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Evaluating Iron Overload in Survivors of Pediatric Cancer

By

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Master of Science in Clinical Research

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Evaluating Iron Overload in Survivors of Childhood Cancer

By

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Mercer University School of Medicine, 2012

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Evaluating Iron Overload in Survivors of Pediatric Cancer

By

Ashley C. Eason, MD

Background: Iron overload is a known complication in patients who receive multiple erythrocyte transfusions for chronic hematologic disease. Due to their disease process and treatment, some pediatric cancer patients develop anemia requiring supportive transfusions at frequent intervals. Currently the Children's Oncology Group (COG) has established guidelines to obtain a ferritin for iron overload screening in patients who have undergone hematopoietic stem cell transplantation (HSCT); however, no guidelines exist for other patients. Since treatment options are available, successful screening may help prevent end organ damage from iron accumulation.

Objective: To analyze our childhood cancer survivor population to identify factors which increase the risk for development of iron overload.

Methods: We performed a retrospective cohort study of pediatric cancer patients inclusive of all malignant diagnoses from 2009-2015. Patients were identified from the cancer registry at Children's Healthcare of Atlanta and the outcome iron overload was defined as ferritin value ≥ 500 ng/mL.

Results: We identified 2486 children with malignant diagnoses during our study period. Of these participants, 75% (1866/2486) received an erythrocyte transfusion and 11% (134/1186) of eligible patients had a screening ferritin level performed following completion of therapy. Iron overload was noted in 47% (61/130) of the screened patients at follow up. Increased number of erythrocyte transfusions (aOR 1.33 [95% CI: 1.19-1.49]) and older age at diagnosis (aOR 1.15 [95% CI: 1.04-1.32]) were associated with development of iron overload. In addition, higher intensity treatment rating (ITR) (aOR 26.91 [95%CI 3.04-239.20]) and earlier time to ferritin (aOR 0.95 [95%CI: 0.91-0.98]) after completion of therapy were found to be associated with the development of iron overload when controlling for additional covariates of interest.

Conclusion: Patients with greater treatment intensity and more erythrocyte transfusions are at risk to develop iron overload. We found that ITR is a useful clinical tool to identify patients who would benefit from iron overload screening. A minority of patients had confirmatory imaging with MRI Ferriscan (24/61), however, all imaging found excess iron accumulation in the liver. This demonstrates our institutional imaging threshold is too high and we are missing patients who would benefit from treatment for iron overload.

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A. Introduction

Although cancer in children is rare, approximately 15,000 children and adolescents are diagnosed with a malignancy each year. With continued advances in treatment, overall survival for children with cancer is approaching 85%. This progress is quite significant when considering in the mid-1970s, only 58% of children (ages 0 to 14 years) and 68% of adolescents (ages 15 to 19 years) diagnosed with cancer survived at least 5 years [1]. Current survival rates are highest in Acute Lymphoblastic Leukemia, Hodgkin Lymphoma, and Wilms Tumor [Appendix 1]. With the improvements in survival, a growing emphasis has been placed on long term health outcomes for survivors of pediatric cancer. Recent estimates suggest over 400,000 survivors of childhood cancer are alive in the United States today [2].

At Children's Healthcare of Atlanta, we have the largest combined pediatric cancer and blood disorders center in the country. We have a dedicated multidisciplinary survivor clinic which includes oncology, endocrinology, and psychology teams who focus on specific medical problems which may result from cancer therapy. Health problems which occur in the years following cancer therapy are known as late effects. The most frequently encountered late effects include growth and developmental delay, learning disabilities, diseases of the heart and lungs, vision and hearing problems, secondary cancers, and infertility. Iron overload is a recognized late effect with growing interest in the survivor community which has been under investigated to date. Excess iron is stored in organs which are already at risk of toxicity and damage from cancer treatment. The development of iron overload has been well described in patients with benign hematologic diseases which require frequent erythrocyte transfusions. Pediatric patients with malignant diseases may also receive frequent supportive erythrocyte transfusions during their treatment. However, the prevalence, significance, and long-term outcomes of iron overload in this population are less well described. Prior studies have reported an increased risk of iron

overload in pediatric patients with hematologic malignancies following allogeneic hematopoietic stem cell transplantation (HSCT) [3, 4]. Presently, based on these data, the Children's Oncology Group (COG) Long Term Follow Up Guidelines recommend a screening ferritin level be obtained at baseline entry into long term follow up care in patients who have undergone HSCT as part of their treatment [5]. More information is needed to establish additional criteria warranting evaluation for iron overload in pediatric oncology patients as well as specific parameters for initiating treatment for iron overload. When iron overload is identified, treatment options are available, including phlebotomy and oral iron chelation, to reduce iron burden and end organ damage. The goal of this single institution retrospective cohort study was to analyze our childhood cancer survivor population to identify factors which increase the risk for development of iron overload in order to establish guidelines to effectively screen our childhood cancer survivor population.

B. Background

Iron is an essential bio-element with the primary role of transporting oxygen throughout the body. It is primarily absorbed in the first part of the small intestine, the duodenum, and the amount of iron absorbed can vary based on body iron stores and rate of erythropoiesis. Iron metabolism is tightly regulated through proteins such as hepcidin and ferroportin. Ferritin is the primary protein involved in iron storage and is produced in the liver.

On average one unit of blood contains 250 mg of iron, a significant increase from the average absorption of 1-2 mg per day [6]. The body does not have a physiologic mechanism to regulate iron excretion and excess iron can deposit in several organ systems. The development of iron overload has been studied in patients with illnesses which require frequent erythrocyte transfusions, such as thalassemia major and sickle cell anemia. Without a physiologic mechanism for iron excretion, repeated transfusions can lead to brisk iron accumulation. Elevated iron concentration can be seen after as few as 10-20 erythrocyte transfusions in these patients and is known as transfusional siderosis or transfusional iron overload [7-9]. In the case of transfusional iron overload, iron accumulates in reticuloendothelial macrophages first and then moves into parenchymal cells. Iron is ultimately stored in solid organs, such as the liver, heart, pancreas, and spleen, with the liver being most common. This iron loading can result in tissue damage and fibrosis through the production of reactive oxygen species [10]. The contribution of iron overload to morbidity and mortality in these patients is also well established [11-14]. For example, cardiomyopathy because of iron overload is the leading cause of mortality in thalassemia major patients [15].

Liver biopsy is the gold standard for detection of iron overload; yet, due to its invasive nature, it is not frequently performed [16]. Magnetic Resonance Imaging (MRI) with dedicated Ferriscan protocol is now quickly gaining ground as a standardized and non-invasive technique for assessing liver iron concentration (LIC) [17, 18]. However, the MRI Ferriscan is not an easy screening exam, since it is expensive, requires sedation for small children, and is not widely available. A serum ferritin levels is commonly obtained as a screening tool to assess iron stores in the body due to its low cost, ease of collection, and ability to trend. However, ferritin is known to be an acute phase reactant protein and can be elevated in states of both chronic and acute inflammation which is an important consideration when interpreting ferritin levels. The correlation between ferritin level and MRI findings is imperfect and can make ferritin thresholds difficult to determine. Despite its limitations, serum ferritin is the easiest and cheapest screening method for iron overload in children and is currently performed as a first line screening test for patients at risk of iron overload.

Previous studies evaluating iron overload in childhood cancer survivors have had limitations. A 2013 study from St Jude Children's Research Hospital reviewed the transfusion burden among patients treated for hematological malignancies over a 40-year period (1962-2004) [4]. They noted more recent survivors received increasing numbers of erythrocyte transfusions subsequently increasing the risk for iron overload. They also reported a significantly greater number of transfusions among patients who received HSCT compared to those receiving chemotherapy alone. Additionally, they demonstrated patients who received higher intensity treatment received a greater number of transfusions; however, they were not able to correlate these findings with measures of iron overload such as ferritin or MRI Ferriscan [4]. Similarly, a retrospective 2011 study from Children's Hospital of Los Angeles (CHLA) also explored erythrocyte transfusion volumes as well as treatment intensity. They used the Intensity Treatment Rating Scale, a validated method for comparing the magnitude of diagnosis and

strength of treatment, and demonstrated a projected increase in iron burden based on increasing intensity of treatment received [23]. However, they also did not have available markers of iron overload and used a mathematical calculation to determine projected iron overload in this population. Some authors have only evaluated patients with a history of acute lymphoblastic leukemia (ALL) as it is the most common type of cancer in children. Each of these studies found number of erythrocyte transfusions correlated with elevated ferritin although different ferritin thresholds were used in each study [19-21].

More recently, several small prospective studies have been conducted to further explore this issue. A cross sectional study of 75 patients, who were on average 4 years following completion of therapy, noted a positive correlation between volume of erythrocyte transfusion and LIC as measured by MRI Ferriscan. The same study found an association between older age and increased LIC [24]. A prospective study including 61 patients from Minnesota demonstrated patients treated with HSCT were more likely to have iron overload compared to survivors treated with chemotherapy alone. They were unable to demonstrate an association between serum ferritin levels and organ dysfunction [25]. Both studies were limited by incomplete transfusion records due to some patients receiving care at other institutions. In 2018, Trovillion, et. al published a prospective study of 116 childhood cancer survivors who were screened for iron overload at a minimum of 24 months following completion of therapy [26]. In this study, only 3% of patients were found to have iron overload; however, they were all teenagers at the time of diagnosis demonstrating a similar age pattern as prior studies. Longer time to screening may have contributed to low prevalence in this group.

Overall, these results begin to determine the scope of the problem in childhood cancer survivors; however, larger studies are needed to validate the results.

In addition to treatment and transfusion variables, it is important to assess demographic variables that may impact iron overload in order to establish screening protocols. Age at diagnosis was a specific demographic factor of interest for our study. As suggested by the previously described studies, older age at diagnosis was associated with increased LIC [24, 26]. The rationale for considering this as a factor of interest is that growth and development likely plays a pivotal role in expenditure of excess iron stores. As children grow, they expand their blood volume, which requires iron. It is also important to consider that specific cancer diagnoses and treatments are more prevalent in the adolescent and young adult population which may also contribute to risk for iron overload. Gender is an additional variable which has the potential to mediate iron overload, since adolescent females have blood loss due to menstruation, which leads to increased utilization of iron. We assessed these variables in our patient population to evaluate their role in iron overload.

The risk for iron overload may also vary based on race/ethnicity. Hemochromatosis is a genetic disorder which allows for increased and unregulated iron absorption in the body and subsequent development of end organ damage from iron toxicity. This disorder is found most commonly in European populations and genetic mutations which lead to hemochromatosis have been identified. These genes may additionally play a role in iron accumulation in our population and may lead to the finding that ethnicity also contributes to risk of iron overload. Although we did not have genetic information in our study, we collected race/ethnicity data on each patient.

The COG has developed guidelines for screening and monitoring pediatric cancer patients following completion of treatment. Many long-term complications have been identified as consequences of exposure to specific chemotherapeutic agents or radiation therapy, and patients are followed closely to prevent and lessen these adverse effects as much as possible. Despite gains in knowledge regarding late effects, the significance of iron overload from supportive erythrocyte transfusions during therapy has not been well studied. Iron accumulation can

impact multiple organ systems which are already at risk from treatment related toxicity and thus may have a greater role in outcomes than is currently appreciated. Treatment options for iron overload are available which could reverse toxicity and improve health outcomes for affected patients. Current screening recommendations are limited to obtaining a serum ferritin level in HSCT survivors, and further knowledge is needed to appropriately extend these guidelines to other at risk patients.

Our background information led us to a design a study with the following questions in mind:

Research Questions:

1. What are current institutional screening practices and how do they differ between treatment teams?
2. What childhood cancers survivors are at greatest risk to develop iron overload?
3. What is the relationship between screening ferritin and LIC on MRI Ferriscan?

C. Methods

Research Objectives:

1. Characterize the institutional screening practice and estimate the prevalence of iron overload in screened survivors of pediatric cancer using serum ferritin and/or MRI Ferriscan.
2. Determine the demographic factors including age at diagnosis, gender, and race/ethnicity which may predispose to iron overload in survivors of pediatric cancer.
3. Estimate the association of treatment intensity and cumulative erythrocyte transfusion events with the development of iron overload in survivors of pediatric cancer.
4. Investigate the relationship between serum ferritin and MRI Ferriscan in survivors of pediatric cancer with both markers of iron status.

Hypotheses:

1. In pediatric cancer survivors, higher treatment intensity and increased erythrocyte transfusion events during treatment increase the prevalence of iron overload.
2. When controlled for age at diagnosis, gender and race/ethnicity, the treatment intensity and number of erythrocyte transfusion events are associated with development of iron overload.

Study Design:

A retrospective single institution cohort study was conducted using the Children's Healthcare of Atlanta (CHOA) cancer registry and electronic medical record. Eligible patients who had been diagnosed with an oncologic disease from January 1, 2009 through December 30, 2015 were identified through the institutional cancer registry. Within the oncology patient cohort, we identified patients who had received at least one erythrocyte transfusion during their treatment and were evaluated for iron overload after completion of therapy using serum ferritin and/or MRI Ferriscan. Electronic medical records were retrospectively examined from the time of initial presentation until the conclusion of the follow up period, December 31, 2017. Study data were collected and managed using REDCap® electronic data capture tools hosted at CHOA. The study was approved by the CHOA Institutional Review Board and informed consent was waived prior to initiation.

Study Population:

To characterize our institutional screening practices, inclusion criteria included all patients diagnosed in the above-mentioned timeframe with a malignant diagnosis who had completed their initial cancer treatment, specifically chemotherapy and/or radiation, including patients who underwent HSCT, prior to the conclusion of the follow up period. Patients must have received at least one erythrocyte transfusion during their treatment.

Exclusion criteria included patients who received part of their therapy outside of CHOA with the potential for incomplete records. Additionally, patients who died during our study period or remained on therapy at the end of our study period were excluded.

When evaluating iron overload, included patients received at least one erythrocyte transfusion during their treatment and had at least one ferritin level obtained following completion of therapy. For patients who suffered a relapse of their disease, we included initial diagnosis and treatment as well as any relapse therapy completed before the first iron overload evaluation.

Exclusion criteria included patients who received multiple erythrocyte transfusions for another indication including patients with history of solid organ transplantations, HSCT for non-malignant indications, and non-malignant hematologic diseases requiring chronic transfusions. We also excluded patients with co-existing rheumatologic disorders as these patients may have elevated ferritin levels due to chronic inflammation. As only patients with full treatment at CHOA were included, all patients had complete information for variables of interest.

Data Collection and Measurements:

After obtaining approval from the CHOA IRB, electronic medical records were reviewed for demographic, clinical, and laboratory information. To evaluate screening practices, our outcome of interest was screening ferritin obtained following completion of therapy. In patients with more than one ferritin value, only the first value following completion of therapy was evaluated. Additional clinical and demographic variables evaluated were age at diagnosis, sex, race/ethnicity, malignancy type, intensity of treatment rating (ITR), HSCT status, and number of erythrocyte transfusions. Malignancy type was categorized as follows: brain tumor, leukemia, lymphoma, myelodysplastic syndrome, and solid tumor. The ITR was assessed using the ITR, version 3. This scale is used to categorize treatment intensity based on disease, treatment types (i.e., chemotherapy, radiation, HSCT), and stage/risk group. It was developed using a two-part questionnaire completed by pediatric oncologists across the country and has high inter-rater reliability. Previous studies have used this scale in predicting iron accumulation and it is a validated tool for classifying treatment intensity among a variety of diagnoses and treatments received [4, 22-24]. Using this scale, ITR category was manually assigned for each patient by reviewing diagnosis and treatment data available in the electronic medical record [Appendix 2]. This abstraction and classification was performed by the principal investigator.

For iron status analysis, iron overload was our primary outcome. We defined this as a ferritin level greater than or equal to 500 ng/ml and/or MRI Ferriscan with LIC greater than normal

(0.2– 2 mg of iron per gram of dry liver [Fe/g]). Our primary exposure variable was number of erythrocyte transfusions during therapy. Additional variables of interest were age at diagnosis, sex, race/ethnicity, malignancy type, ITR, HSCT status, and time from completion of therapy to screening. Ferritin levels and MRI Ferriscans were included through December 31, 2017.
For patients who met our definition of iron overload based on screening ferritin and were subsequently evaluated with MRI Ferriscan, additional variables including liver function tests, echocardiogram results, liver biopsy results, and iron overload treatment modality were collected when available.

Statistical Analyses:

Summary statistics were calculated to characterize the cohort. Descriptive analyses involved calculation of proportions (frequencies) for categorical data, and mean and range for normally distributed continuous data. Institutional screening practices were examined by dividing patients into groups with a screening ferritin to assess iron overload and those who were not screened then comparing characteristics of each group. Comparisons between groups were obtained using Chi-square or Fisher's Exact tests for categorical variables or two-sample T-tests for normally distributed continuous variables.

When evaluating iron status, patients with a screening ferritin were divided into two groups using our definition of iron overload: iron overload (ferritin ≥ 500ng/mL) and no iron overload (ferritin < 500 ng/mL). Chi Square analysis and Fisher's Exact Test were performed to compare categorical variables between groups. T-tests were performed for age at diagnosis, number of erythrocyte transfusions, and time since completion of therapy to ferritin as continuous variables. Pearson correlation coefficient was performed to assess the linear relation between serum ferritin value and number of erythrocyte transfusions. Multivariable analysis via logistic regression modeling was used to determine relationship between predictor variables and outcome variable, iron overload, incorporating statistically significant independent variables on

univariate analysis and clinically significant variables documented in the literature. Separate analyses were performed using ITR as a predictor variable and erythrocyte transfusion as predictor variable.

With MRI Ferriscan used as our gold standard, sensitivity and specificity analyses were conducted to compare the accuracy of serum ferritin to that of MRI Ferriscan in detecting iron overload. For patients with MRI Ferriscan imaging, Pearson correlation coefficient was performed to determine linear correlation between LIC and ferritin level. Chi square, Fisher's Exact Test and T-tests were again used to compare characteristics among patients with iron overload who then had definitive imaging with MRI Ferriscan versus those who did not proceed to MRI Ferriscan. For patients with MRI Ferriscan, descriptive characteristics of interest were evaluated including median LIC, demonstration of abnormal liver function tests or echocardiogram, performance of liver biopsy, and treatment for iron overload.

Only patients with complete information were included for analysis. Thus, no accommodations were needed for missing data. For all tests described, a p value < 0.05 was considered statistically significant. All computations were performed using SAS System v9.4 (2012, SAS Institute, Cary, NC, USA).

D. Results

Demographic and Clinical Characteristics for Cohort:

Between January 1, 2009 and December 31, 2015, 2486 pediatric patients with malignancy diagnoses were identified through the institutional cancer registry at CHOA. Figure 1 demonstrates the CONSORT diagram for subject eligibility. Next, 75% (1866/2486) patients were found to receive an erythrocyte transfusion as supportive care during their treatment. After removing patients who were deceased, treated at another institution for any part of therapy, and still on therapy at the end of our study period, the remaining patients 1186 met our eligibility criteria for screening ferritin analysis. Table 1 provides a summary of the demographic and clinical characteristics of all subjects eligible for screening ferritin. Median age at diagnosis was 7.8 years (range 0-22 years). There were similar numbers of males (n=615) and females (n=571). The relative percentages of each race and ethnicity closely approximated the distribution seen in the state of Georgia population. Subjects were categorized into the following malignancy subtypes brain tumor 21.9% (260/1186), leukemia 31.0% (368/1186), lymphoma 9.5% (113/1186), myelodysplastic syndrome 1.3% (15/1186), and solid tumor 36.3% (430/1186). These percentages mirrored the overall distribution of new cancer diagnoses at CHOA. For intensity of treatment ratings, the majority of patients were classified in the middle range categories with 36.4% (431/1186) meeting criteria for ITR 2 and 46.0% (546/1186) in ITR 3. A small percentage of patients, 9.4% (111/1186), required HSCT as part of their therapy. The mean number of erythrocyte transfusions per patient was 5.8 (range 1-65).

Analysis of Ferritin Screening Practices:

In our cohort, only 11% (134/1186) of patients had a screening ferritin obtained following completion of therapy. When comparing characteristics between screened patients and those who were not screened, age at diagnosis (p=0.36), sex (p=0.41), and race/ethnicity (p=0.69) were not statistically different between the groups (Table 2). Statistically significant differences were found for malignancy type with patients with brain tumors or solid tumors screened less frequently (p< 0.01). Higher ITR (p< 0.01), HSCT as part of therapy (p <0.01), and higher number of erythrocyte transfusions (p <0.01) were significantly associated with obtaining a screening ferritin.

Analysis of Iron Status:

Of the 134 patients with a screening ferritin, four of these patients met exclusion criteria including sickle cell anemia (n=1), liver transplant (n=2), and rheumatologic disease, Juvenile Idiopathic Arthritis (n=1). Of 130 patients eligible for analysis, 47% (61/130) met our definition of iron overload. Table 3 details the differences between those with iron overload versus those without iron overload groups. Sex (p=0.18), malignancy (p=0.08), and age at diagnosis (p=0.06) were not statistically different between groups (Table 3, Figure 2). Higher ITR (p< 0.01), HSCT as part of therapy (p< 0.01), higher number of erythrocyte transfusions (p< 0.01), and longer time since completion of therapy to obtaining screening ferritin (p< 0.01) were significantly associated with the development of iron overload. (Table 3, Figure 3, Figure 4). When estimating the association between ferritin level and number or erythrocyte transfusions, a positive linear relationship was identified (r=0.73, p< 0.01) (Figure 5).

Multivariable analysis with logistic regression was used to evaluate the associations between predictor variables and the outcome of iron overload. Race/Ethnicity, ITR, time since therapy completion to ferritin, and number of erythrocyte transfusions were significantly associated with

iron overload development on univariate analysis and were included in the final models. In addition, sex and age at diagnosis were felt to be clinically significant variables which were also included. As post-pubertal females lose blood and thus iron due to menstruation, the interaction of age and gender was estimated and not found to be significant (P=0.49). Due to covariation between ITR and number of erythrocyte transfusions, these variables were included in separate models to assist with determining the clinical value of using ITR as a risk factor for iron overload independent of number of erythrocyte transfusions. As malignancy type and HSCT status are included criteria in the classification rating for ITR, they were not included in the final models. The final multivariable logistic regression model including ITR was as follows:

$$\textbf{Logit P(Iron Overload)} = \beta_0 + \beta_1 \textbf{ITR} + \beta_2 \textbf{AGE} + \beta_3 \textbf{SEX} + \beta_4 \textbf{RACE} + \beta_5 \textbf{TIME}$$

As seen in Table 4, increased time since completion of therapy to ferritin (p = 0.004) and ITR category (p = 0.01) were associated with development of iron overload. Although overall race/ethnicity was not statistically significant, Hispanic patients were estimated to be 5.03 times more likely to develop iron overload than white patients (95% CI:1.08-23.36) after adjusting for ITR, age at diagnosis, sex, and time since therapy completion to screening ferritin.

The final multivariable logistic regression model including number of erythrocyte transfusions was as follows:

$$\textbf{Logit P(Iron Overload)} = \beta_0 + \beta_1 \textbf{TRANSFUSION} + \beta_2 \textbf{AGE} + \beta_3 \textbf{SEX} + \beta_4 \textbf{RACE} + \beta_5 \textbf{TIME}$$

In this model, the number of erythrocyte transfusions (aOR 1.33 [95% CI: 1.19-1.49]) and older age at diagnosis (aOR 1.15 [95%CI: 1.04-1.32]) were associated with the development of iron overload (Table 5). Again, Hispanic patients were more likely to develop iron overload than White patients (aOR 5.41 [95% CI: 0.97-30.11]).

When assessing for potential bias in obtaining screening ferritin by ITR, patients who had a screening ferritin were estimated to have higher numbers of erythrocyte transfusions that were consistent among each ITR (Figure 6).

Analysis of MRI Ferriscan versus Ferrition for Screening:

MRI Ferriscan was obtained in 39% (24/61) of patients who were found to have iron overload on screening ferritin and no patients without iron overload. All 24 patients had a LIC on MRI Ferriscan which was indicative of iron overload, i.e, > 2 mg of Fe/g (100% sensitivity, 100% specificity). When comparing which patients proceeded to MRI Ferriscan, no differences were noted between age at diagnosis, malignancy type, HSCT status, ITR, or number of erythrocyte transfusions (Table 6). Ferritin values between those screened with MRI Ferriscan and those not screened were statistically different with a higher mean ferritin in those patients who had a MRI Ferriscan (2789.7 vs 1704.4, p=0.05).

When evaluating additional clinical characteristics of interest for patients with MRI Ferriscan, 75% (18/24) of patients were treated for iron overload. The mean LIC was 8.25 (Normal range: 0.2 mg Fe/g – 2 mg Fe/g). Few patients were found to have abnormal cardiac function (1/24) or abnormal liver function tests (3/24) (Table 7). There was a weak linear correlation between Ferritin value and LIC (r=0.4, p=0.05) (Figure 7).

E. Discussion

Despite the potential for organ toxicity, iron overload has not been thoroughly studied in survivors of childhood cancer. While emphasis on pediatric survivor late effects is increasing, minimal guidelines exist to guide providers on screening for iron overload. The liver is the most frequently considered site of iron toxicity; however, excess iron can also accumulate in the heart and numerous endocrine organs. These sites are also at risk for toxicity from chemotherapy and radiation, and iron has the potential to potentiate the late effects of cardiomyopathy and infertility. To date, our understanding of the contributions of iron overload to pediatric cancer survivor late effects has been incomplete, and current institutional practices are provider dependent with few guidelines to determine who and when to screen for iron overload. Based on knowledge regarding iron overload in chronic anemia patients and previous work in survivors of childhood cancer, we have evaluated risk factors to better determine which oncology patients are at high risk for developing iron overload. The analysis of this retrospective cohort provides the largest data described in the literature thus far using screening ferritin to identify patients at risk of iron overload and further identifies risk factors to assist in the development of screening guidelines.

Due to the lack of screening guidelines in place, few patients were evaluated for iron overload following the completion of their cancer treatment. In our institution, only 11% (134/1186) of eligible survivors were screened for iron overload using ferritin. As COG currently provides recommendations for HSCT patients, it is not surprising that 58% (79/134) of patients with screening ferritin had undergone HSCT as part of their therapy. However, only 71% of patients who underwent HSCT had a screening ferritin and only 5% (55/1075) of those treated without HSCT had a ferritin evaluated following the completion of therapy.

Although a significant difference was noted among malignancy types when comparing screening practices ($p < 0.01$), this difference is likely attributed to underlying HSCT status. Leukemia and myelodysplastic syndrome patients had higher rates of screening than those in the other categories; however, these patients undergo HSCT more frequently as part of therapy. In addition, the highest ITR category (4) which takes into account both malignancy category and treatment modality had the highest percentage of screened patients (85%), and screened patients had higher mean number of erythrocyte transfusions (12.68 vs 5.00, $p< 0.01$).

When comparing demographic and clinical characteristics amongst patients with iron overload and those without iron overload, no significant differences were noted between sex or malignancy type. Again, patients with higher treatment intensity, HSCT as part of therapy, and increased number of erythrocyte transfusions were more likely to have iron overload. Age at diagnosis showed a trend toward patients who were older at diagnosis developing iron overload though not statistically significant (9.51 vs 7.57, $p=0.06$). This finding is similar to prior studies that suggest younger patients may utilize excess iron during pubertal maturation and growth.

Race/ethnicity showed a surprising difference between the groups with 81.3% (13/16) Hispanic patients having iron overload. It remained significant in multivariable models adjusting for ITR and number of erythrocyte transfusions. Although the overall number of Hispanic patients in our study was small, this finding suggests a potential biologic factor at play increasing the risk of iron accumulation. While genetic polymorphisms which can predispose to iron overload are well described in the Caucasian population, no similar genetic findings have been reported in people with Hispanic ethnicity. This is a limited finding but warrants additional exploration in future studies.

Within our cohort, we were able to identify risk factors associated with iron overload. When controlling for other covariates, we found for every additional erythrocyte transfusion received, the odds of developing iron overload increased by 33% (aOR 1.33, 95% CI 1.19-1.49, $p < 0.001$). As we know each erythrocyte transfusion provides an additional 250 mg of iron, this is an expected finding. In addition, age at diagnosis was a statistically significant predictor with the odds of developing iron overload increased by 15% for each 1 year in age at diagnosis. Because older girls begin to lose blood through menstruation, the interaction of age at diagnosis and gender was estimated and not found to have statistical significance. However, clinically this is likely to be an important consideration when evaluating and treated iron overload.

ITR was evaluated separately from number of erythrocyte transfusions to explore its clinical utility as a predictor of iron overload. When controlling for covariates, it was found to have significant association with iron overload ($p< 0.01$). In this model, time since therapy completion to ferritin was also significant (aOR 0.95, [95%CI: 0.91-0.98]). ITR demonstrated similar association with iron overload compared to number of erythrocyte transfusions when controlling for the same clinical variables of interest. ITR has promising clinical utility when evaluating risk for this late effect as it is a straight forward classification system which physicians can quickly assign to patients following diagnosis and treatment review. The number of erythrocyte transfusions per patient is difficult to quantify and is especially cumbersome when patients have care in multiple institutions.

Our analysis of MRI Ferriscan was limited by small patient numbers. Only 39% (24/61) of patients meeting our definition of iron overload by ferritin value proceeded to MRI Ferriscan. All MRI Ferriscan results had LIC values greater than the normal range confirming excess iron accumulation in the liver. Ferritin value was the only

characteristic found to significantly differ between the patients who had MRI Ferriscan and those who did not with grossly abnormal mean ferritin values in both groups (2789.7 vs 1704.4, p=0.05). Of note the median LIC for our cohort was 8.25 mg Fe/g which is 4x the upper limit of normal.

Advantages and Limitations:

Our study had several advantages; including the use of a clinically relevant research question and the availability of effective treatment modalities when iron overload is identified. As CHOA is the largest combined pediatric cancer and blood disorders center in the country, we were able to conduct the largest retrospective study to date using screening ferritin to project iron burden. Additionally, we identified ITR as a clinically useful surrogate to number of erythrocyte transfusions when assessing risk for iron overload.

Our study was limited due to its retrospective design with the inability to control for potential unknown confounders and difficulty in proving causal associations. Due to limited guidelines, there is the inherent risk of selection bias amongst providers when determining which patients to screen for iron overload. As a result, a small number of patients were screened, and a significant number of at-risk patients were not evaluated. Ferritin is a controversial marker of iron status because it is also an acute phase reactant. While we attempted to control for this by only including off therapy values of ferritin, additional unknown factors at the time of testing could allow for falsely elevated values. In addition, our definition of iron overload based on ferritin value > 500 ng/mL is conservative, though used similarly in other studies. Ferritin's ability to correlate with iron burden identified on imaging is imperfect thus prompting us to use a slightly lower cutoff value. MRI Ferriscan is the definitive test to identify iron overload. However, it was utilized in very few patients with all patients undergoing imaging having positive

results. Certainly, this finding demonstrates that our institutional imaging threshold is too high, and we are missing patients who would benefit from treatment for iron overload.

Summary:

Our study contributes to the findings in the existing literature and further demonstrates the need for guidelines to assist providers in deciding which patients should be screened for iron overload. In our population, ITR was found to be a clinically useful surrogate for number of erythrocyte transfusions and should be considered when identifying patients at risk of iron overload. Although in small studies to date the overall prevalence of iron overload has been low, identifying and evaluating high risk patients has the potential to improve long term outcomes for survivors of childhood cancer by helping to prevent treatment-related complications.

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F. References

1. Ward, E., et al., *Childhood and adolescent cancer statistics, 2014.* CA Cancer J Clin, 2014. **64**(2): p. 83-103.
2. Robison, L.L. and M.M. Hudson, *Survivors of childhood and adolescent cancer: life-long risks and responsibilities.* Nat Rev Cancer, 2014. **14**(1): p. 61-70.
3. Chotsampancharoen, T., et al., *Iron overload in survivors of childhood leukemia after allogeneic hematopoietic stem cell transplantation.* Pediatr Transplant, 2009. **13**(3): p. 348-52.
4. Nottage, K., et al., *Trends in transfusion burden among long-term survivors of childhood hematological malignancies.* Leuk Lymphoma, 2013. **54**(8): p. 1719-23.
5. Chow, E.J., et al., *Late Effects Surveillance Recommendations among Survivors of Childhood Hematopoietic Cell Transplantation: A Children's Oncology Group Report.* Biol Blood Marrow Transplant, 2016. **22**(5): p. 782-95.
6. Remacha, A., et al., *Guidelines on haemovigilance of post-transfusional iron overload.* Blood Transfus, 2013. **11**(1): p. 128-39.
7. Andrews, N.C., *Disorders of iron metabolism.* N Engl J Med, 1999. **341**(26): p. 1986-95.
8. Olivieri, N.F., *Progression of iron overload in sickle cell disease.* Semin Hematol, 2001. **38**(1 Suppl 1): p. 57-62.
9. Inati, A., et al., *Iron overload indices rise linearly with transfusion rate in patients with sickle cell disease.* Blood, 2010. **115**(14): p. 2980-1; author reply 2981-2.
10. Fleming, R.E. and P. Ponka, *Iron overload in human disease.* N Engl J Med, 2012. **366**(4): p. 348-59.
11. Wood, J.C., et al., *Organ iron accumulation in chronically transfused children with sickle cell anaemia: baseline results from the TWiTCH trial.* Br J Haematol, 2016. **172**(1): p. 122-30.

12. Brown, K., et al., *Hepatic iron overload in children with sickle cell anemia on chronic transfusion therapy*. J Pediatr Hematol Oncol, 2009. **31**(5): p. 309-12.

13. Fung, E.B., et al., *Morbidity and mortality in chronically transfused subjects with thalassemia and sickle cell disease: A report from the multi-center study of iron overload*. Am J Hematol, 2007. **82**(4): p. 255-65.

14. Harmatz, P., et al., *Severity of iron overload in patients with sickle cell disease receiving chronic red blood cell transfusion therapy*. Blood, 2000. **96**(1): p. 76-9.

15. Farmakis, D., et al., *Heart failure in haemoglobinopathies: pathophysiology, clinical phenotypes, and management*. Eur J Heart Fail, 2016.

16. Kohgo, Y., et al., *Body iron metabolism and pathophysiology of iron overload*. Int J Hematol, 2008. **88**(1): p. 7-15.

17. St Pierre, T.G., et al., *Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance*. Blood, 2005. **105**(2): p. 855-61.

18. Wood, J.C., et al., *MRI R2 and R2* mapping accurately estimates hepatic iron concentration in transfusion-dependent thalassemia and sickle cell disease patients*. Blood, 2005. **106**(4): p. 1460-5.

19. Halonen, P., et al., *Iron overload in children who are treated for acute lymphoblastic leukemia estimated by liver siderosis and serum iron parameters*. Pediatrics, 2003. **111**(1): p. 91-6.

20. Sait, S., et al., *Transfusion related iron overload in pediatric oncology patients treated at a tertiary care centre and treatment with chelation therapy*. Pediatr Blood Cancer, 2014. **61**(12): p. 2319-20.

21. Unal, S., et al., *Number of erythrocyte transfusions is more predictive than serum ferritin in estimation of cardiac iron loading in pediatric patients with acute lymphoblastic leukemia*. Leuk Res, 2014. **38**(8): p. 882-5.

22. Kazak, A.E., et al., *A revision of the intensity of treatment rating scale: classifying the intensity of pediatric cancer treatment*. Pediatr Blood Cancer, 2012. **59**(1): p. 96-9.

23. Ruccione, K.S., et al., *Association of projected transfusional iron burden with treatment intensity in childhood cancer survivors*. Pediatr Blood Cancer, 2012. **59**(4): p. 697-702.

24. Ruccione, K.S., et al., *Characterization of transfusion-derived iron deposition in childhood cancer survivors*. Cancer Epidemiol Biomarkers Prev, 2014. **23**(9): p. 1913-9.

25. Schempp, A., et al., *Iron Overload in Survivors of Childhood Cancer*. J Pediatr Hematol Oncol, 2016. **38**(1): p. 27-31.

26. Trovillion, E.M., L. Schubert, and A.C. Dietz, *Iron Overload in Survivors of Childhood Cancer*. J Pediatr Hematol Oncol, 2018. **40**(5): p. 396-400.

G. Tables and Figures

Figure 1. Consort Diagram of Patients Included in Iron Overload Analyses

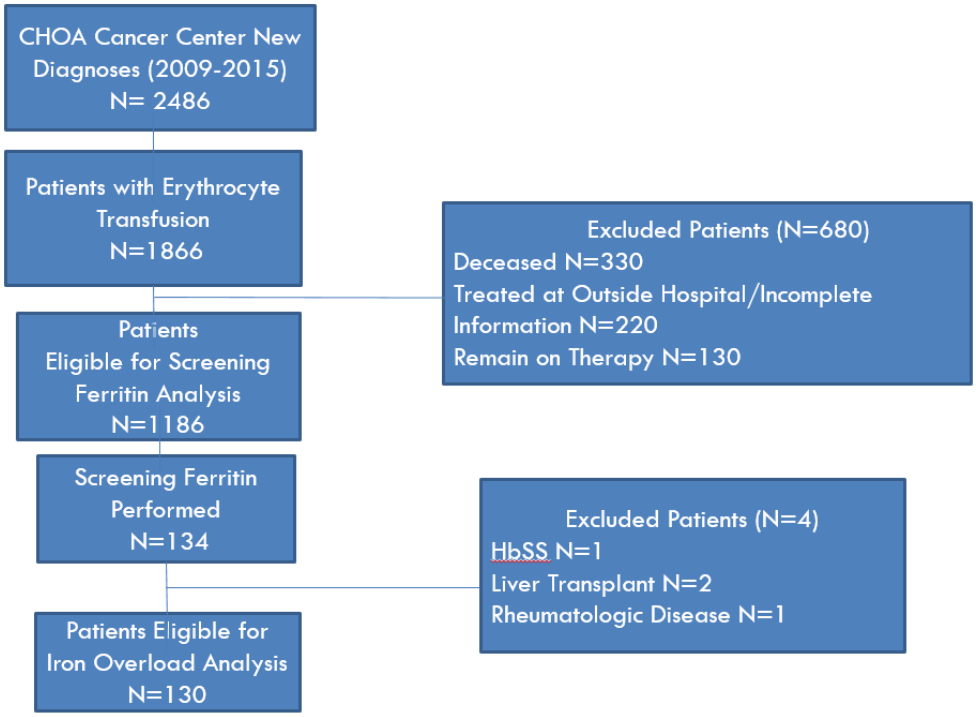


Table 1. Demographic and Clinical Characteristics for Cohort of Patients Eligible for Screening Ferritin*

Demographics	**Results (N=1186)**
Age at Diagnosis, mean (range)	7.8 (0-22)
Sex, N (%)	
Male	571 (48.1)
Female	615 (51.9)
Race/Ethnicity, N (%)	
White, Non-Hispanic	617 (52.0)
Black, Non-Hispanic	335 (28.3)
Hispanic	171 (14.4)
Other	63 (5.3)
Malignancy, N (%)	
Brain Tumor	260 (21.9)
Leukemia	363 (31.0)
Lymphoma	113 (9.5)
Myelodysplastic Syndrome	15 (1.3)
Solid Tumor	430 (36.3)
Intensity of Treatment Rating, N (%)	
1	58 (4.9)
2	431 (36.4)
3	546 (46.0)
4	151 (12.7)
Hematopoietic Stem Cell Transplant, N (%)	
Yes	111 (9.4)
No	1075 (90.6)
Number of Erythrocyte Transfusions, mean (range)	5.8 (1-65)

*Diagnosed from 2009-2015, received erythrocyte transfusion, completed therapy

Table 2. Comparison of patients who received and did not receive a Screening Ferritin to assess for Iron Overload

Demographics	Screened (N=134)	Not Screened (N=1052)	P Value
Age at Diagnosis, mean (range)	8.26 (0-20)	7.78 (0-22)	0.36
Sex, N (%)			0.41
Female	60 (10.5)	511 (89.5)	
Male	74 (12.0)	541 (88.0)	
Race/Ethnicity, N (%)			0.69
White, Non-Hispanic	68 (11.0)	549 (89.0)	
Black, Non-Hispanic	43 (12.8)	292 (87.2)	
Hispanic	16 (9.4)	155 (90.6)	
Other	7 (11.1)	56 (88.9)	
Malignancy, N (%)			**< 0.01**
Brain Tumor	15 (5.8)	245 (94.2)	
Leukemia	60 (16.3)	308 (83.7)	
Lymphoma	16 (14.2)	97 (85.8)	
Myelodysplastic Syndrome	5 (33.3)	10 (66.7)	
Solid Tumor	38 (8.8)	392 (91.2)	
Intensity of Treatment Rating, N (%)			**< 0.01**
1	0 (0.0)	58 (100.0)	
2	22 (5.1)	409 (94.9)	
3	27 (4.9)	519 (95.1)	
4	8.5 (56.3)	66 (43.7)	
Hematopoietic Stem Cell Transplant, N (%)			**< 0.01**
Yes	79 (71.2)	32 (28.8)	
No	55 (5.1)	1020 (94.9)	
Number of Erythrocyte Transfusions, mean (range)	12.68 (1-65)	5.00 (1-41)	**< 0.01**

Table 3. Comparison of Screened Patients with and without Iron Overload

Demographics	Iron Overload* (N=61)	No Iron Overload (N=69)	P Value
Age at Diagnosis, mean (range)	9.51	7.57	0.06
Sex, N (%)			0.18
Female	31 (53.4)	27 (46.6)	
Male	30 (41.7)	42 (58.3)	
Race/Ethnicity, N (%)			**0.02**
White, Non-Hispanic	27 (40.9)	39 (59.1)	
Black, Non-Hispanic	19 (46.3)	22 (53.7)	
Hispanic	13 (81.3)	3 (18.7)	
Other	2 (28.6)	5 (71.4)	
Malignancy, N (%)			0.08
Brain Tumor	6 (40.0)	9 (60.0)	
Leukemia	31 (51.7)	29 (48.3)	
Lymphoma	3 (18.8)	13 (81.2)	
Myelodysplastic Syndrome	4 (80.0)	1 (20.0)	
Solid Tumor	17 (50.0)	17 (50.0)	
Intensity of Treatment Rating, N (%)			**< 0.01**
1	0 (0.0)	0 (0.0)	
2	0 (0.0)	19 (100.0)	
3	12 (44.4)	15 (55.6)	
4	49 (58.3)	35 (41.7)	
Hematopoietic Stem Cell Transplant, N (%)			**< 0.01**
Yes	79 (71.2)	32 (28.8)	
No	55 (5.1)	1020 (94.9	
Number of Erythrocyte Transfusions, mean (range)	7.67 (1-22)	18.48 (3-65)	**< 0.01**
Time Since Therapy Completion to Screening Ferritin (months), mean (range)	11.6 (0.2-42)	21.4 (2.3-67)	**< 0.01**

*Iron Overload defined as ferritin ≥ 500 mg/mL

Figure 2. Distribution of Age at Diagnosis by Iron Overload in Those with and Without Iron Overload

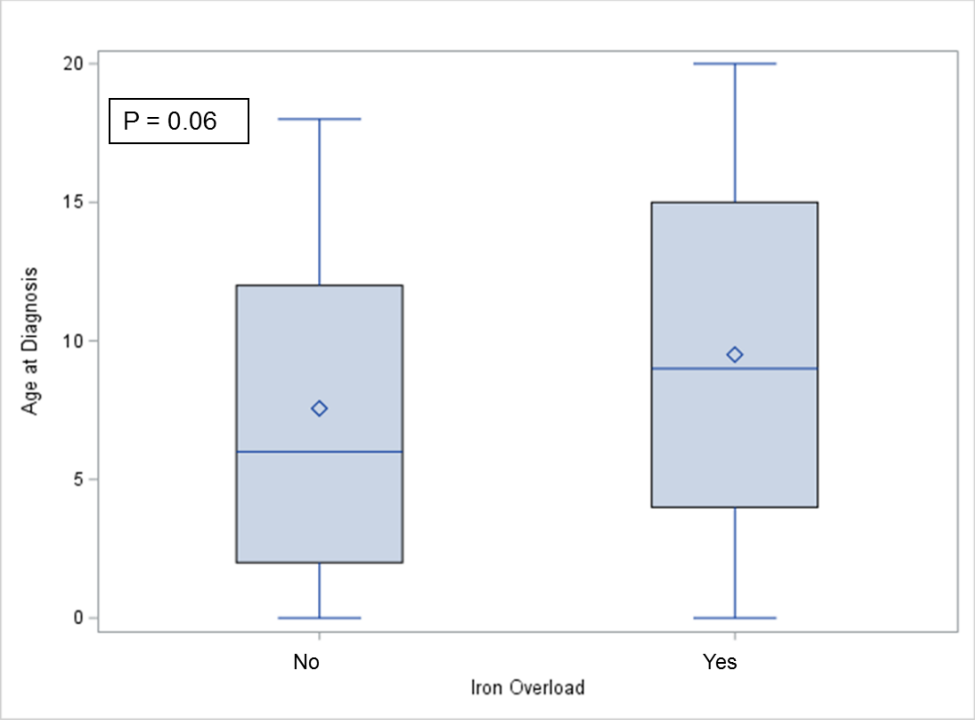


Figure 3. Distribution of Number of Erythrocyte Transfusions in those with and without Iron Overload

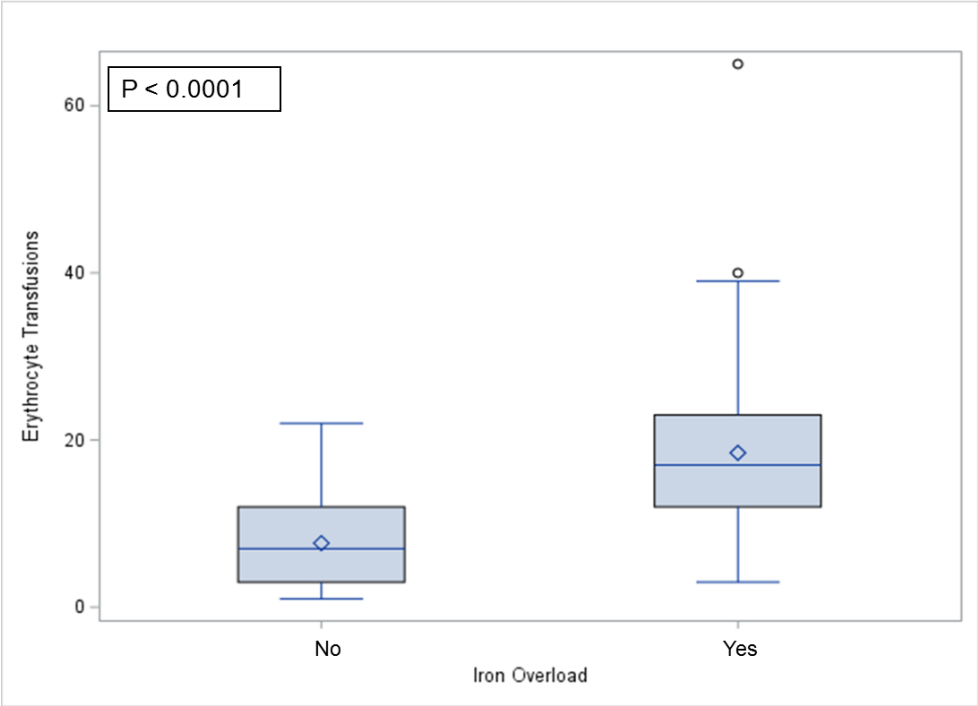


Figure 4. Distribution of Time Since Therapy Completion to Screening Ferritin in those with and without Iron Overload

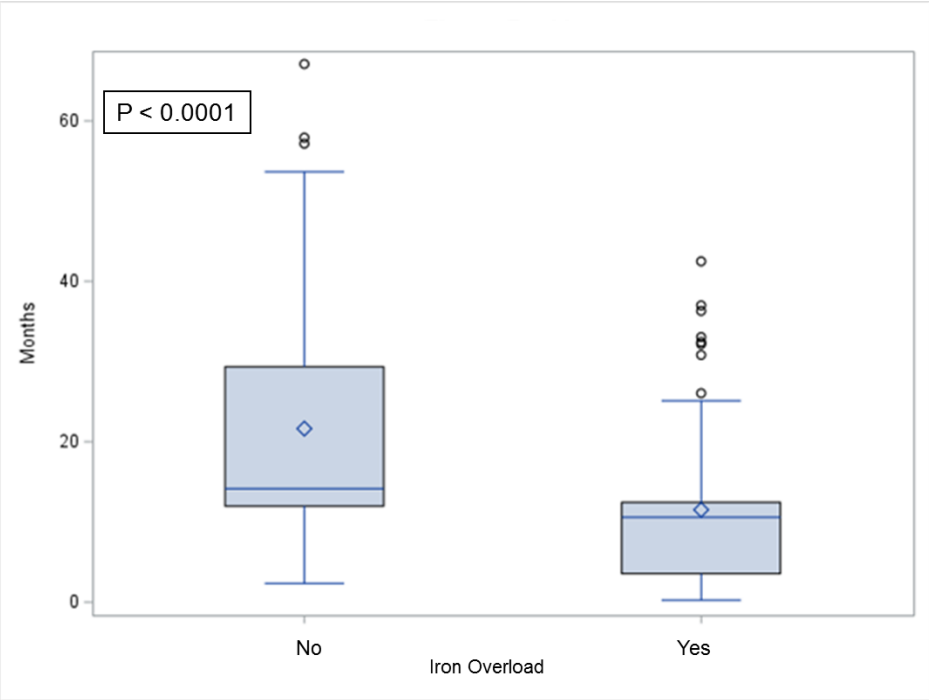


Figure 5. Relationship of Ferritin Value versus Number of Erythrocyte Transfusions in those with Screening Ferritin

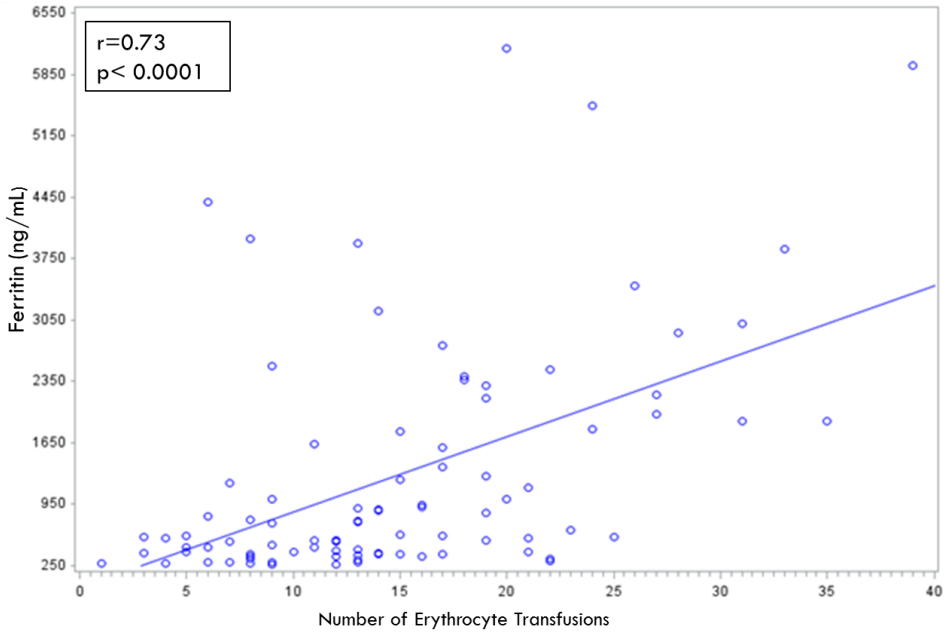


Table 4. Multivariate Analysis of Factors Associated with Iron Overload Focused on Treatment Intensity

Demographics	**OR (95% CI)**	**P value**	**aOR (95% CI)**	**P Value**
ITR		**0.01**		**0.01**
2	Ref		Ref	
3	14.4 (1.76-123.87)		13.53(1.41-130.19)	
4	25.2 (3.21-197.69)		26.91 (3.04-239.20)	
Age at Diagnosis	1.06 (1.0-1.13)	0.06	1.05 (0.98-1.13)	0.19
Sex, N (%)		0.18		0.23
Male	Ref		Ref	
Female	0.62 (0.31-1.25)		0.59 (0.25-1.40)	
Race/Ethnicity, N (%)		0.06		0.20
White, Non-Hispanic	Ref		Ref	
Black, Non-Hispanic	1.25 (0.57-2.74)		1.18 (0.45-3.05)	
Hispanic	6.26 (1.63-24.09)		5.03 (1.08-23.36)	
Other	0.58 (0.10-3.20)		0.66 (0.08-5.39)	
Time to Screening Ferritin (months)	0.94 (0.91-0.97)	**< 0.01**	0.95 (0.91-0.98)	**< 0.01**

Table 5. Multivariate Analysis of Factors Associated with Iron Overload Focused on Number of Erythrocyte Transfusions

Demographics	OR (95% CI)	P value	aOR (95% CI)	P Value
Number of Erythrocyte Transfusions	1.23 (1.15-1.34)	**< 0.01**	1.33 (1.19-1.49)	**< 0.01**
Age at Diagnosis	1.06 (1.0-1.13)	0.06	1.15 (1.04-1.32)	**< 0.01**
Sex, N (%)		0.18		0.23
Male	Ref		Ref	
Female	0.62 (0.31-1.25)		0.86 (0.30-2.44)	
Race/Ethnicity, N (%)		0.06		0.20
White, Non-Hispanic	Ref		Ref	
Black, Non-Hispanic	1.25 (0.57-2.74)		1.08 (0.35-3.34)	
Hispanic	6.26 (1.63-24.09)		5.41 (0.97-30.1)	
Other	0.58 (0.10-3.20)		0.77 (0.07-8.43)	
Time to Screening Ferritin (months)	0.94 (0.91-0.97)	**< 0.01**	0.96 (0.93-1.00)	0.07

Figure 6. Number of Transfusion Events by Intensity of Treatment Rating (ITR) in Patients Screened versus Not Screened for Iron Overload

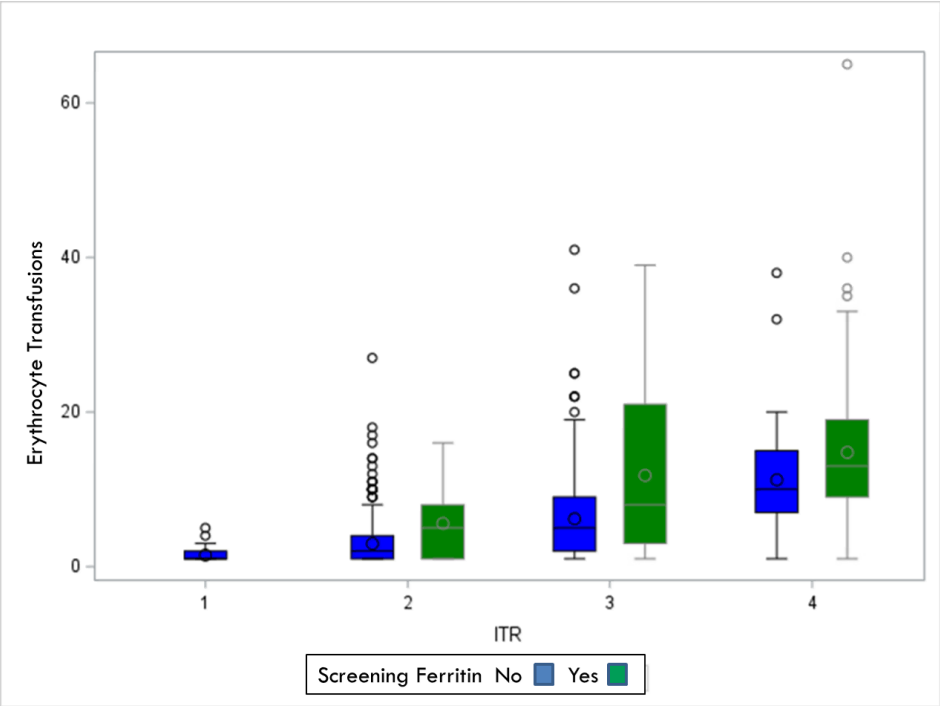


Table 6. Analysis of patients who had MRI Ferriscan to Quantify Iron Overload

Demographics	Ferriscan (N=24)	No Ferriscan (N=37)	P Value
Ferritin, Mean (Range)	2789.7.7 (574-10,000)	1704.4 (521-10,000)	**0.05**
Age at Diagnosis, mean (range)	10.8 (2-20)	8.7 (2-20)	0.19
Malignancy, N(%)			0.96
Brain Tumor	2 (33.3)	4 (66.7)	
Leukemia	13 (41.9)	18 (58.1)	
Lymphoma	1 (33.3)	2 (66.6)	
Myelodysplastic Syndrome	1 (25.0)	3 (75.0)	
Solid Tumor	7 (41.2)	10 (58.8)	
ITR, N (%)			0.25
2	0	0	
3	5 (41.7)	7 (58.3)	
4	19 (32.8)	30 (67.2)	
HSCT, N (%)			0.94
Yes	19 (39.6)	29 (60.4)	
No	5 (38.5)	8 (61.5)	
Number of Erythrocyte Transfusions, mean (range)	20.5 (3-65)	17.2 (4-39)	0.24

Figure 7. Ferritin Value versus Liver Iron Content (N=24)

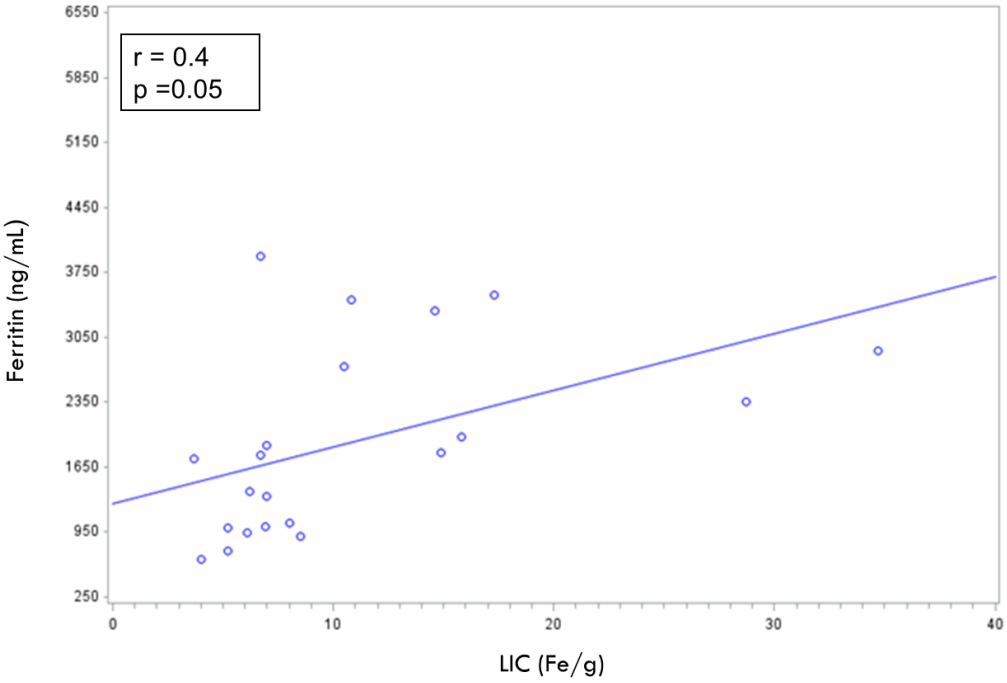
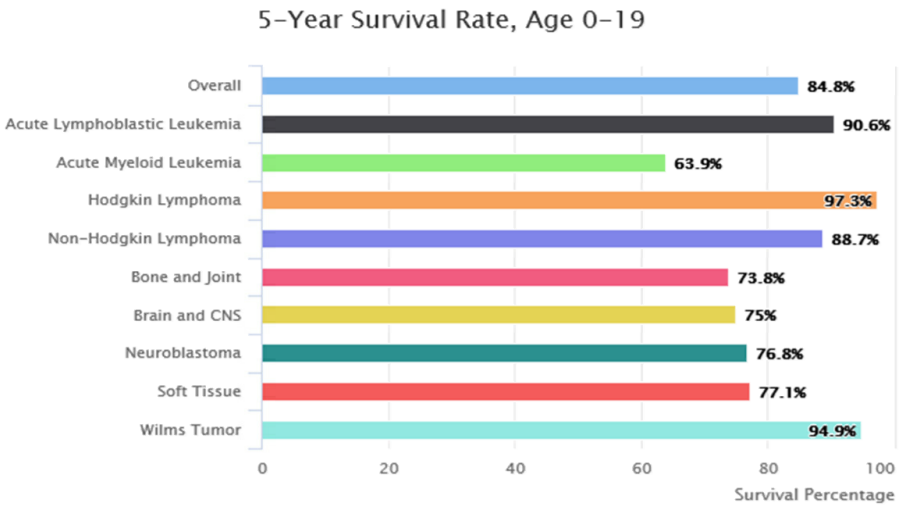


Table 7. Iron Overload Related Outcomes in Patients with MRI Ferriscan

Outcomes	**Results (N=24)**
LIC, median (IQR)	8.25 (6.5-15.4)
Abnormal Liver Function Tests, N (%)	3 (12.5)
Abnormal Echocardiogram, N (%)	1 (4.2)
Liver Biopsy, N (%)	2 (8.3)
Treatment for Iron Overload, N (%)	18 (75%) Phlebotomy: 11 Chelation: 2 Combination:5

***Abnormal Liver Function Tests defined as 3x the upper limits of normal**

Appendix 1:



Source: Surveillance, Epidemiology, and End Results (SEER) Program (seer.cancer.gov)

Appendix 2.

Intensity of Treatment Rating (ITR-3)

Directions: Please review carefully the criteria at the bottom of the page that lists examples of diseases and treatment modalities under each of the four levels of intensity. Based on the information regarding each patient's disease and treatment, use the criteria at the bottom of this page to circle one number to indicate the intensity of treatment (1, 2, 3, 4). Make your ratings based on the specified criteria, rather than your own clinical judgment.

ABSTRACTION INFORMATION							INTENSITY RATING
ID #	Diagnosis, including if relapsed	Stage or Risk Level	Treatment Modalities: Surgery?	Chemo?	Radiation?	Transplant?	
			y n	y n	y n	y n	1 2 3 4
			y n	y n	y n	y n	1 2 3 4
			y n	y n	y n	y n	1 2 3 4
			y n	y n	y n	y n	1 2 3 4
			y n	y n	y n	y n	1 2 3 4
			y n	y n	y n	y n	1 2 3 4
			y n	y n	y n	y n	1 2 3 4
			y n	y n	y n	y n	1 2 3 4

Level 1: Least Intensive Treatments

Includes the least intensive treatments, for these diseases or treatment modalities:

- Surgery Only – All tumor types except brain tumors
- Retinoblastoma - Enucleation (unilateral disease) without chemotherapy
- Wilms' Tumor (Stages 1, 2)
- Chronic Myeloid Leukemia - any chemotherapy, including tyrosine kinase inhibitors
- LCH, surgery or steroid injection only

Level 2: Moderately Intensive Treatments

Includes moderately intensive treatments, for these diseases or treatment modalities:

- Acute Lymphoblastic Leukemia (Low, Standard, or Intermediate Risk; precursor B cell)
- Brain Tumor - One treatment modality, not including biopsy
- Germ Cell Tumors - With chemotherapy or radiation
- Hepatoblastoma - With chemotherapy and surgical resection, no metastatic disease
- Hodgkin Lymphoma (Low/Intermediate risk: all stages except IIIB, IVB)
- Langerhans Cell Histiocytosis (LCH) with chemotherapy
- Neuroblastoma/Ganglioneuroblastoma (Stages 1, 2 w/chemotherapy and Stage 4S)
- Non-Hodgkin Lymphoma (Stages 1, 2, 3 and Groups A, B)
- Retinoblastoma - With chemotherapy
- Rhabdomyosarcoma (Stages 1, 2)
- Thyroid cancer
- Tumor, other – either chemo or radiation alone

Level 3: Very Intensive Treatments

Includes very intensive treatments, for these diseases or treatment modalities:

- Relapse Protocols for Hodgkins & Wilms' Tumor (first relapse) Only
- Acute Lymphoblastic Leukemia (ALL) (High Risk, Very High Risk, T-cell)
- Acute Myeloid Leukemia and Down Syndrome
- Acute Promyelocytic Leukemia (APL)
- Biphenotypic leukemia – treated like ALL
- Brain Tumor - Two or more treatment modalities
- Carcinoma NOS – Two or more treatment modalities
- Ewings Sarcoma
- Hepatoblastoma- With metastatic disease
- Hemophagocytic lymphohistiocytosis (HLH), chemo alone
- Hodgkin Lymphoma (Stages 3B or 4/High Risk)
- Juvenile Mylomonocytic Leukemia (JMML) – Pretransplant/chemo only
- Nasopharyngeal Carcinoma
- Neuroblastoma/Ganglioneuroblastoma (Stages 3, 4) - Without transplant
- Non-Hodgkin Lymphomas (Group C or Stage 4)
- Osteosarcoma
- Rhabdomyosarcoma (Stages 3, 4)
- Soft Tissue Sarcoma – Two or more treatment modalities
- Wilms' Tumor (Stages 3, 4) – Three treatment modalities
- Tumor, other – 2 or 3 treatment modalities

Level 4: Most Intensive Treatments

Includes the most intensive treatments, for these diseases or treatment modalities:

- Relapsed Disease - Excluding Hodgkin Lymphoma, first relapse of Wilms' Tumor, LCH with systemic treatment, or CML with a different tyrosine kinase inhibitor
- Hematopoietic Stem Cell Transplant (HSCT) - All diseases
- Acute Myeloid Leukemia (AML)
- Biphenotypic leukemia – treated like AML
- Juvenile Myleomonocytic Leukemia (JMML) - With transplant
- Brain tumor – with HSCT

***In case of more than one tumor, rate the tumor that falls into the highest level.**

Kazak, A.E., et al., A revision of the intensity of treatment rating scale: classifying the intensity of pediatric cancer treatment. Pediatr Blood Cancer, 2012. 59(1): p. 96-9.