017; Taylor et al., 2018; Yang et al., 2011). This study is hence an important step towards refining clinical decision making related to drug choice and doses. Improved prediction of at-risk children and pattern of PEG-asparaginase-related toxicity is important to inform recommendations for follow-up and surveillance and to develop new preventive and rescue approaches.

Hypothesis, Research Question, Specific Aims or Objectives

Hypothesis for aim 1:

The incidence of PEG-asparaginase-related toxicities varies by demographic and clinical factors, including self-reported race and ethnicity, age of diagnosis, gender, and risk-subgroups. Also, variation in the incidence and frequency of the four toxicities can be partly explained by clinical and demographic factors. The purpose of this aim is to evaluate the association of clinical and demographic factors with four individual PEG-asparaginase-related toxicities and co-occurrence of the four toxicities.

Aim 1:

To describe the incidence of and clinical factors associated with PEG-asparaginase-related toxicities in recently diagnosed pediatric acute lymphoblastic leukemia.

Hypothesis for aim 2:

The four most common PEG-asparaginase-related toxicities are hypersensitivity, pancreatitis, hyperbilirubinemia, and venous thromboembolic events (VTE). We hypothesize that there are latent groups of patients who are systematically more similar than others with respect to the

occurrence of these four PEG-asparaginase-related toxicities. The purpose of this aim is to statistically classify and describe these groups.

Aim 2:

To classify subgroups of newly diagnosed ALL patients based on the co-occurrence of PEG-associated toxicities during pediatric acute lymphoblastic leukemia treatment.

Hypothesis for aim 3:

Genetically-determined ancestry, defined by the proportion of European, African, and Native American ancestry, is associated with the individual and observed groups of PEG-asparaginase-related toxicities. The variation in the frequency of the four individual toxicities can be explained by the biological differences based on race and ethnicity as defined by genetic ancestry. The purpose of this aim is to evaluate the association of genetic ancestry with the four individual PEG-asparaginase-related toxicities and co-occurrence of the four toxicities.

Aim 3:

To evaluate the role of genetic ancestry in the development of PEG-asparaginase-related toxicities in recently diagnosed pediatric acute lymphoblastic leukemia patients.

METHODS

Study Subjects

The study included data from 548 children and young adults between 1 to 22 years of age with newly diagnosed ALL from September 2011 to December 2017 at Texas Children's Hospital, Houston, Texas. For this retrospective cohort study, the main exposure is self-reported ethnicity

and genetically defined ancestry, with the main outcome being the incidence of PEG-asparaginase-related toxicities. The clinical information and data to determine exposure and outcome status were abstracted from medical records.

Data Analysis

Exposure

- *Self-reported ethnicity*

During admission, the children or children's parents were asked to self-report their race and ethnicity. It was categorized as Hispanic, non-Hispanic white, non-Hispanic black, and non-Hispanic other, with non-Hispanic white as the reference group.

- *Genetically-defined race and ethnicity.*

The genetic ancestry was defined using STRUCTURE software (details to follow).

Outcome

PEG-asparaginase-related toxicity incidence

As discussed in the background, we only considered the PEG-asparaginase-related toxicities that were clinically significant and required interruption or termination of PEG-asparaginase. Table 3 details how individual toxicity were defined for the purpose of this study.

Table 3:Definitions of PEG-asparaginase-related toxicities

PEG-toxicity	Diagnosis
Hypersensitivity	Indication of <i>Erwinia</i> asparaginase
Pancreatitis	Lipase > 2.0 UNL (upper normal limit)
Hyperbilirubinemia	Bilirubin> 3.0 UNL
Thromboembolic events	documented cases of deep venous thrombosis and pulmonary embolism or indication of Lovenox and or warfarin

Covariates

We hypothesized that the clinical characteristics that have been associated with survival in leukemia patients also will be associated with PEG-asparaginase-related toxicities and have been selected *a priori* as covariates – age at diagnosis (1-5, 5-10, 10 -15, and 15+ years); gender (male or female); and risk group (standard- and high-risk). Age at diagnosis was measured as the number of completed years prior to the diagnosis of ALL. Since young adults ranging from age 19 to 22 years are treated using pediatric protocols and have a similar ALL profile as children, they were included in the analysis.

The determination of risk group depends on several factors such as white cell blood count at the time of diagnosis, the presence of testicular involvement in males, and central nervous system involvement. The decision of a risk group is necessary to determine the course of treatment and was decided by the oncologist.

Specific Aim 1:

To describe the incidence of and clinical factors associated with PEG-asparaginase-related toxicities in recently diagnosed pediatric acute lymphoblastic leukemia.

This aim had two parts: 1) to analyze the association of clinical and demographic factors with four individual PEG-asparaginase-related toxicities, and 2) to analyze the association of clinical and demographic factors with co-occurrence of four toxicities (no toxicities, any one toxicity, any two toxicities, any three toxicities, and all four toxicities).

Statistical Analysis

Descriptive analysis was performed to compare 4 groups of race-ethnicity – non-Hispanic white, non-Hispanic black, Hispanic, and others using chi-square tests. Correlations among the

independent variables were checked to ensure the assumption of independence is satisfied. As independent variables were self-reported ethnicity, age of diagnosis, gender, and risk group for treatment, we did not have any collinearity. Association of individual covariates with individual PEG-asparaginase-related toxicities and co-occurrence of toxicities were performed using multivariable logistic regression, and ordinal logistic regression respectively. Irrespective of the significance of individual associations with the outcome, all covariates were included in the final model as decided *a priori* for their clinical significance. The final multivariable logistic regression models determined the relationship of the clinical and demographic factors with individual PEG-asparaginase-related toxicities, and the ordinal logistic regression determined the relation of clinical and demographic factors with co-occurring PEG-asparaginase-related toxicities.

Specific Aim 2:

To classify subgroups of newly diagnosed ALL patients based on the co-occurrence of PEG-associated toxicities during pediatric acute lymphoblastic leukemia treatment.

For aim 2, we classified subgroups of patients based on toxicity profiles using the k-modes algorithm. K-modes algorithm is considered an extension of a k-means hierarchical agglomerative clustering method for categorical data (Huang, 1997; Huang, 1998). However, unlike k-means, k-modes does not use Euclidian distance, but it identifies clusters based on the matching attributes between two or more data points. The k-modes method is an appropriate approach for clustering categorical data of event occurrence (Loree. J, 2018).

Statistical analysis

Steps to obtain clusters which are statistically similar and clinically significant are as follows:

1. Assign the number of clusters (or modes) (K):

The study focused on four toxicities, hence the maximum number of clusters possible is 16. To determine the optimal number of clusters, preliminary cluster analysis was done with k of 2-16. The goodness of a cluster configuration was assessed using the purity of clusters and the number of individuals in a given cluster. Purity is an external measure of a cluster; defined as the ratio between the dominant class in the cluster and the size of the cluster. The optimal number of clusters was selected with purity greater than 0.80 and a maximum of one cluster with less than five individuals.

2. Calculate the dissimilarity measure:

This step calculates the dissimilarity measure between each of the remaining objects (i.e. cases) from the k chosen modes. The dissimilarity measure between two cases X and Y is defined as the sum of total mismatches of corresponding attribute categories (i.e. toxicities) between two cases. The higher the number of mismatches, the more dissimilar the two cases are. For instance, if case X has hypersensitivity, pancreatitis, and hyperbilirubinemia, case Y has hypersensitivity only, and case Z has hypersensitivity and pancreatitis; then case X and case Y have more mismatches compared to case X and case Z.

Mathematically,

Equation 1: Dissimilarity measure

$$X = \begin{pmatrix} X_1 \\ X_2 \\ \vdots \\ X_m \end{pmatrix} \text{ and } Y = \begin{pmatrix} Y_1 \\ Y_2 \\ \vdots \\ Y_m \end{pmatrix}$$
$$\text{Dissimilarity measure} = d(X, Y) = \sum_{j=1}^m \delta(x_j, y_j)$$

Where m = number of attributes (i.e. toxicities) and

$$\delta(x_j, y_j) = \begin{cases} 0 & (x_j = y_j) \\ 1 & (x_j \neq y_j) \end{cases}$$

The program allocates a case to the cluster with the nearest mode according to the dissimilarity measure and updates the mode of the cluster after each case allocation.

3. Assign the case to the mode whose dissimilarity score is smallest:

Once all the cases in the dataset have been assigned to clusters, the program retests the dissimilarity score of the cases against the current modes. If a case is found to be closer to another cluster compared to the current cluster, then the case is reassigned to the new cluster followed by updating the modes for both clusters. With successive iterations, different cases are clustered together, and the modes are updated. Changing modes with consecutive iteration is known as a moving frequency-based method.

4. Repeat steps 1-3:

Steps 1-3 are repeated until no case changes cluster.

The R package 'klaR' was used for cluster analysis (Christian Roever, Nils Raabe, Karsten Luebke, Uwe Ligges, Gero Szepannek, Marc Zentgraf, 2018; R Core Team, 2014).

Specific Aim 3:

To evaluate the role of genetic ancestry in the development of PEG-asparaginase-related toxicities in recently diagnosed pediatric acute lymphoblastic leukemia patients.

Genotype calling and quality control

The study included 167 children with recently diagnosed acute lymphoblastic leukemia treated on one of the following COG developed protocols: AALL0932, AALL1231, AALL1131, AALL0434, AALL1122, and AALL0031. The participants were genotyped using the Illumina OmniExpress array per the manufacturer's instructions. DNA was isolated from peripheral blood taken at the time of remission for most of the patients or saliva for the remaining patients.

For accurate genetic ancestry analysis, genotyping quality control measures were followed. The genotyping quality was assessed using per-individual quality control followed by per-marker quality control. Per-individual quality control comprised excluding individuals having a high number of missing call rates (sample call rate <95%) and identification of duplicated individuals. The next step in quality control was per-marker quality control comprising exclusion of SNPs which had a high missing call rate (SNP call rate <98%) and minor allele frequency <1%. Also, the exclusion of markers with significant deviation from Hardy-Weinberg equilibrium ($< 10^{-5}$) (Anderson et al., 2010; Laurie et al., 2010).

Genetically defined ancestral groups by STRUCTURE analysis

To characterize the genetically defined ancestral groups, we used STRUCTURE software (V 2.3.4) (Hubisz, Falush, Stephens, & Pritchard, 2009). The length of the burn-in period and the number of simulations was set to 10,000 as recommended by the developers. 1000 Genome phase 3 samples of North European, West Africans, East Asians, and Native Americans were used as reference populations. The Hispanic population is highly diverse with significant population differences. For instance, Dominicans and Puerto Ricans have the highest level of African ancestry and low levels of Native American ancestry, compared to the Mexican population which shows the lowest level of African ancestry and highest level Native American

ancestry (Katarzyna Bryc et al., 2010). Since our study population has a predominance of Mexican Hispanics, the data were analyzed to evaluate the proportion of Native American global ancestry as a continuous variable.

Statistical Analysis

Similar to the analysis to evaluate the association of clinical factors to and PEG-asparaginase-related toxicities, logistic regression models were used to evaluate the role of the genetic ancestry of the development of PEG-asparaginase-related toxicities in recently diagnosed pediatric acute lymphoblastic leukemia. The main exposure variable was genetically defined ancestry, a continuous variable, and the covariates included age at diagnosis, risk group, and gender.

Human Subjects, Animal Subjects, or Safety Considerations

The analyses for this study were conducted on de-identified data abstracted from electronic medical records at Texas Children's Hospital, Houston, Texas for patients diagnosed between September 2011 and December 2017.

JOURNAL ARTICLE 1: INCIDENCE AND PATTERNS OF PEG-ASPARAGINASE-RELATED TOXICITIES IN AN ETHNICALLY DIVERSE COHORT OF ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer with an incidence of 3.4 per 100,000 persons in the U.S. (Ward et al., 2014). The survival of children with ALL has improved drastically due to multiagent treatment (Rivera, Pinkel, Simone, Hancock, & Crist, 1993). Contemporary ALL treatment protocols include polyethylene glycol asparaginase (PEG-asparaginase) as a first-line drug (Withycombe et al., 2019). Since asparaginase was approved by the US Food and Drug Administration (FDA) in the 1970s, it has been a critical drug instrumental in long-term survival for pediatric ALL patients (Avramis et al., 2002). Although PEG-asparaginase is an effective and essential part of treatment for ALL, it is commonly associated with four drug-related toxicities: hypersensitivity, hyperbilirubinemia, pancreatitis, and venous thromboembolism (VTE) (Conrich, 2019; Dinndorf, Patricia Anne et al., 2007; Parmentier et al., 2015).

The reported incidence of several PEG-asparaginase-related toxicities varies considerably in children and young adults. For example, hypersensitivity affects 3%-24% (Henriksen et al., 2015; Vrooman et al., 2010) while VTE may occur in 5%-12% (Klaassen et al., 2019; Mateos et al., 2019). Furthermore, most reports focus on individual toxicities and the cumulative burden of PEG toxicity remains unknown, particularly in ethnically diverse, understudied populations. Because PEG-asparaginase-related toxicities may necessitate treatment interruption, potentially jeopardizing long-term survival, additional information on the cumulative incidence and clinical predictors of toxicity are needed to identify patients at highest risk of adverse

treatment responses (Amylon et al., 1999; Clavell, Luis A. et al., 1986). To address this need, the study aims to describe the incidence and co-occurrence of PEG-related toxicities and evaluate potential clinical and demographic risk factors for the occurrence PEG-asparaginase related toxicities in a multi-ethnic cohort of pediatric ALL patients.

METHODS

This is a retrospective study that included 548 children and young adults (ages 1-22) newly diagnosed with ALL at Texas Children's Hospital in Houston, Texas between September 1, 2011 and December 31, 2017. Young adults (18-22 years old) were included in the study, as they were treated as per pediatric protocols. Cases of infant leukemia (<1 year of age at diagnosis) were excluded. The study was performed at Baylor College of Medicine in collaboration with and The University of Texas Health Science Center at Houston (UTHealth) with Institutional Review Board approval from both institutions.

The primary outcomes were the four PEG-asparaginase related toxicities: hypersensitivity, hyperbilirubinemia, pancreatitis, and venous thromboembolism. Per protocol, grade 3+ hypersensitivity was diagnosed as a requirement to switch from first-line PEG-asparaginase to second-line *Erwinia* asparaginase formulation. As per the Common Terminology Criteria for Adverse Events (CTCAE v5.0), grade 3+ hyperbilirubinemia and pancreatitis were defined as 5 times the upper normal levels of bilirubin and 3 times the upper normal levels of lipase, respectively. VTE was defined as either a physician-led diagnosis or indication of Levenox/low molecular weight heparin. The predictors included in the analysis, age at diagnosis, body mass index (BMI), self-reported race/ethnicity, gender, and ALL risk group (standard-risk or high-risk), were abstracted from electronic medical records from Texas Children's Hospital.

Statistical Analysis

Descriptive statistics were calculated for the predictor variables (age at diagnosis, BMI, gender, self-reported race/ethnicity, ALL risk group). Patients younger than 10 years of age were categorized as 1-5 years and 6-10 years old, and patients older than 10 years of age were categorized as ≥ 10 years old. For patients < 2 years of age BMI percentile z-score was calculated as per CDC guidelines by substituting height by the length in centimeters (Roy et al., 2016). For patients from 2 to 19 years old BMI percentile z-score was calculated using the CDC BMI calculator for children and teens (BMI calculator for child and teen.2019). BMI for young adults (>20 years old) was calculated using the standard BMI formula ($\text{weight(kg)}/[\text{height(m)}]^2$). Individuals were categorized as underweight or normal weight if their BMI percentile <85 (if < 20 years of age) or BMI $< 25 \text{ kg/m}^2$ (if ≥ 20 years of age) and as overweight or obese if their BMI percentile was ≥ 85 (if < 20 years of age) or BMI $\geq 25 \text{ kg/m}^2$ (if ≥ 20 years of age). Self-reported race/ethnicity of individuals was categorized as non-Hispanic white, Hispanic, non-Hispanic black, and other. The group 'others' included patients who were Asian and Pacific Islanders. The ALL risk group (standard- or high-risk) was determined by the oncologist.

Cumulative incidence was calculated for individual PEG-asparaginase-related toxicities. To examine the association of the predictors with individual PEG-asparaginase-related toxicities, multivariable logistic regression was used. To further evaluate the association of the clinical factors with risk of co-occurrence of toxicities in a multi-ethnic cohort, two approaches were used: three multivariable logistic models (no toxicities vs any one toxicity, no toxicity vs any two toxicities, and no toxicity vs any three or more toxicities) and ordinal logistic regression.

Pairwise slope analysis fulfilled the proportional odds ratio assumption, justifying the use of

ordinal logistic regression analyses. All analyses were performed on R (version 3.6.1) (R Core Team, 2014)

RESULTS

Five hundred forty-eight patients were included in the study. Individuals were followed from diagnosis of ALL to the end of the post-induction phase of therapy. The demographics and clinical characteristics of the study population are presented in Table 1.1. The mean age of ALL was 7.6 years (range: 1.1 to 22.2 years). Most patients in the study population were males (55.8%) of Hispanic ethnicity (60.4%) and treated with standard-risk treatment protocols (55.7%).

Eighty-one (14.7%) of the patients experience PEG-asparaginase-related hypersensitivity. Individuals being treated as per high-risk treatment protocols were more susceptible to PEG-asparaginase-related hypersensitivity compared to individuals treated with standard risk treatment protocols when adjusting for other clinical factors (Adjusted odds ratio [OR]: 2.25, 95% confidence interval [CI]: 1.25 – 4.05) (Table 1.2). Pancreatitis was seen in 5.3%, hyperbilirubinemia in 9.6%, and VTE in 9.7% of the patients. When adjusted for other clinical and demographic factors, older patients (≥ 10 years) were at a higher risk of pancreatitis, hyperbilirubinemia, and VTE compared to patients 1-5 years old and 5-10 years old.

In our study population, 68.9% of patients experienced no toxicity, 24.6% had any one toxicity, and 4.7% any of the two PEG-asparaginase-related toxicities (Table 1.3). There were only 9 patients (1.6%) with 3 or more co-occurring toxicities. The percentage of patients older than 10 years increased with the increase in the number of PEG-asparaginase-related toxicities.

Compared to patients between 1-5 years old, patients older than 10 years had a higher risk of

co-occurring PEG-asparaginase-related toxicities (Adjusted OR: 3.28, 95% CI: 1.97 -5.47).

Patients with higher BMI (overweight and obese) had a higher frequency of co-occurrence of toxicities compared to patients with lower BMI (underweight and normal). Other covariates including race/ethnicity, age at diagnosis and ALL risk group were not significantly associated with co-occurring PEG-asparaginase-related toxicities compared to no toxicities.

DISCUSSION

To the best of our knowledge, this is the first study to evaluate demographic and clinical factors associated with the co-occurrence of PEG-asparaginase-related toxicities in patients with newly diagnosed ALL. The incidence of PEG-asparaginase-related hypersensitivity (14.7%) lies in the range published in previous reports between 3-24% (Henriksen et al., 2015; Vrooman et al., 2010). In our study, hypersensitivity is significantly associated with high-risk treatment protocol (Adjusted OR: 2.25, 95% CI: 1.25-4.05). Browne et al. compared patients with low- vs standard/high-risk disease and reported that standard/high-risk patients had a higher risk of hypersensitivity compared to the low-risk group (53.4% vs 46.6%), although not statistically significant (Browne et al., 2017). Higher number of doses in high-/very high-risk treatment protocols could explain the higher probability of hypersensitivity (Abbott et al., 2015; Burke et al., 2018).

Hypersensitivity reaction to PEG-asparaginase is a direct indicator of the presence of antibodies against the drug and decrease in plasma asparaginase levels (Willer et al., 2011; Zalewska-Szewczyk, Gach, Wyka, Bodalski, & Młynarski, 2009). For PEG-asparaginase to be an effective anti-neoplastic agent, sufficient asparaginase plasma concentration (≥ 100 U/L) is required. The decrease in plasma asparaginase levels decreases the activity which may lead to worse event-

free survival in patients with ALL (Tong et al., 2014). Grade 3 or higher hypersensitivity reactions also necessitate a switch from PEG-asparaginase to *Erwinia* asparaginase (Raetz & Salzer, 2010; Silverman, Lewis B. et al., 2001; Vrooman et al., 2010). Due to its shorter half-life than PEG-asparaginase, *Erwinia* asparaginase is administered more frequently. Although, it does not have cross-reactivity with PEG-asparaginase, 5-33% (Silverman et al., 2001; Vrooman et al., 2010; Woo et al., 2016) experience hypersensitivity as well.

In an *in vivo* experiment, Tong et al. found that the plasma glutamine levels in patients treated with *Erwinia* asparaginase were lower compared to patients treated with PEG-asparaginase due to higher glutaminase activity of *Erwinia* asparaginase (Tong et al., 2014). Some studies state that glutaminase activity of asparaginase analogues have been linked to dysfunction of protein-rich organs such as the liver and pancreas, clinically manifested as hyperbilirubinemia and pancreatitis, respectively (Avramis & Panosyan, 2005; Narta, Kanwar, & Azmi, 2007; Parmentier et al., 2015; Reinert et al., 2006).

The incidence of PEG-asparaginase-related hyperbilirubinemia (9.7%) was higher compared to the published data (4-5%) (Avramis et al., 2002; Dinndorf, Gootenberg, Cohen, Keegan, & Pazdur, 2007; Kurre et al., 2002); whereas, the incidence of PEG-asparaginase-related pancreatitis (4.7%) in our population was consistent with that seen in previously published reports (4-5%) (Denton, Rawlins, Oberley, Bhojwani, & Orgel, 2018; Kearney et al., 2009). The risk of both hyperbilirubinemia and pancreatitis was higher in older patients (≥ 10 years) (Adjusted OR 3.83, 95% CI 1.64- 8.95 and adjusted OR 3.72, 95% CI 1.29 - 10.68) compared to patients between the age 1 to 5 years old. These results are consistent with previous studies. For example, a retrospective cohort with 262 patients treated with contemporary Children's

Oncology Group (COG) treatment regimens found a statistically significant difference in mean ages of patients with toxicity and without toxicity (hyperbilirubinemia: mean age 10.3 + 5.5 vs no hyperbilirubinemia: mean age 7.9 + 5.4 ($p = 0.002$) and pancreatitis: mean age 12.5 + 4.9 vs no pancreatitis: mean age 8.1 + 5.4 ($p = 0.001$)) (Denton et al., 2018). This relationship could partly explain the higher incidence of toxicities in adults with ALL treated with pediatric regimens (de Bont et al., 2004; Hallböök, Gustafsson, Smedmyr, Söderhäll, & Heyman, 2006; Stock et al., 2008).

In a recent study of 778 ALL patients, Klaassen et al. reported that age at diagnosis ≥ 7 years old had 2.7 times the odds of experiencing VTE when compared to patients < 7 years of age (Klaassen et al., 2019). Consistent with Klaassen et al. and other studies, (Ghanem et al., 2017; Jh & Aj, 2007; Truelove, Fielding, & Hunt, 2013) age at diagnosis was an important risk factor for PEG-asparaginase related VTE (OR 4.65, 95% CI 1.96 -11.02) in the current study.

Our study has uniquely investigated the cumulative burden of four common PEG-asparaginase-related toxicities. In our population, 9.7% of patients experienced hyperbilirubinemia, and 58.4% of these patients have more than one toxicity. Similarly, 50.9% of patients experiencing VTE have more than one toxicity. As the development of PEG-asparaginase-related toxicities may be interdependent, it is necessary to evaluate the burden of co-occurrence of the toxicities. For example, dysfunction of protein synthesis due to glutamine depletion is indicated as a mechanism of development of liver dysfunction (Avramis & Panosyan, 2005; Narta et al., 2007; Parmentier et al., 2015; Reinert et al., 2006). The liver plays an important role in the blood coagulation system, and liver dysfunction is often associated with coagulation impairment and can partly explain the development of VTE (Caldwell et al., 2006; Roberts &

Cederbaum, 1972). Notably, patients with higher BMI (overweight and obese) had an increased odds of experiencing each of the four individual PEG-asparaginase-related toxicities ($p > 0.05$). In particular, patients with higher BMI had a significantly higher risk of developing more than one toxicity (OR 1.76, 95% CI 1.20-2.57).

In conclusion, age at diagnosis is the only factor significantly associated with the development of hyperbilirubinemia, pancreatitis, VTE, and treatment with a high-risk protocol is the only factor significantly associated with hypersensitivity in a multivariable model. Further, older (>10 years) and heavier patients (overweight and obese) may be at higher risk of co-occurrence of PEG-asparaginase-related toxicities. Future research is necessary to understand the cumulative burden of toxicities to improve the guidelines and recommendations for the prevention of PEG-asparaginase-related toxicities.

Table 1.1: Clinical and demographic characteristics of patients treated on acute lymphoblastic leukemia protocols

	Study Population (N = 548) N(%)
Age at diagnosis	
1-5 years old	224 (40.9%)
5-10 years old	148 (27.0%)
≥ 10 years old	176 (32.1%)
BMI	
Underweight and Normal	284 (51.8%)
Overweight and Obese	264 (48.2%)
Race/Ethnicity	
Non-Hispanic white	142 (25.9%)
Hispanic	331 (60.4%)
Non-Hispanic black	38 (6.9%)
Others	37 (6.8%)
Gender	
Male	306 (55.8%)
Female	243 (44.3%)
ALL risk group	
Standard risk	305 (55.7%)
High risk	243(44.3%)

Table 1.2: Results of multivariable logistic regression of individual PEG-asparaginase-related toxicities

Variable	Odds ratios (95% CI)			
	(N = 548)			
	Hypersensitivity (N = 81)	Hyperbilirubinemia (N = 53)	Pancreatitis (N = 29)	VTE (N = 53)
Race/ethnicity				
Non-Hispanic White	Ref	Ref	Ref	Ref
Hispanic	1.08 (0.60 -1.94)	1.16 (0.55 -2.41)	0.5 (0.20 -1.20)	1.06 (0.52 -2.16)
NHB	1.20 (0.42 -3.37)	1.45 (0.45 -4.70)	0.92 (0.22 -3.81)	0.72 (0.18 -2.83)
Others	1.42 (0.51 -3.95)	0.71 (0.14 -3.47)	1.41 (0.34 -5.7)	0.62 (0.12-2.99)
Age at diagnosis				
1-5 years old	Ref	Ref	Ref	Ref
5-10 years old	1.15 (0.59 - 2.22)	1.43 (0.59 -3.50)	0.92 (0.26 -3.24)	1.44 (0.60 - 3.52)
≥ 10 years old	1.37 (0.72 -2.59)	3.83 (1.64 -8.95)*	3.72 (1.29 - 10.68)*	4.65 (1.96 -11.02)*
BMI categories				
Underweight or normal	Ref	Ref	Ref	Ref
Overweight or Obese	1.47 (0.91 - 2.41)	1.74 (0.96 -3.18)	2.06 (0.92 - 4.61)	1.72 (0.94 -3.15)
ALL Risk Group				
Standard risk	Ref	Ref	Ref	Ref
High-risk	2.25 (1.25 -4.05)*	1.09 (0.52 - 2.31)	1.08 (0.41 -2.82)	0.77 (0.37 -1.65)
Gender				
Male	Ref	Ref	Ref	Ref
Female	0.81 (0.50 -1.34)	0.84 (0.45 -1.55)	1.17 (0.52 - 2.60)	0.71 (0.38 -1.33)

*p< 0.05; VTE: Venous Thromboembolism

Table 1.3: Association of risk factors with co-occurrence of PEG-asparaginase-related toxicities

	No Toxicity 378 (68.9 %)	1 toxicity 135(24.6 %)	2 toxicities 26(4.7%)	≥3 toxicities 9 (1.6%)	Ordinal Logistic OR (95% CI)
Age at diagnosis					
1 -5 years old	179 (47.4%)	37 (27.4%)	7 (26.9%)	1 (11.1%)	Ref
5-10 years old	113 (29.9%)	30 (22.2%)	4 (15.4%)	1 (11.1%)	1.28 (0.77 -2.13)
≥ 10 years old	86 (22.8%)	68 (50.4%)	15 (57.7%)	7 (77.8%)	3.28 (1.97 -5.47)
BMI categories					
Underweight or Normal	213 (56.3%)	58(43.0%)	12 (46.2%)	1 (11.1%)	Ref
Overweight or Obese	165 (43.7%)	77 (57.0%)	14 (57.7%)	8 (88.9%)	1.76 (1.20 -2.57)
Race/ethnicity					
Non-Hispanic white	100 (26.5%)	35 (25.9%)	5 (19.2%)	2 (22.2%)	Ref
Hispanic	224 (59.3%)	84 (62.2%)	19 (73.1%)	4 (44.4%)	0.97 (0.62 -1.51)
Non-Hispanic black	26 (6.9%)	9 (6.7%)	2 (7.7%)	1 (11.1%)	0.94 (0.42 -2.12)
Others	28 (7.4%)	7 (5.2%)	0 (0.0%)	2 (22.2%)	0.86 (0.36 -2.04)
Gender					
Male	205 (54.2%)	79 (58.5%)	16 (61.5%)	5 (55.6%)	Ref
Female	173 (45.8%)	56 (41.5%)	10(38.5%)	4 (44.4%)	0.83 (0.56 -1.21)
ALL risk group					
Standard risk	239 (63.2%)	54 (40.0%)	9 (34.6%)	3 (33.3%)	Ref
High Risk	139 (36.8%)	81 (60.0%)	17 (65.4%)	6 (66.7%)	1.50 (0.95 - 1.36)

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JOURNAL ARTICLE 2: CLASSIFYING PEG-ASPARAGINASE-RELATED TOXICITIES IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA USING K-MODES CLUSTER ANALYSIS

INTRODUCTION

Between 2011 and 2014, the overall incidence of pediatric ALL was 3.4 per 100,000 children (Siegel et al., 2017), corresponding to 3,500 incident cases of pediatric ALL annually in the U.S. Incremental progress in chemotherapy regimens has resulted in steady improvements in treatment outcomes for children with ALL. With current treatment approaches, upwards of 90% of children diagnosed with ALL are expected to achieve long-term survival (Hunger, S. P. & Mullighan, 2015; Matasar et al., 2006; Stephen P. Hunger et al., 2012). In particular, asparaginase is an important component of curative chemotherapy regimens and studies have shown that intensive and prolonged treatment with asparaginase is associated with better response rates and treatment outcomes in pediatric ALL as compared with shorter/less intense use of PEG-asparaginase (Abshire, Pollock, Billett, Bradley, & Buchanan, 2000; Silverman et al., 2001).

Three types of asparaginase analogs are currently used to treat ALL: L-asparaginase, derived from *Escherichia coli*; a pegylated form of L-asparaginase (PEG-asparaginase); and Erwinia asparaginase, derived from *Erwinia chrysanthemi* (Asselin, 1999). In 2004, the U.S. Food and Drug Administration (FDA) approved PEG-asparaginase as a first-line drug for pediatric ALL over L-asparaginase due to its longer half-life and lower immunogenicity (Clavell, L. A. et al., 1986; Jones et al., 1977; Panetta et al., 2009; Vrooman et al., 2010). Although PEG-asparaginase has been instrumental in improving ALL outcomes, it is associated with four primary toxicities. Hypersensitivity, with an incidence of 30% (Abbott et al., 2015; Vrooman et al., 2010), is the

most common PEG-asparaginase-related toxicity, primarily due to the immune response to bacterial proteins in PEG-asparaginase. Additionally, hyperbilirubinemia and pancreatitis (Denton et al., 2018; Raja et al., 2012); (Burke et al., 2018; R Core Team, 2014; Treepongkaruna et al., 2009) each occur in approximately 4-5% of patients, and venous thromboembolism (VTE) in 10-12% (Ghanem et al., 2017; Jh & Aj, 2007; Truelove et al., 2013).

Asparaginase acts by depleting extracellular asparagine, a non-essential amino acid used in the biosynthesis of proteins, and extracellular glutamine, a non-essential amino acid and precursor for the synthesis of proteins, peptides, and amino sugars. Primarily, asparaginase hydrolyzes asparagine to aspartic acid and ammonia. Leukemic cells lack asparagine synthetase, the enzyme needed for *de novo* asparagine synthesis, resulting in a deficiency of intracellular asparagine and subsequent cell death (Avramis et al., 2002; Parmentier et al., 2015). However, asparagine may also disrupt the metabolic homeostasis of healthy organ systems, potentially resulting in toxicity to the liver, pancreas, and coagulation system (Conrich, 2019; Dinndorf, P. A. et al., 2007; Parmentier et al., 2015).

Given the common mechanism of action for these PEG-asparaginase-related adverse events, it is biological plausible that some individuals may be susceptible to multiple asparaginase-associated toxicities. However, to date, most studies have evaluated PEG-asparaginase toxicities in isolation. We hypothesized that pediatric patients who experience PEG-asparaginase-related toxicities have latent similarities to each other, with respect to the occurrence of hypersensitivity, hyperbilirubinemia, pancreatitis, and VTE. Therefore, the purpose of this study is to characterize latent classes of patients with similar toxicity profiles and compare clinical and demographic factors between these groups.

METHODS

Study Design

We conducted a single-site, retrospective cohort study of newly diagnosed ALL cases treated at Texas Children's Hospital between September 1, 2011 and December 31, 2017, who were treated on or according to one of the following Children's Oncology Group (COG) protocols: AALL1131, AALL0932 and AALL1231, AALL0434, AALL0031, AALL9404, and AALL0232. All protocols had PEG-asparaginase as a first-line drug administered on day 4 of induction and between 35 and 54 days of post-induction as per protocol (Borowitz et al., 2015; Salzer et al., 2018; Schultz et al., 2014; Winter et al., 2018; Zheng et al., 2018). The study protocol was reviewed and approved by institutional review boards at Baylor College of Medicine and The University of Texas Health Science Center at Houston (UTHealth).

Baseline characteristics including date of birth, date of diagnosis, gender, self-reported race/ethnicity, and ALL risk group were abstracted from electronic medical records. Age at diagnosis was calculated as the difference between the date of diagnosis and date of birth. For children between the ages of 2-19 years, body mass index (BMI) z-scores were calculated based on the Center for Disease Control (CDC) age- and sex-specific growth curves using height and weight at the time of diagnosis (BMI calculator for child and teen.2019). For children less than 2 years old, age-appropriate BMI z-scores were calculated from length and weight measurements (Roy et al., 2016). Height (in meters) and weight (in kilograms) at the time of diagnosis were used to calculate BMI (kg/m^2) for individuals ≥ 20 years of age. Individuals with BMI z-score < 85 (< 20 years of age) or BMI $< 25 \text{ kg}/\text{m}^2$ (≥ 20 years of age) were categorized as underweight or

normal, and individuals with BMI z-score ≥ 85 (<20 years of age) or BMI ≥ 25 kg/m² (≥ 20 years of age) were categorized as overweight or obese.

Toxicities

The patients were followed until the end of the post-induction phase to determine the incidence of the four PEG-asparaginase toxicities: hypersensitivity, hyperbilirubinemia, pancreatitis, and VTE. Toxicities were defined based on the Common Terminology Criteria for Adverse Events (CTCAE) v 5.0, with incident cases meeting grade 3+ definitions considered events in the current study (US Department of Health and Human Services, National Institutes of Health, National Cancer Institute, 2017). Briefly, grade 3+ hypersensitivity was defined as prolonged recurrence of transient flushing or rash and fever >100.4 degrees F, which were not rapidly responsive to symptomatic treatment. According to treatment protocols, grade 3+ hypersensitivity necessitates a switch from PEG-asparaginase to *Erwinia* asparaginase (Raetz & Salzer, 2010; Silverman et al., 2001; Vrooman et al., 2010). Bilirubin (conjugated/unconjugated) levels greater than 3 times the upper normal limit were defined as grade 3+ hyperbilirubinemia. Similarly, lipase levels greater than twice the upper normal limits were defined as grade 3+ pancreatitis (US Department of Health and Human Services, National Institutes of Health, National Cancer Institute, 2017). Patients were considered to have VTE when they were administered Lovenox or noted to have VTE by the healthcare professionals treating the patient.

Statistical Analysis

Age at diagnosis was categorized in 5-year intervals. Statistical group comparisons for age, BMI category, gender, race/ethnicity and ALL risk group were performed using Fisher Exact test or χ^2 test. To assess the possible latent common clinical and demographic characteristics in

patients who experience PEG-asparaginase related toxicities, we performed k-modes clustering. K-modes clustering was applied instead of hierarchical clustering using Euclidean distances because the toxicity variables were categorical. K-modes clustering is an extension of k-means clustering where modes are used instead of standard distances (Papachristou et al., 2018; Yuhua Qian, Feijiang Li, Jiye Liang, Bing Liu, & Chuangyin Dang, 2016).

The study focused on four PEG-asparaginase-related toxicities; hence the maximum possible clusters were 16. For $k = 1$, all individuals will be included in one group; therefore, we conducted cluster analyses using k of 2 to 16 clusters. K-modes clustering used a weighted algorithm with a maximum of 10^8 iterations. We used two criteria to determine optimal clusters: purity and number of clusters with fewer than five individuals. Purity is an external validation metric for the quality of the clusters, computed as the proportion of all individuals in a cluster who have the most common (i.e. mode) toxicity profile included in the given cluster) (Uddin J., Ghazali R., Deris M.M., 2016). The optimal number of clusters was selected with purity greater than 0.85 and maximum of one cluster with less than five individuals. The R package 'klaR' was used for cluster analysis (Christian Roever, Nils Raabe, Karsten Luebke, Uwe Ligges, Gero Szepannek, Marc Zentgraf, 2018; R Core Team, 2014).

The obtained clusters were then described with respect to age at diagnosis, BMI categories, gender, self-reported race/ethnicity and ALL risk group. Univariable logistic regression was used to examine the association of clinical and demographic factors with the obtained clusters. Unadjusted odds ratios were computed using separate simple logistic regression models to assess the association of each clinical and demographic variable with cluster, using cluster 1 (i.e. no toxicity) as the referent. For example, we evaluated the unadjusted odds of belonging to

cluster 2 (individuals with isolated hypersensitivity) compared to cluster 1 (no toxicities) by age at diagnosis. To further examine if significant unadjusted odds ratios (< 0.05) show similar patterns of significance when adjusting for age at diagnosis, BMI, ALL risk group, race/ethnicity, and gender, we performed multivariable logistic regression.

RESULTS

In total, 548 patients were included in the analysis. Table 2.1 presents the distribution of ethnicities of the patients: 331 Hispanics, 142 non-Hispanic whites, 38 non-Hispanic blacks and 37 patients in the mixed group of Asian, Pacific Islanders, and Native Americans. In our study population, 40.2% of patients were between 1 to 5 years old, 55.8% were males, and 55.7% were treated with low/standard-risk treatment protocols. Of the 548 patients, 49.5% were categorized as underweight or normal weight, 22.8% were overweight, and 25.4% were obese. The incidence of any hypersensitivity in our data was 14.8% over the induction and post-induction phase, that of VTE was 10.0%, hyperbilirubinemia 9.7%, and pancreatitis 5.6% (Table 2.2).

The k-modes cluster analyses evaluated 14 observed toxicity combinations because there were no individuals with hypersensitivity, pancreatitis, and hyperbilirubinemia. Figure 2.1 and Table 2.3 describe the purity and the number of clusters with fewer than 5 individuals for each cluster configuration for a given value of k. We selected the cluster configuration for $k=8$, based on its high purity (0.96) and having zero clusters with less than 5 individuals.

The distribution of toxicities for the 8 clusters is presented in Table 2.4. The clusters are distributed as follows:

- Cluster 1: individuals with no toxicities ($n = 378$)

- Cluster 2: individuals with isolated hypersensitivity (n= 61)
- Cluster 3: individuals with isolated hyperbilirubinemia (n=31)
- Cluster 4: individuals with combination of hypersensitivity and hyperbilirubinemia (n = 5)
- Cluster 5: individuals with pancreatitis with or without hyperbilirubinemia and/or hypersensitivity (n = 20)
- Cluster 6: individuals with VTE and hyperbilirubinemia with or without hypersensitivity (n= 32)
- Cluster 7: individuals with VTE with or without hypersensitivity (n = 11)
- Cluster 8: individuals with VTE and pancreatitis with or without additional toxicities. (n = 9)

The distribution of clinical and demographic factors in the 8 identified clusters is presented in Table 2.5. Cluster 1 (no toxicities) had the highest percentage of patients between the ages of 1 to 5 years (47.4% vs 40.9% overall) and lowest percentage of patients older than 10 years of age (22.8% vs 32.1% overall). Similarly, in the cluster with no toxicities, 63.2% of patients were treated with a low/standard-risk treatment protocol, while the majority of patients in clusters with toxicities were treated on high- or very high-risk treatment protocols ($p<0.001$). Compared to individuals with no toxicity, individuals in the clusters with toxicity were consistently more likely to be classified as overweight or obese at diagnosis ($p=0.03$). For example, there were 56.3% of patients who were underweight or healthy weight in the cluster with no toxicities (cluster 1), compared to only 10.1% of patients in cluster 8 (VTE and pancreatitis with or without additional toxicities).

Table 2.6 presents the results of univariable logistic regression. When compared to patients between 1-5 years old, older patients (≥ 10 years) generally show greater odds for being in a cluster with toxicities than in cluster 1 (no toxicities). Except for cluster 4 (individuals with a combination of hypersensitivity and hyperbilirubinemia), odds ratios for all other clusters were statistically significant. However, the 95% CI tended to be imprecise, given the low numbers within each cluster (e.g. cluster 8, $N = 9$). The magnitude of the unadjusted odds ratio for older patients (>10 years) is highest for cluster 7 (individuals with VTE with or without hypersensitivity; OR 8.34, 95% CI 1.7-40.0). Similar results were obtained in the multivariate analyses that included all covariates (data not presented). The results for cluster 3 (individuals with hyperbilirubinemia alone; adjusted OR 3.91, 95% CI 1.3-11.8), cluster 5 (individuals with pancreatitis with or without hyperbilirubinemia and/or hypersensitivity; adjusted OR 4.76, 95% CI 1.3-17.0), cluster 6 (individuals with VTE and hyperbilirubinemia with or without hypersensitivity; adjusted OR 5.14, 95% CI 1.7-15.9), and cluster 7 (individuals VTE with or without hypersensitivity; adjusted OR 6.44.91, 95% CI 1.0-40.3) showed similar patterns of magnitude and significance after adjusting for BMI, ALL risk group, self-reported race/ethnicity, and gender.

As compared to under/normal weight, the estimated odds ratio for patients categorized as overweight/obese was greater for those belonging to cluster 8 (individuals with VTE and pancreatitis with or without additional toxicities; OR 10.32, 95% CI 1.3 – 83.4). This association remained in multivariable logistic regression adjusting for age at diagnosis, self-reported race/ethnicity, ALL risk group and gender (adjusted OR 12.55, 95% CI 1.4 – 110.4).

Treatment with a high-/very high-risk treatment protocol was significantly associated with cluster 2 (individuals with hypersensitivity alone; OR 3.28, 95% CI 1.9-5.8), cluster 5 (individuals with pancreatitis with or without hyperbilirubinemia and/or hypersensitivity; OR 3.19, 95% CI 1.2-8.2), and cluster 7 (individuals with VTE with or without hypersensitivity; OR 4.58, 95% CI 1.2-17.6) in univariable logistic regression models; however, the results were not statistically significant in multivariable logistic regression, adjusting for clinical and demographic factors.

DISCUSSION

PEG-asparaginase is an important drug in the treatment of pediatric patients with ALL (Dinndorf, Patricia Anne et al., 2007). However, optimal use of PEG-asparaginase is sometimes limited by the incidence of toxicity, including hypersensitivity, hyperbilirubinemia, pancreatitis, and VTE (Dinndorf, Patricia Anne et al., 2007; Klaassen et al., 2019; Silverman et al., 2001; Vrooman et al., 2010). While the incidence of each of these toxicities has been independently evaluated in previous reports, in the current study we leveraged a novel method (k-modes clustering) to identify latent subgroups of patients with similar toxicity profiles.

To our knowledge, this is the first study to use the k-modes analysis approach to evaluate and describe subgroups of ALL patients with distinct PEG-asparaginase-related toxicity profiles.

Using k-modes clustering, we were able to reduce the 16 possible toxicity profiles to 8 mutually exclusive clusters based on their PEG-asparaginase toxicity profiles. Notably, 69% of patients did not experience any PEG-asparaginase toxicity (cluster 1). The individuals with toxicities typically occurred in patterns consistent with hypersensitivity alone (cluster 2), hyperbilirubinemia alone (cluster 3), or some combination of multiple toxicities (clusters 4-8).

The occurrence of PEG-asparaginase-associated toxicities appears to vary across age, treatment

risk group, and weight status. For example, 47.4% of patients with no toxicity (cluster 1) were between 1 and 5 years of age at diagnosis, compared to <33% of patients in the clusters with toxicities (clusters 2-8). These results are consistent with previous reports that found that children >10 years of age have >2-fold increased risk of PEG-asparaginase-related pancreatitis compared to children younger than 10 years (Alvarez & Zimmerman, 2000; Barry et al., 2007; Knoderer et al., 2007; Samarasinghe et al., 2013) as well as a prior report that of an increased risk of VTE among children ≥ 7 years of age as compared to children <7 years of age (Klaassen et al., 2019). Similarly, in a study of 262 patients diagnosed with ALL, age ≥ 10 years was significantly associated with hepatotoxicity and pancreatitis compared to patients < 10 years old adjusting for Hispanic ethnicity, gender, BMI, and ALL risk group (Denton et al., 2018). Additionally, overweight and obesity were consistently associated with higher odds of toxicities in the current study, with the strongest association observed with cluster 8 (VTE and pancreatitis with or without other toxicities). Denton et al. previously reported that higher BMI (overweight/obese) was significantly associated with hepatotoxicity and pancreatitis during ALL treatment (Denton et al., 2018). Finally, receiving high-risk or very high-risk therapy was associated with an increased likelihood of toxicity, particularly among clusters with individuals experiencing PEG-asparaginase-related hypersensitivity (Table 2.6). A study by Browne et al. found that standard-/high-risk group patients had a higher risk of hypersensitivity compared to the low-risk group (53.4% vs 46.6%), although this difference was not statistically significant (Browne et al., 2017). The increased risk of toxicity for individuals treated on high- and very high-risk protocols likely reflects the greater number of doses of PEG-asparaginase these patients receive compared to low-/standard-risk treatment.

One of the strengths of the study is that the k-modes approach statistically defines clusters using frequency-based distance metrics (i.e., groups with high frequency were clustered together). For example, a group of 378 patients with no toxicities was included in one cluster. The varying frequencies of the four PEG-asparaginase-related toxicities determined the size and constitution of the clusters. This method is useful when identifying latent groups for preliminary analysis and can be utilized for multivariable modeling of the factors related to these clusters. Although we used a novel methodology (k-modes clustering) to identify latent subgroups of patients, there are some shortcomings in our approach. First, the selection of the optimal number of clusters is subjective and, hence, difficult to determine standardized criteria. Second, some groups of clusters can be challenging to interpret. For example, for k=3 all three clusters had at least one individual who experienced toxicity, making it challenging to identify a reference cluster (results not shown). The k-modes cluster analysis used in this study uncovered latent subgroups of patients with similar toxicity experiences, providing new insight into distinct toxicity profiles. For example, individuals with hypersensitivity and hyperbilirubinemia may occur individually or in combination with any of the other toxicities. However, VTE and pancreatitis seem to occur in the context of other toxicities rather than individually. Further, we also found that older patients (≥ 10 years) and patients with high BMI (overweight/obese) were more likely to experience co-occurring PEG-asparaginase-related toxicities. If obesity is a risk factor for toxicity, clinically important preventive measures to manage BMI can be developed to help mitigate the burden of PEG-asparaginase-related toxicities. On the other hand, isolated hypersensitivity (cluster 2) represented 75% of all patients who experienced hypersensitivity (alone or in combination with any other toxicities). Developing guidelines for anaphylaxis

preventive measures can decrease the number of patients experiencing hypersensitivity and prevent treatment modification.

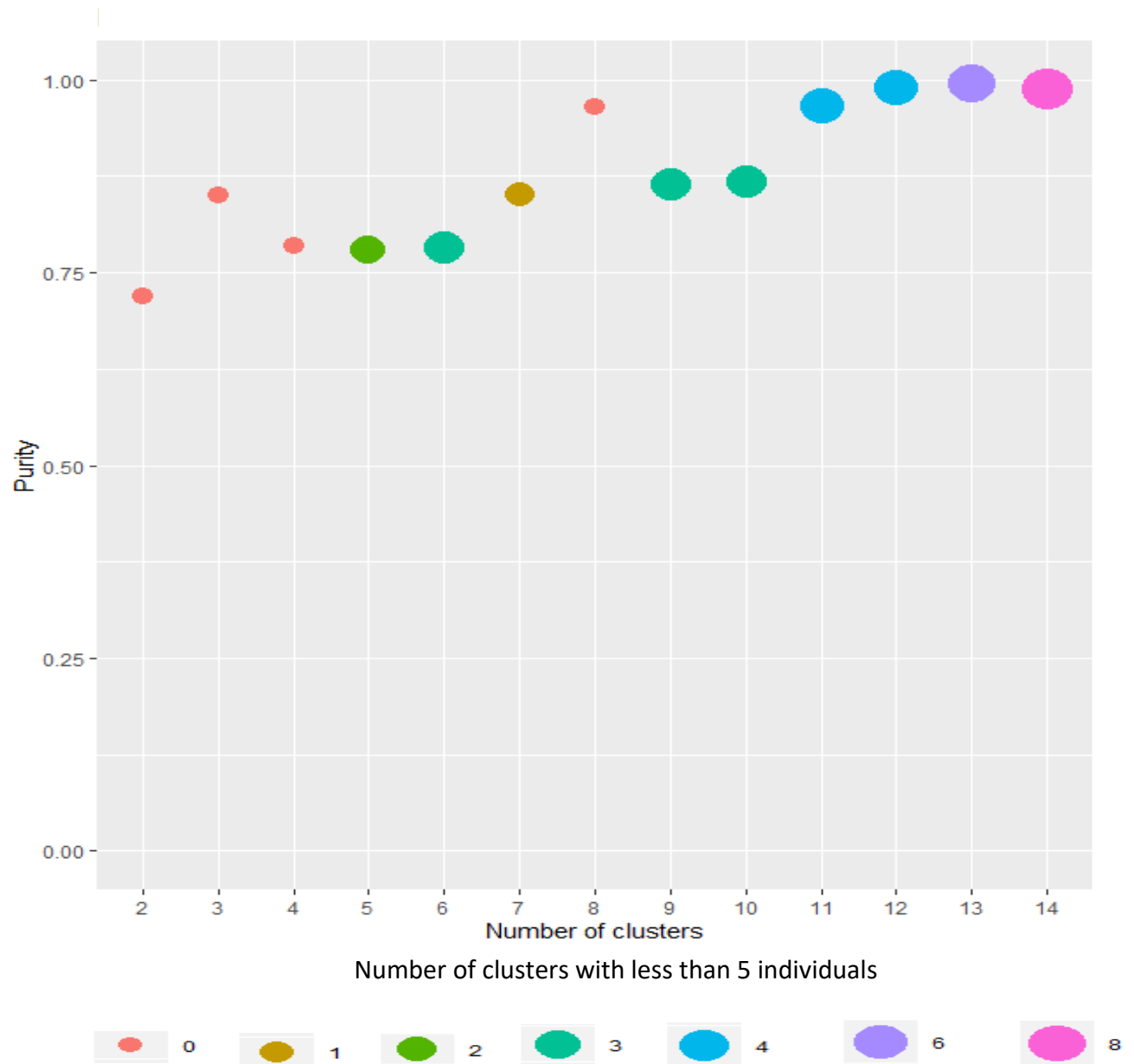
Table 2.1: Distribution of clinical and demographic factors in the study population

	Overall (N=548)
Race/Ethnicity	
Hispanic	331 (60.4%)
Non-Hispanic black	38 (6.9%)
Non-Hispanic white	142 (25.9%)
Others	37 (6.8%)
Age categories	
1-5 years old	224 (40.9%)
5-10 years old	148 (27.0%)
≥ 10 years old	176 (32.1%)
Gender	
Female	242 (44.2%)
Male	306 (55.8%)
BMI categories	
Underweight/Healthy	284 (51.8%)
Overweight/Obese	264 (48.2%) (22.8%)
ALL Risk Group	
Low/Standard-risk	305 (55.7%)
High-risk/Very high-risk	243 (44.3%)

Table 2.2: Cumulative Incidence of PEG-asparaginase-related toxicities in patients with ALL

	Hypersensitivity	Hyperbilirubinemia	Pancreatitis	VTE
Yes	81 (14.78%)	53 (9.67%)	29 (5.58%)	52 (9.49%)
No	467 (85.22%)	495 (90.33%)	519 (94.42%)	496 (90.51%)

Figure 2.1: Plot of purity and number of clusters with fewer than 5 individuals



Color and the size indicate the number of clusters with less than 5 individuals. The x-axis is k (modes from 2-14) and the y-axis is the purity for k.

Table 2.3: Purity of clusters and number of clusters with less than 5 individuals

Number of clusters	The purity of the clusters	Number of clusters with less than 5 individuals
2	0.719	0
3	0.850	0
4	0.785	0
5	0.779	2
6	0.783	3
7	0.852	1
8	0.964	0
9	0.865	3
10	0.869	3
11	0.967	4
12	0.991	4
13	0.995	6
14	0.989	8

Table 2.4: Cluster modes and distribution of PEG-asparaginase related toxicities

HS	HB	PT	VTE	Modes	Cluster number and name							
					1	2	3	4	5	6	7	8
					No toxicities ¹	Only HS ¹	Only HB ¹	HB+HS ¹	PT +/- HB / HS ¹	VTE + HB +/- HS ¹	VTE +/- HS ¹	VTE+PT +/- HB/HS ¹
-	-	-	-	1	378	-	-	-	-	-	-	-
1	-	-	-	2	-	61	-	-	-	-	-	-
-	1	-	-	3	-	-	31	-	-	-	-	-
-	-	1	-	4	-	-	-	-	16	-	-	-
-	-	-	1	5	-	-	-	-	-	-	27	-
1	1	-	-	6	-	-	-	5	-	-	-	-
1	-	1	-	7	-	-	-	-	2	-	-	-
1	-	-	1	8	-	-	-	-	-	-	5	-
-	1	1	-	9	-	-	-	-	2	-	-	-
-	1	-	1	10	-	-	-	-	-	9	-	-
-	-	1	1	11	-	-	-	-	-	-	-	2
1	1	1	-	12	-	-	-	-	-	-	-	-
1	1	-	1	13	-	-	-	-	-	2	-	-
1	-	1	1	14	-	-	-	-	-	-	-	4
-	1	1	1	15	-	-	-	-	-	-	-	2
1	1	1	1	16	-	-	-	-	-	-	-	1

¹Cluster 1 comprised of individuals with no toxicities (n = 378); cluster 2 consisted of individuals with hypersensitivity; cluster 3 consisted of individuals with hyperbilirubinemia alone; cluster 4 consisted of individuals with combination of hypersensitivity and hyperbilirubinemia; cluster 5 consisted of individuals with pancreatitis with or without hyperbilirubinemia and/or hypersensitivity; cluster 6 consisted of individuals with VTE and hyperbilirubinemia with or without hypersensitivity; cluster 7 consisted of individuals with VTE with or without hypersensitivity, and cluster 8 consisted of individuals with VTE and pancreatitis with or without additional toxicities.

HS: Hypersensitivity; HB: Hyperbilirubinemia; PT: Pancreatitis; VTE: Venous Thromboembolism

Table 2.5: Distribution of age at diagnosis, risk of ALL, race/ethnicity, gender and BMI by cluster

	Cluster number and name								Total (N=548)	p value*
	1	2	3	4	5	6	7	8		
	No toxicities ¹	Only HS ¹	Only HB ¹	HB+HS ¹	PT +/- HB /HS ¹	VTE + HB +/- HS ¹	VTE +/- HS ¹	VTE + PT +/- HB/HS ¹		
	(N=378)	(N=61)	(N=31)	(N=5)	(N=20)	(N=32)	(N=11)	(N=9)		
	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)	
Race/Ethnicity										0.808
Non-Hispanic white	100 (26.5%)	15 (24.6%)	6 (19.4%)	1 (20.0%)	8 (40.0%)	7 (21.9%)	4 (36.4%)	1 (11.1%)	142 (25.9%)	
Hispanic	224 (59.3%)	37 (60.7%)	21 (67.7%)	4 (80.0%)	9 (45.0%)	24 (75.0%)	6 (54.5%)	5 (55.6%)	331 (60.4%)	
Non-Hispanic black	26 (6.9%)	5 (8.2%)	2 (6.5%)	0 (0.0%)	2 (10.0%)	1 (3.1%)	1 (9.1%)	1 (11.1%)	38 (6.9%)	
Others	28 (7.4%)	4 (6.6%)	2 (6.5%)	0 (0.0%)	1 (5.0%)	0 (0.0%)	0 (0.0%)	2 (22.2%)	37 (6.8%)	
Gender										0.914
Male	205 (54.2%)	35 (57.4%)	19 (61.3%)	3 (60.0%)	10 (50.0%)	20 (62.5%)	7 (63.6%)	6 (66.7%)	306 (55.8%)	
Female	173 (45.8%)	26 (42.6%)	12 (38.7%)	2 (30.0%)	10 (50.0%)	12 (37.5%)	4 (36.4%)	3 (33.3%)	242 (44.2%)	
Age at diagnosis										< 0.001
1 -5 years old	179 (47.4%)	20 (32.8%)	8 (25.8%)	1 (20.0%)	5 (25.0%)	7 (21.9%)	2 (18.2%)	2 (22.2%)	224 (40.9%)	
5-10 years old	113 (29.9%)	14 (23.0%)	7 (22.6%)	1 (20.0%)	3 (15.0%)	8 (25.0%)	1 (9.1%)	1 (11.1%)	148 (27.0%)	
≥ 10 years old	86 (22.8%)	27 (44.3%)	16 (51.6%)	3 (60.0%)	12 (60.0%)	17 (53.1%)	8 (72.7%)	6 (66.7%)	176 (32.1%)	
ALL risk group										< 0.001
Low-/Standard-risk	239 (63.2%)	21 (34.4%)	14 (45.2%)	2 (30.0%)	7 (35.0%)	15 (46.9%)	3 (27.3%)	4 (44.4%)	305 (55.7%)	
High-/Very high-risk	139 (36.8%)	40 (65.6%)	17 (54.8%)	3 (60.0%)	13 (65.0%)	17 (53.1%)	8 (72.7%)	5 (55.6%)	243 (44.3%)	
BMI										0.030
Underweight/healthy	213 (56.3%)	28 (45.9%)	13 (41.9%)	1 (20.0%)	9 (45.0%)	14 (43.8%)	5 (45.5%)	1 (11.1%)	284 (51.8%)	
Overweight/obese	165 (43.7%)	33 (54.1%)	18 (58.1%)	4 (80.0%)	11 (55.0%)	18 (56.2%)	6 (54.5%)	8 (88.9%)	264 (48.2%)	

*Using chi-square test to compare the differences in the distribution of clinical and demographic factors by the identified clusters

¹Cluster 1 comprised of individuals with no toxicities (n = 378); cluster 2 consisted of individuals with hypersensitivity; cluster 3 consisted of individuals with hyperbilirubinemia alone; cluster 4 consisted of individuals with combination of hypersensitivity and hyperbilirubinemia; cluster 5 consisted of individuals with pancreatitis with or without hyperbilirubinemia and/or hypersensitivity; cluster 6 consisted of individuals with VTE and hyperbilirubinemia with or without hypersensitivity; cluster 7 consisted of individuals with VTE with or without hypersensitivity, and cluster 8 consisted of individuals with VTE and pancreatitis with or without additional toxicities.

HS: Hypersensitivity; HB: Hyperbilirubinemia; PT: Pancreatitis; VTE: Venous Thromboembolism

Table 2.6: Results of univariable logistic regression for 8 identified clusters

	Cluster 2		Cluster 3		Cluster 4		Cluster 5		Cluster 6		Cluster 7		Cluster 8	
	ONLY HS (N=61) ¹		Only HB (N=31) ¹		HS + HB (N= 5) ¹		PT+HS/ HB (N=21) ¹		VTE +/- HS (N =32) ¹		VTE+HB +/- HS (N=11) ¹		VTE + PT + ≥3 toxicities (N=9) ¹	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Age at diagnosis														
1-5 years old	Ref		Ref		Ref		Ref		Ref		Ref		Ref	
5-10 years old	1.11	0.54 - 2.28	1.38	0.49-3.93	1.58	0.10-25.57	0.95	0.22-4.05	1.81	0.63-5.12	0.79	0.07-8.84	0.79	0.07-8.83
≥ 10 years old	2.81	1.49 - 5.29	4.16	1.75 -10.11	8.32	0.91-75.61	4.99	1.70-14.62	5.05	2.02-12.64	8.34	1.73-40.04	6.24	1.23-31.57
BMI														
Underweight/Normal	Ref		Ref		Ref		Ref		Ref		Ref		Ref	
Overweight/Obese	1.52	0.88 - 2.62	1.79	0.85 -3.75	6.64	0.75-55.78	1.58	0.64-3.89	1.65	0.80-3.44	1.55	0.46-5.16	10.32	1.27-83.39
ALL risk group														
Low-/Standard-risk	Ref		Ref		Ref		Ref		Ref		Ref		Ref	
High-/Very high-risk	3.28	1.9 - 5.8	2.08	0.99-4.36	3.4	0.62-19.01	3.19	1.24-8.19	1.94	0.94-4.02	4.58	1.19-17.56	2.14	0.56-8.13
Race/Ethnicity														
Non-Hispanic white	Ref		Ref		Ref		Ref		Ref		Ref		Ref	
Hispanic	1.10	0.58 -2.10	1.56	0.61-3.99	2.23	0.25-19.35	0.50	0.18-1.33	1.53	0.64-3.66	0.67	0.18-2.42	2.23	0.35-19.35
Non-Hispanic black	1.28	0.43 -3.85	1.28	0.24-6.72	NA	NA	0.96	0.19-4.80	0.54	0.06-4.66	0.96	0.10-8.97	3.84	0.23-63.57
Others	0.95	0.29 - 3.10	1.19	0.22-6.22	NA	NA	0.44	0.05-3.72	NA	NA	NA	NA	7.14	0.62-81.68
Gender														
Male	Ref		Ref		Ref		Ref		Ref		Ref		Ref	
Female	0.88	0.51 - 1.52	0.75	0.35-1.59	0.6	0.11-3.27	1.18	0.48-2.91	0.71	0.33-1.49	0.67	0.19-2.35	0.59	0.14-2.4

¹Cluster 1 comprised of individuals with no toxicities (n = 378); cluster 2 consisted of individuals with hypersensitivity; cluster 3 consisted of individuals with hyperbilirubinemia alone; cluster 4 consisted of individuals with combination of hypersensitivity and hyperbilirubinemia; cluster 5 consisted of individuals with pancreatitis with or without hyperbilirubinemia and/or hypersensitivity; cluster 6 consisted of individuals with VTE and hyperbilirubinemia with or without hypersensitivity; cluster 7 consisted of individuals with VTE with or without hypersensitivity, and cluster 8 consisted of individuals with VTE and pancreatitis with or without additional toxicities.

HS: Hypersensitivity; HB: Hyperbilirubinemia; PT: Pancreatitis; VTE: Venous Thromboembolism

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JOURNAL ARTICLE 3: ROLE OF NATIVE AMERICAN ANCESTRY IN INCIDENCE OF PEG-ASPARAGINASE-RELATED TOXICITIES IN ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

INTRODUCTION

Polyethylene glycol-asparaginase (PEG-asparaginase) is a first-line drug for the treatment of acute lymphoblastic leukemia (ALL) in pediatric and young adult patients (Dinndorf, P. A. et al., 2007). Uninterrupted and prolonged treatment with PEG-asparaginase has been linked to better event-free survival rates (Abshire et al., 2000); however, PEG-asparaginase is associated with four common adverse events: hypersensitivity, hyperbilirubinemia, pancreatitis, venous thromboembolism (VTE) (Dinndorf, P. A. et al., 2007). The incidence of PEG-asparaginase-related toxicities often requires delays and interruption in treatment, which can result in lower survival (Amylon et al., 1999; Clavell, Luis A. et al., 1986).

While older age at diagnosis, elevated body mass index (BMI), and treatment intensity have been linked to the occurrence of PEG-asparaginase-related toxicities, studies have been conducted in predominately non-Hispanic white populations, leaving potential racial and ethnic differences in toxicity largely unexplored (Barry et al., 2007; Denton et al., 2018; Klaassen et al., 2019; Samarasinghe et al., 2013). Notably, compared to non-Hispanic whites, Hispanic patients treated on pediatric ALL protocols are at increased risk of methotrexate neurotoxicity (Taylor et al., 2018). Similarly, Moriyama and colleagues identified genetic variants in the *NUDT15* gene that influence thiopurine-associated myelosuppression in pediatric ALL patients (Moriyama et al., 2017). The frequency of these

risk variants is highest in individuals of Hispanic or Asian descent, suggesting susceptibility to specific drug toxicities varies across ancestry (Moriyama et al., 2017).

Hispanics are a genetically diverse population, with considerable genetic admixture often consisting of varying proportions of Native American, European, and African genetic ancestry (Gravel et al., 2013; Rodriguez et al., 2014). However, much of the published work evaluating disparities in treatment outcomes among Hispanic children with ALL have relied on self-reported ethnicity (Abrahao et al., 2015; Dores et al., 2012; Hunger, S. P. & Mullighan, 2015; Siegel et al., 2017; Wang, Bhatia, Gomez, & Yasui, 2015). Adopting a different approach, Yang *et al.* found that a higher proportion of Native American genetic ancestry was associated with an increased risk of relapse among both Hispanic and non-Hispanic patients (Yang et al., 2011). Given that the established risk factors for PEG-asparaginase-related toxicities do not fully explain the variation observed in the incidence, evaluating the role of genetic ancestry in the development of these toxicities may provide new insight into potential biologic sources of variability. The purpose of the current study was to evaluate the association of genetic ancestry and PEG-asparaginase-related toxicities in an ethnically diverse retrospective cohort of pediatric ALL patients.

METHODS

Patients included in this analysis were diagnosed with ALL at Texas Children's Hospital between September 1, 2011, and December 31, 2017. Eligible patients were between 1 and 22 years of age at diagnosis and treated on or according to Children's Oncology Group

(COG) clinical trial protocols (AALL0932, AALL1231, AALL1131, AALL0434, AALL1122, and AALL0031). The study was reviewed and approved by institutional review boards at Baylor College of Medicine and The University of Texas Health Science Center at Houston (UTHealth).

The primary outcomes for this analysis included four common PEG-asparaginase toxicities: hypersensitivity, hyperbilirubinemia, pancreatitis, and venous thromboembolism. When possible, the incidence of toxicities was defined as events meeting the Common Terminology Criteria for Adverse Events (CTCAE v5.0) grade ≥ 3 toxicity. For example, hyperbilirubinemia was defined as levels of bilirubin exceeding five times the upper normal limit. Events met the definition of pancreatitis when lipase exceed three times the upper normal limit. Hypersensitivity was diagnosed as allergic reactions requiring a switch from PEG-asparaginase to *Erwinia* asparaginase, while cases of VTE were either a physician-led diagnosis or indication for the administration of Lovenox or low molecular weight heparin. The clinical and demographic factors were abstracted from electronic medical records. Factors evaluated in the analysis included age at diagnosis, body mass index (BMI), gender, and ALL risk group (low-/standard-risk or high-/very high-risk). The BMI z-scores for patients 2-18 years of age were calculated according to the Centers for Disease Control and Prevention (CDC) recommendations (BMI calculator for child and teen.2019). For patients younger than 2 years of age, the length of the child replaced height to calculate BMI as per CDC guidelines. The BMI for young adults (≥ 20 years) was calculated using the adult BMI

formula (weight (kg) / [height (m)]²). Individuals with a sex- and age-specific BMI percentile <85 (<20 years) or BMI <25 kg/m² (≥20 years) were categorized as a underweight/normal weight, while individuals with percentiles ≥85(<20 years) or BMI ≥25 kg/m² (≥ 20 years) were categorized as overweight or obese.

DNA was extracted from peripheral blood samples, which were obtained at first remission. Samples were genotyped using the Illumina OmniExpress or Global Screening array. After merging genotyped data from our study sample with the genotype data from 1000 Genome Phase 3 samples consisting of individuals of known European, African, East Asian, and Native American ancestry as reference populations (Abecasis et al., 2010), we retained genome-wide coverage of 1,083,368 single nucleotide polymorphisms (SNPs) across all platforms. The genotyping quality was assessed at the individual (i.e., sample) and the marker (i.e., SNP) level. Individual-level quality control consisted of excluding samples with a high proportion of missing calls (sample call rate <95%) and removal of genetic duplicates. Marker level quality control included removing SNPs with low call rates (SNP call rate <95%), minor allele frequency <1%, and those exhibiting significant deviations from Hardy-Weinberg equilibrium ($< 10^{-5}$) (Anderson et al., 2010; Laurie et al., 2010) (Figure 3.1). After the quality control, 98,369 autosomal SNPs passed filtering, common across all genotyping platforms, were available for potential inclusion in the ancestry analysis. For the estimation of genetic ancestry, 1000 Genome Phase 3 samples consisting of individuals of known European, African, East Asian, and Native American ancestry were included as

reference populations (Abecasis et al., 2010). After merging the genotyped data from the study and reference populations, we filtered the variants to previously identified ancestry informative markers and performed linkage disequilibrium (LD) pruning (R^2 of 0.5) to arrive at a subset of 941 markers for genetic ancestry estimation using STRUCTURE software (V 2.3.4) (Hubisz, Falush, Stephens, & Pritchard, 2009). The length of the burn-in period and the number of simulations were set to 10,000 as recommended by the developers.

Statistical analysis

Descriptive statistics were calculated for clinical and demographic variables (i.e., age at diagnosis, gender, BMI z-score, and treatment risk group). The association between each PEG-asparaginase-related toxicity and Native American genetic ancestry was evaluated in multivariable logistic regression models adjusting for relevant clinical and demographic variables. Similarly, the association with multiple PEG-asparaginase-related toxicities (i.e., no toxicity, any 1 toxicity, any 2 toxicities, or 3 or more toxicities) was evaluated using ordinal logistic regression. Pairwise slope analysis validated the proportional odds assumption for the ordinal logistic regression model. All analyses were performed using the MASS package in R (version 3.6.1) at a two-sided significance level of 0.05 (Brian Ripley et al., 2002; Klaassen et al., 2019).

RESULTS

A total of 170 patients with toxicity data were enrolled in the parent study and provided DNA samples for genetic analyses (Table 3.1). Most participants included in this analysis

were diagnosed with ALL <10 years of age (63.5%), underweight or normal weight at diagnosis (55.9%), male (55.9%), and treated with low- or standard-risk therapy (52.9%).

The mean proportion of Native American genetic ancestry did not differ significantly across age group, BMI category, gender, or treatment risk group (Figure 3.2). On average, self-reported non-Hispanic whites were largely of European genetic ancestry, self-reported non-Hispanic blacks were predominantly of African genetic ancestry, and Hispanics had the highest proportions of Native American genetic ancestry. Although the mean proportion of Native American genetic ancestry among self-reported Hispanic participants was approximately 73%, across individual, self-reported Hispanic patients the estimated proportion of Native American genetic ancestry ranged from 0.3% to 99.6% (Table 3.2).

Overall, 37 (21.7%) children experienced hypersensitivity, 16 (9.4%) experienced hyperbilirubinemia, 10 (5.9%) experienced pancreatitis, and 11 (6.5%) experienced venous thromboembolism (Table 3.3). The mean proportions of Native American genetic ancestry by PEG-asparaginase-related toxicities are depicted in Figure 3.3. The proportions of Native American genetic ancestry were somewhat higher in individuals who experienced hypersensitivity (63.3% vs 61.6%; p-value=0.37) and hyperbilirubinemia (68.1% vs 61.0%, p-value=0.41) compared to those who do not, although neither difference was statistically significant (i.e. $p>0.05$). The proportion of Native American genetic ancestry was similar among patients who did and those who did not experience pancreatitis (61.1% vs 60.8%; p-value=0.97) or venous thromboembolism (61.6% vs 63.63%; p-value=0.84).

Although in multivariable logistic models (Table 3.3), the proportion of Native American genetic ancestry was not significantly associated with any of the four toxicities evaluated after accounting for clinical factors, we found that clinical factors – age at diagnosis, BMI category, and ALL risk group – were significantly ($p < 0.05$) associated with PEG-asparaginase-related toxicities. Children treated on high-risk treatment protocols had increased odds of hypersensitivity (adjusted odds ratio [OR]=5.23, 95% confidence interval [CI]: 1.91-14.29) but lower odds of venous thromboembolism (adjusted OR=0.11, 95% CI: 0.01-0.80) compared to those treated with a standard-risk treatment protocol. The odds of venous thromboembolism and hyperbilirubinemia were increased among individuals who were overweight or obese at diagnosis (OR=19.53; 95% CI: 2.12-179.24 and OR=3.07; 95% CI: 1.01-9.39, respectively). Age ≥ 10 years at diagnosis was also associated with an increased odds of venous thromboembolism (OR=10.81; 95% CI: 1.40-83.17).

A total of 59 (34.7%) participants experienced at least one toxicity, including 11 (6.5%) who experienced two or more toxicities (Table 3.4). Proportions of individual genetic ancestry estimates with respect to the cumulative frequency of PEG-asparaginase-related toxicities are represented in Figure 3.4. Notably, the proportion of estimated Native American ancestry increased with an increasing number of toxicities. However, the results of ordinal logistic regression (Table 3.4) suggests that this finding is not statistically significant (adjusted OR 1.32, 95% CI 0.56-3.11). On the other hand, older (≥ 10 years old) (OR 2.72,

95% CI 1.23 -6.03) and heavier (overweight or obese) children (OR 19.53, 95% CI 2.12 – 179.24) were at higher risk of co-occurrence of PEG-asparaginase-related toxicities.

DISCUSSION

PEG-asparaginase-related toxicity is a clinical challenge in the treatment of pediatric ALL, often resulting in treatment interruptions or delays. In this study, we aimed to examine the association of Native American genetic ancestry and clinical factors with individual and co-occurrence of PEG-asparaginase-related toxicities. To our knowledge, this is the first study to evaluate the association of Native American genetic ancestry with the cumulative burden of PEG-asparaginase-related toxicities. Although we did not identify a statistically significant association between the proportion of Native American genetic ancestry and the cumulative frequency of PEG-asparaginase-related toxicities during pediatric ALL treatment, the estimated proportion of Native American ancestry with an increased toxicity burden. In our study population, 34.7% of children developed at least one PEG-asparaginase-related toxicity, and 6.4% (11/170) of children had multiple toxicities. The risk of co-occurrence of toxicities was higher in older (≥ 10 years old) and heavier (BMI z-score at diagnosis ≥ 85 percentile) compared to younger (< 10 years) and underweight or normal (BMI z-score at diagnosis < 85 percentile) children. We also noted that the proportion of Native American genetic ancestry gradually increased with the increasing burden of toxicities (Figure 3.3). Although, not a statistically significant result, we believe this is a notable and novel finding that is worthy of further evaluation in future studies. Similarly, in a cohort of 2,534 children,

Yang *et al.* found that the cumulative incidence of relapse was significantly associated with higher Native American ancestry (Yang et al., 2011). Correlation of higher Native American ancestry with a higher number of PEG-asparaginase-related toxicities may partly explain the association between Native American genetic ancestry and relapse, given that toxicities often result in treatment modification or treatment delays, which increase the chances of relapse.

There are some limitations inherent in our study. The sample size for the PEG-asparaginase-related toxicities is limited, particularly the group with 3 or more toxicities. Thus, the current study was likely underpowered to adequately evaluate the role of genetic ancestry in PEG-asparaginase toxicity. Although this was among the first multi-ethnic cohorts used to study the cumulative burden of the PEG-asparaginase-related toxicities, our population predominantly had European and Native American genetic ancestry. A larger population may be needed to replicate our findings, while a more diverse cohort may be necessary to generalize this work to the broader pediatric ALL population. We believe that increased genetic research among minority populations is needed to examine critical questions among populations that appear to be at risk of adverse outcomes.

In summary, although this study did not show a significant association of Native American genetic ancestry with PEG-asparaginase-related toxicity, we did observe increased proportions of Native American genetic ancestry with an increasing PEG-asparaginase-related toxicity burden. This finding, if replicated in future larger studies, may lead to the

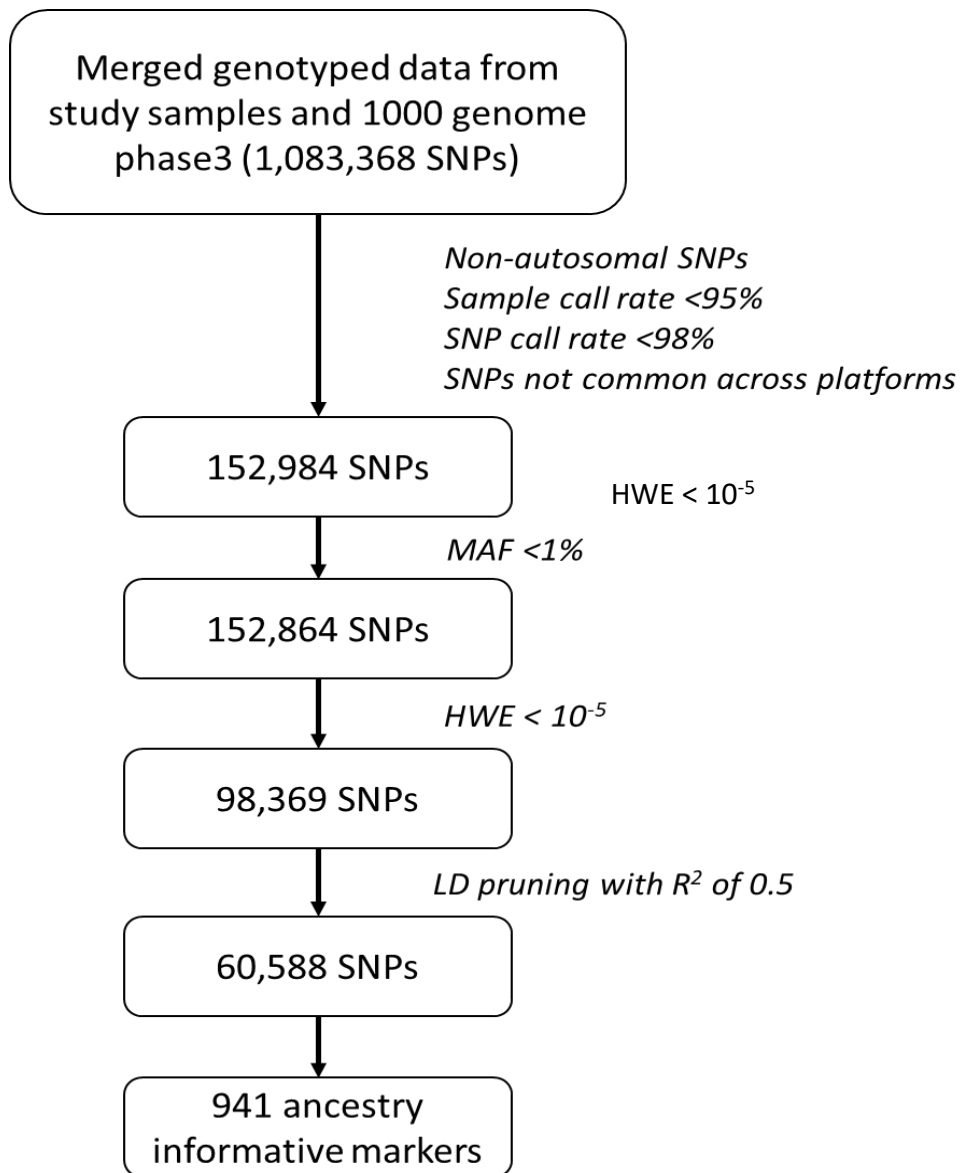
development of guidelines for PEG-asparaginase-related toxicity surveillance or prophylaxis, particularly among patients with very high proportions of Native American genetic ancestry. While the highest proportion of Native American genetic ancestry is seen among Hispanic patients; Native American ancestry in our non-Hispanic white population ranged from 0.2% to 39.7%. Given the evidence that the Native American genetic ancestry is associated with a higher risk of relapse in the non-Hispanic population as well (Yang et al., 2011), it is imperative to examine the association of Native American genetic ancestry on the development of toxicities in this group. For a comprehensive understanding of the racial/ethnic disparities in the development of PEG-asparaginase-related toxicities, we feel that it is necessary to evaluate the role of genetic ancestry in addition to self-reported race and ethnicity.

Table 3.1: Distribution of clinical and demographic factors by Native American ancestry estimates

		Genetic Ancestry	Self-reported ethnicity				
		Native American estimate	Hispanic	Non-Hispanic black	Non-Hispanic white	Others	p-value*
	N(%)	Mean (SD)	N(%)	N(%)	N(%)	N(%)	
Age of diagnosis							
Age <10 years old	108 (63.5%)	0.43 (0.39)	58 (57.4%)	7 (53.8%)	39 (76.5%)	4 (80.0%)	0.32
Age ≥ 10 years old	62 (36.5%)	0.50 (0.39)	43 (42.6%)	6 (46.2%)	12 (23.5%)	1 (20.0%)	
BMI							
Underweight or Normal	95(55.9%)	0.44 (0.40)	56 (55.4%)	5 (38.5%)	30 (58.8%)	4 (80.0%)	0.68
Overweight or Obese	75 (44.1%)	0.47 (0.39)	45 (44.6%)	8 (61.5%)	21 (41.2%)	1 (20.0%)	
Gender							0.14
Male	95 (55.9%)	0.50(0.37)	58 (57.4%)	6 (46.2%)	28 (54.9%)	3 (60.0%)	
Female	75 (44.1%)	0.40(0.39)	43 (42.6%)	7 (53.8%)	23 (45.1%)	2 (40.0%)	
ALL risk group							0.31
Low/Standard Risk	90 (52.9%)	0.43 (0.40)	49 (48.5%)	6 (46.2%)	32 (62.7%)	3 (60.0%)	
High/Very high risk	80 (47.1%)	0.50 (0.38)	52 (51.5%)	7 (53.8%)	19 (37.3%)	2 (40.0%)	

*p-value: calculated for the difference in the clinical and demographic factors with respect to Native American genetic ancestry

Figure 3.1: Genotyping quality check



SNPs: Single nucleotide polymorphisms

Figure 3.2: Individual genetic ancestry estimates (n=170)

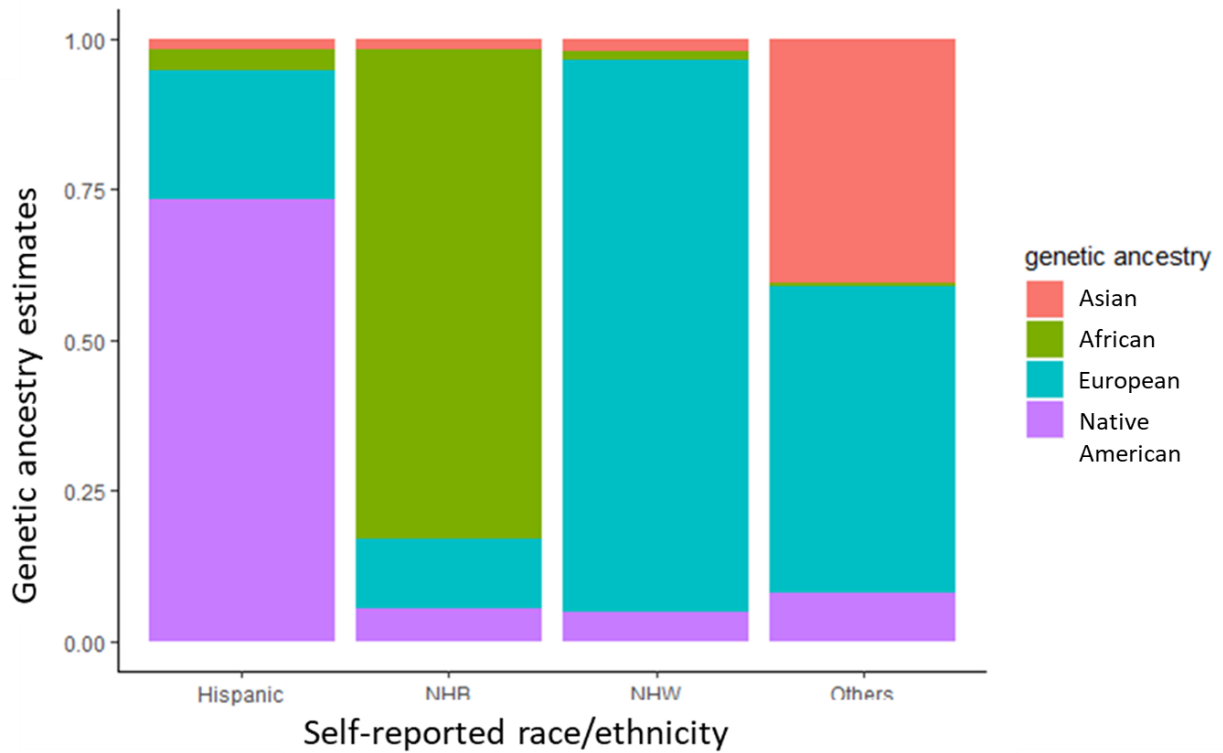
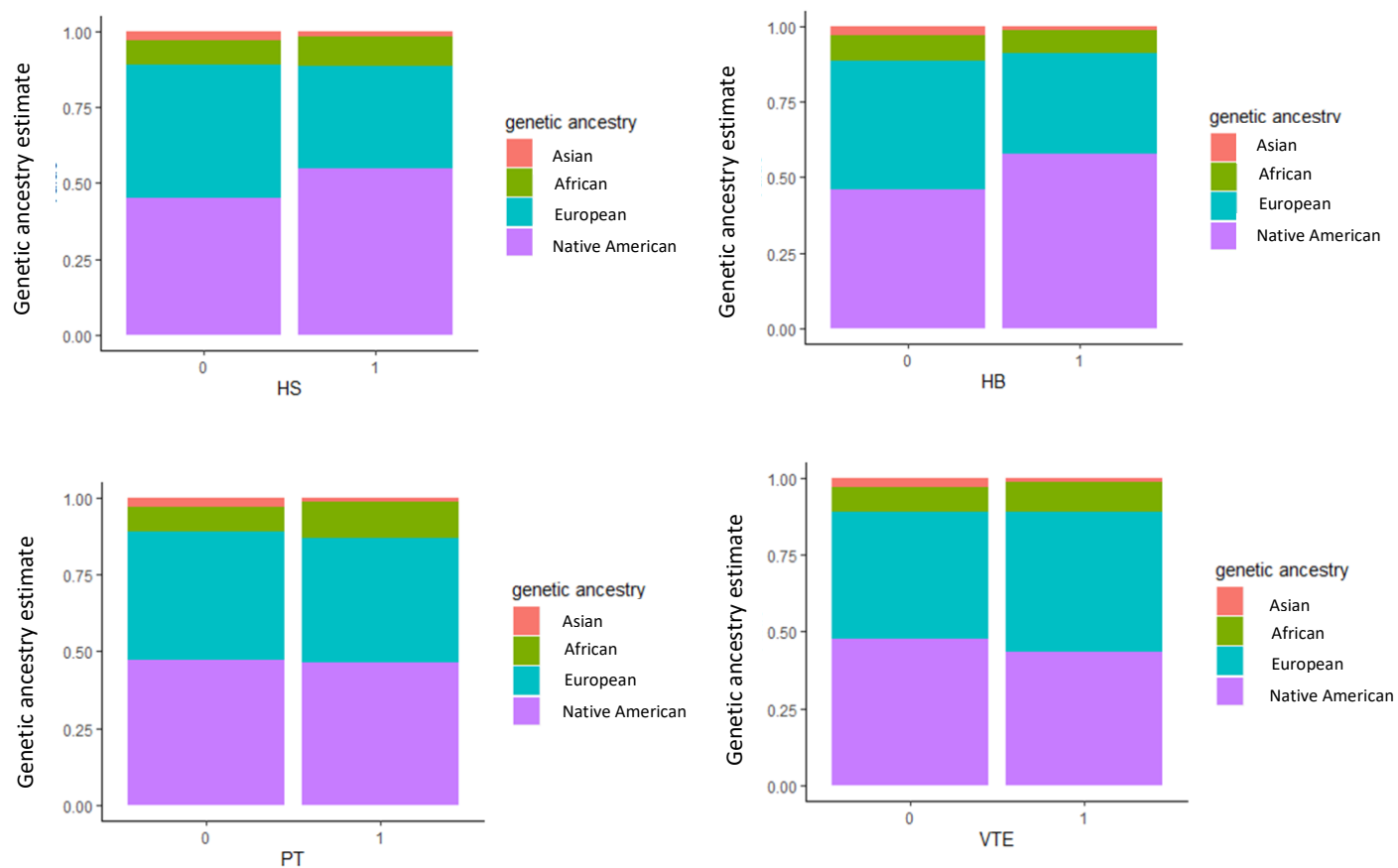


Table 3.2: Native America ancestry proportions and self-reported race/ethnicity

Self-reported race/ethnicity	Native American ancestry proportion Mean (Range)
Non-Hispanic white	0.047 (0.002 - 0.397)
Hispanic	0.733 (0.003 - 0.996)
Non-Hispanic black	0.054 (0.002 - 0.485)
Others	0.080 (0.004 - 0.339)

Figure 3.3: Genetic ancestry estimates by individual PEG-asparaginase-related toxicities



The x-axis and y-axis represent PEG-asparaginase-related toxicities and genetic ancestry estimates respectively. HS: Hypersensitivity, HB: Hyperbilirubinemia, PT: Pancreatitis, VTE: Venous thromboembolism

Figure 3.4: Genetic ancestry estimation by an increasing number of co-occurrence of PEG-asparaginase-related toxicities

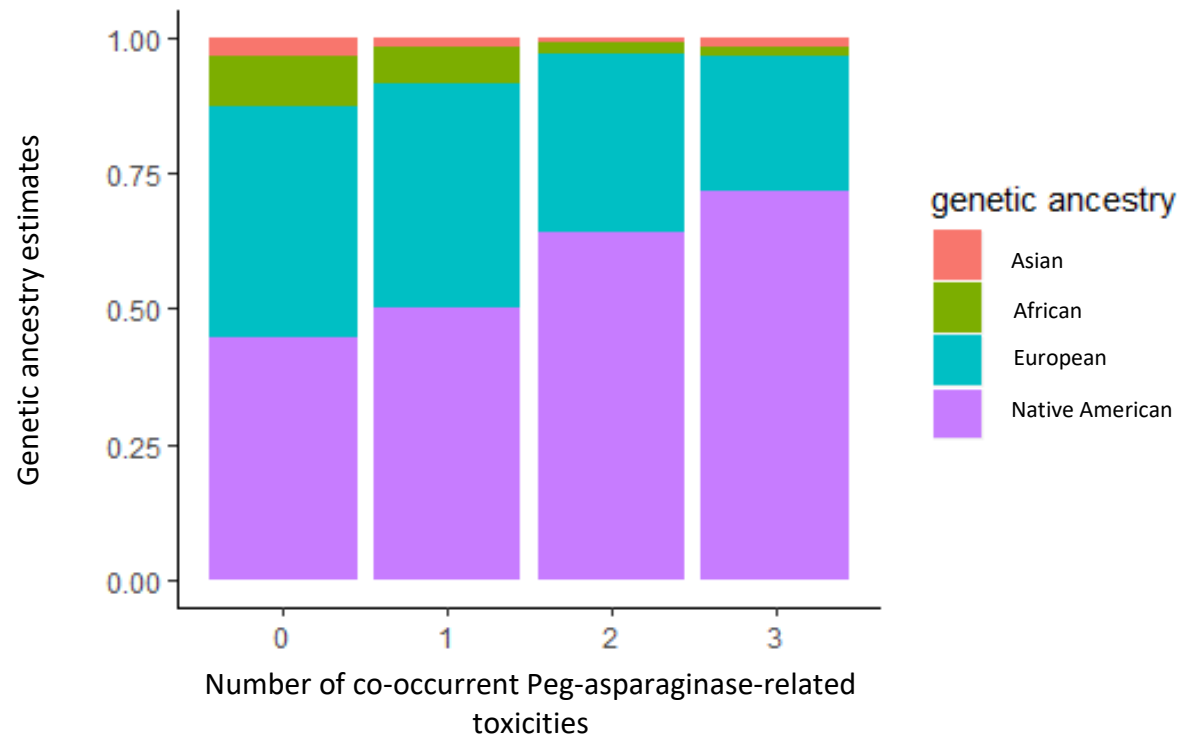


Table 3.34: Multivariable logistic regression of individual PEG-asparaginase-related toxicities

Variable	Odds ratios (95% CI)			
	(N = 170)			
	HS (N = 37)	HB (N = 16)	PT (N = 10)	VTE (N = 11)
Native American Ancestry	1.23 (0.45 -3.39)	1.68 (0.46- 6.90)	0.99 (0.17 – 5.63)	0.75 (0.12 -4.61)
Age at diagnosis				
<10 years old	Ref	Ref	Ref	Ref
≥10 years old	1.31 (0.53 -3.27)	2.24 (0.58 -8.67)	4.13 (0.78 -21.65)	10.81* (1.40 – 83.17)
BMI categories				
Underweight or Normal	Ref	Ref	Ref	Ref
Overweight or Obese	1.77 (0.78 -3.78)	3.07* (1.01 – 9.39)	2.02 (0.54 – 7.65)	19.53* (2.12 – 179.24)
ALL risk group				
Low/Standard risk	Ref	Ref	Ref	Ref
High/Very high risk	5.23* (1.91 – 14.29)	0.66 (0.16 – 2.54)	0.48 (0.09 – 2.50)	0.11* (0.01 – 0.89)
Gender				
Male	Ref	Ref	Ref	Ref
Female	0.50 (0.21 -1.11)	1.01 (0.34 -2.99)	1.15 (0.30 – 4.40)	0.54 (0.12 -2.38)

(*p<0.05) HS: Hypersensitivity, HB: Hyperbilirubinemia, PT: Pancreatitis, VTE: Venous thromboembolism

Table 3.4: Ordinal logistic regression of co-occurring PEG-asparaginase-related toxicities

Variable	Odds ratios (95% CI)				Proportional odds ratio (95% CI)
	(N = 170)				(N = 170)
	No toxicities (N =111)	1 toxicity (N = 48)	2 toxicities (N = 8)	≥3 toxicities (N = 3)	Ordinal
Native American Ancestry Mean (SD)	0.43 (0.38)	0.48 (0.40)	0.56 (0.47)	0.714 (0.11)	1.32 (0.56 – 3.11)
Age at diagnosis					
<10 years old	81 (73.0%)	23(47.9%)	3 (37.5%)	1 (33.3%)	Ref
10 years old	30 (27.0%)	25(52.1%)	5(62.5%)	2(66.7%)	2.72* (1.23 -6.03)
BMI categories					
Underweight or Normal	71 (64.0%)	21 (43.8%)	3 (37.5%)	0(0%)	Ref
Overweight or Obese	40 (36.0%)	27(56.2%)	5 (62.5%)	3 (100%)	2.65* (1.36 -5.16)
ALL risk group					
Low/Standard risk	67 (60.4%)	20 (41.7%)	2 (25.0%)	2(66.7%)	Ref
High/Very high risk	44 (39.6%)	28(58.3%)	6(75.0%)	1(33.3%)	1.46 (0.66 – 3.22)
Gender					
Male	58 (52.3%)	31 (64.6%)	4 (50.0%)	2(66.7%)	Ref
Female	53 (47.7%)	17 (35.4%)	4 (50.0%)	1 (33.3%)	0.64 (0.32 -1.26)

(*p <0.05) HS: Hypersensitivity, HB: Hyperbilirubinemia, PT: Pancreatitis, VTE: Venous thromboembolism

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CONCLUSION

PEG-asparaginase is a first-line drug used in treatment protocols for pediatric ALL. It has been one of the most important drugs in improving the event-free survival of patients with ALL. However, PEG-asparaginase is associated with four common toxicities: hypersensitivity, hyperbilirubinemia, pancreatitis, and VTE. Treatment-related toxicities usually lead to treatment modifications and delays and potentially contribute to lower survival rates and poor treatment outcomes (Avramis & Panosyan, 2005; Hunger, S. P. & Mullighan, 2015; Narta et al., 2007). Recent studies have reported racial/ethnic disparities in treatment-related toxicities (Taylor et al., 2018), which may partially explain the observed differences in survival for certain groups of children. In this study, we evaluated the clinical (age at diagnosis, BMI, and ALL risk group) and demographic factors (gender and self-reported and genetically defined race/ethnicity) associated with individual PEG-asparaginase-related toxicities and the cumulative burden of co-occurring toxicities. In our study, we also evaluated the influence of self-reported race/ethnicity and proportions of Native American ancestry on individual and co-occurring PEG-asparaginase-related toxicities. We did not find a significant association of self-reported race/ethnicity or Native American genetic ancestry with PEG-asparaginase-related toxicities; however, we did observe higher proportions of Native American ancestry with increasing burden of toxicities. Although the highest proportion of Native American ancestry was seen in the Hispanic population, a similar trend with increasing burden of toxicities was not seen within

the self-reported Hispanic population. This finding supports the need for further evaluation of local Native American genetic ancestry to identify loci which may predispose certain patients to toxicity. Ultimately, this line of investigation may inform the development of guidelines for PEG-asparaginase-related toxicity surveillance and/or prophylaxis, particularly for those with increased susceptibility to PEG-asparaginase toxicity.

Consistent with previous studies, we found that patients diagnosed at an older age (≥ 10 years) and with elevated BMI have a significantly higher risk of hyperbilirubinemia, pancreatitis, and VTE (Denton et al., 2018; Kearney, Susan L. et al., 2009; Klaassen et al., 2019; Levy-Mendelovich et al., 2018; Mateos et al., 2019). In this study, we uniquely found that these patients are also at a higher risk for concurrent PEG-asparaginase-related toxicities. Hence, behavioral interventions to promote a healthy BMI and increased clinical awareness of the factors that contribute to the development of multiple toxicities may help to improve the risk-benefit ratio of PEG-asparaginase.

PEG-asparaginase-related hypersensitivity was associated with treatment on high-/very high-risk treatment protocols. The number of PEG-asparaginase doses on high-/very high-risk treatment protocols is greater than that of patients treated on low-/standard-risk treatment protocols, which likely explains the higher risk of hypersensitivity associated with those protocols. Currently, COG discourages pre-treatment to decrease hypersensitivity as this may mask the presence of antibodies that neutralize asparaginase (Children's Oncology Group, 2016; Children's Oncology Group., 2017). Based on the results of this study, there is

a need to improve the strategies for the prevention of hypersensitivity without masking systemic allergy due to asparaginase-neutralizing antibodies.

Our study is substantive because it is the first study to evaluate the cumulative burden of PEG-asparaginase-related toxicities in a multi-ethnic population. To date, published reports have focused on the incidence of individual PEG-asparaginase-related toxicities among primarily non-Hispanic white study populations. Given the limitations of relying on self-reported race/ethnicity to identify disparities, particularly in populations with considerable genetic admixtures such as Hispanics, this study adds to our understanding of the ethnic differences in PEG-asparaginase toxicity by also considering the contribution of the proportion of Native American genetic ancestry, as has been reported with other treatment-related outcomes. Another strength of the study is the novel use of k-modes cluster analysis to identify latent subgroups of patients with distinct toxicity profiles.

There are some limitations inherent to our study. The sample size was relatively small, particularly for patients with ≥ 3 toxicities; hence, our study is potentially underpowered for evaluating some factors, including Native American genetic ancestry. Therefore, it is important to replicate these findings with a larger study population. Our population is multi-ethnic; however, we had an over-representation of self-reported Hispanics. To better characterize the effects of demographic and clinical factors on the risk of asparaginase-related toxicities among non-Hispanic black and Asian populations, who have a lower incidence of ALL, a larger, more nationally representative study population is required.

To summarize, our study identified several novel and potentially impactful findings related to the development of PEG-asparaginase-related toxicities among children being treated for ALL. These toxicities are highly clinically important as they can delay therapy, therefore impacting chances of long-term survival. Future directions will include expanding the analyses of Native American genetic ancestry, age, and BMI, and developing interventions or novel surveillance protocols to mitigate the risk of these toxicities without compromising treatment efficacy.

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