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RESEARCH ARTICLE

Transfusional hemosiderosis in childhood cancer patients and survivors

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Abstract

Background: Children treated for cancer are at risk for adverse effects of iron due to transfusions administered during prolonged marrow suppression, which may increase exposure to toxic forms of iron, extrahepatic iron accumulation, and long-term organ damage.

Objective: This study aimed to characterize the severity and organ distribution of clinically significant, multisystem iron overload (IO) in an at-risk cohort of pediatric cancer patients.

Methods: This was a retrospective, cross-sectional study of childhood cancer patients who underwent a magnetic resonance imaging (MRI) due to clinical concern for IO. Data regarding cancer type and treatment, transfusion history, MRI and laboratory results, and treatment for IO were collected. Severity of IO was analyzed by non-parametric tests with respect to clinical characteristics.

Results: Of the 103 patients, 98% of whom had a Cancer Intensity Treatment Rating (ITR-3) of 3 or higher, 53% (54/102) had moderate or greater hepatic siderosis, 80% (77/96) had pancreatic siderosis, 4% (3/80) had cardiac siderosis, and 45% (13/29) had pituitary siderosis and/or volume loss. Pancreatic iron was associated with both cardiac ($p = .0043$) and pituitary iron ($p = .0101$). In the 73 off-therapy patients, ferritin levels were lower ($p = .0008$) with higher correlation with liver iron concentration (LIC) ($p = .0016$) than on-therapy patients. Fifty-eight subjects were treated for IO.

Conclusion: In this heavily treated cohort of pediatric cancer patients, more than 80% had extrahepatic iron loading, which occurs with significant exposure to toxic forms of iron related to decreased marrow activity in setting of transfusions. Further studies should examine the effects of exposure to reactive iron on long-term outcomes and potential strategies for management.

KEYWORDS
childhood cancer, iron overload

Abbreviations: AML, acute myeloid leukemia; HSCT, hematopoietic stem cell transplant; IO, iron overload; LIC, liver iron concentration; LPI, labile plasma iron; NTBI, non-transferrin-bound iron; RBC, red blood cell; TIBC, total iron-binding capacity.

1 | INTRODUCTION

Patients with cancer may receive multiple red blood cell (RBC) transfusions. While it is true that humans have no way to excrete excess iron, the high iron levels should block dietary absorption and the excess iron will eventually be utilized over time. However, these transfusions lead to iron accumulation that may persist, particularly in the extrahepatic sites, long after patients have completed their cancer therapy, leading to long-term exposure to toxic forms of iron and the consequences of this exposure are unknown.[1–3] Although iron accumulation based on liver iron concentration (LIC) accurately reflects total body iron, it does not reflect movement of toxic ferrous iron into the heart and endocrine organs. Iron measured by magnetic resonance imaging (MRI) is in the non-reactive ferric state. Transferrin bound non-reactive ferric iron enters cells via the transferrin receptor, a process that is shut off if intracellular iron levels are high.[4,5] Reactive, ferrous iron is present at very low levels in circulation, but increases dramatically when transferrin saturation exceeds 60%, which occurs as soon as erythropoiesis is suppressed by cytotoxic chemotherapy.[6] Ferrous iron can enter cells non-physiologically through divalent calcium and zinc transporters that are not regulated by iron. While it is clear from transfusion-dependent anemias that organ toxicity is related to exposure to ferrous iron, the presence and impact of exposure to toxic iron is not clear in pediatric oncology patients.[3]

Toxicities such as endocrine failure and malignant transformation are related to iron overload (IO),[7,8] and overlap with treatment-induced late effects observed in cancer survivors.[9–11] Considering the existing data that these toxicities are reversible or even preventable by treatment of IO in transfusion-dependent anemia patients,[12,13] it would be reasonable to assume that this would be the case in pediatric oncology patients despite the lack of corresponding data in this population. Organ toxicity from iron is related primarily to the amount of reactive iron in tissue and duration of exposure.[5,14] Due to expected long-term survival of most pediatric cancer patients, mitigation of complications is of increased importance. As these iron-related complications may take decades to become apparent, appropriately designed prospective studies to prove causality is not feasible. Based on the known risks of long-term exposure to excess iron from other disorders[14] and the current ability to easily monitor and safely remove excess iron,[15] correction of IO in pediatric cancer patients should be a clinical priority for pediatric survivorship clinics. However, there are many challenging questions regarding when and how to treat the IO that occurs in cancer patients.

The Iron Overload Program at Children's Hospital Los Angeles (CHLA), which focuses on chronic transfusion-dependent anemia and genetic IO syndromes, started an oncology Iron Overload Clinic in 2016. The diagnostic and treatment approaches are based on the current understanding of principles of iron biology and contemporary oncology treatment. We present a retrospective assessment of organ-specific IO in a diverse sample of pediatric oncology patients and discuss the results in the context of the iron toxicity biology.

2 | METHODS

2.1 | Study design

This was a single-center, retrospective cohort study performed at CHLA, and was approved by the institutional review board with waiver of consent.

2.2 | Study objectives

The primary study objective was to evaluate the severity and organ distribution of clinically significant transfusion-related IO in a cohort of pediatric cancer patients that had an MRI completed for clinical concern of IO. LIC was measured and used as a surrogate marker for total body iron, while measures of cardiac, pancreatic, and pituitary iron deposition were assessed and used as surrogate markers for labile iron exposure. Secondary objectives were to (i) assess associations between cancer type and the level and distribution of IO; (ii) assess associations between hepatic and/or pancreatic IO with pituitary and cardiac iron levels; (iii) assess treatment and follow-up in patients diagnosed with IO; and (iv) assess the correlation between RBC transfusion exposure and serum markers of IO and MRI-confirmed organ siderosis.

2.3 | Study population

Eligible subjects were currently aged 1–24 years old, post or currently receiving treatment for a malignancy, and had undergone evaluation for IO by MRI of the abdomen, heart, and/or pituitary gland. The decision to screen for IO was at the discretion of the treating oncologist, and which MRI to complete was based on patient-level factors and clinical judgment. Since 2015, all patients seen in our cancer survivorship clinic are screened for IO with a serum ferritin test, and referred to IO Clinic for confirmed values greater than 1000. To establish the cohort, all patients with a primary oncologic diagnosis who were seen at CHLA between January 1, 2007 and December 31, 2019 were identified using diagnostic and procedural billing code data from inpatient and clinic visits. Subsequently, this list was cross-referenced with our clinical database to identify patients with a diagnosis of cancer who had at least one MRI completed for IO. Patients were included regardless of disease status or treatment type.

2.4 | Study procedures

An institutional clinical database was utilized to capture study data, and included evaluations for transfusional hemosiderosis with initial MRI and contemporaneous measurements (within 1 month) of serum iron, total iron-binding capacity (TIBC), transferrin saturation, and ferritin. RBC transfusion history was determined by blood dispensed from the CHLA Blood Bank. Subsequent MRI evaluations, initiation, and/or adjustments in therapy with chelation or phlebotomy were also collected. Data regarding the patients' cancer type, date of diagnosis,

TABLE 1 Subject characteristics.

Characteristic	Summary Median	[IQR]
Age at Cancer Diagnosis (yrs)	10.7	[10.0]
Age at MRI (yrs)	14.0	[9.5]
Time from cancer diagnosis (yrs)	3.0	[3.2]
	N	**[%]**
Sex		
Male	60	[58%]
Female	43	[42%]
Ethnicity		
Asian	12	[12]
Black	2	[2]
Hispanic	55	[53]
Non-hispanic white	28	[27]
Other	6	[6]
Oncologic Diagnosis		
Leukemia/Lymphoma	60	[58%]
HR-ALL	32	[27%]
SR-ALL	4	[4%]
AML	20	[19%]
JMML	1	[1%]
CML	1	[1%]
Lymphoma	2	[2%]
Solid Tumor	15	[14.5%]
Brain Tumor	13	[13%]
Neuroblastoma	15	[14.5%]
Treated with HCST		
Total	60	[61%]
Autologous	40	[39%]
Allogeneic	23	[22%]
Completed cancer therapy		
Yes	73	[71%]
No	30	[29%]
Cancer treatment intensity		
2	2	[2%]
3	31	[30%]
4	70	[68%]
RBC Transfusion History		
# transfusions	20	[17]
Total volume (ml/kg)	173	[163]

Note: Subject characteristics (total $n = 103$) including demographics, cancer diagnosis and treatment intensity, and transfusion history. Continuous variables reported as median [interquartile range], and categorical variables as frequency [%].

Abbreviations: AML, acute myeloid leukemia; CML, Chronic myeloid leukemia; HR-ALL, high-risk acute lymphoblastic leukemia; HSCT, hematopoietic stem cell transplant; JMML, juvenile myelomonocytic leukemia; MRI, magnetic resonance imaging; RBC, red blood cell; SR-ALL, standard-risk acute lymphoblastic leukemia.

[a]Non-neuroblastoma, including embryonal and alveolar rhabdomyosarcoma and sarcoma NOS.

[b]Including central nervous system (CNS) tumor NOS, primitive neuroectodermal tumor (PNET).

requirement of hematopoietic stem cell transplant (HSCT), as well as adverse outcomes including relapse and/or death were also collected. Subjects were categorized by cancer therapy using the validated Intensity of Treatment Rating Scale (ITR-3),[16] and defined as being "on therapy" if they were still receiving cancer-directed therapy at the time of initial MRI.

Measures of siderosis using MRI were collected using gradient echo and spin echo pulse sequences on 1.5 Tesla magnets. The relaxation rates R2 and R2* were estimated using custom Matlab routines, where R2 and R2* are the reciprocals of T2 and T2*, respectively. Liver R2* was converted to LIC using the calibration by Wood et al.[17] Severity of LIC, pancreatic R2*, and cardiac T2* was determined by predefined clinically important thresholds.[18–20] LIC by MRI[21] was defined as mild (LIC 3 to <7 mg/g), moderate (7 to <15 mg/g), and severe (≥15 mg/g). Pancreatic R2* from 27 to less than 100 Hz was considered mild to moderate, and 100 Hz or greater indicated severe pancreatic siderosis based on association of R2* greater than 100 with cardiac siderosis and abnormal glucose metabolism.[22] Cardiac T2* 20–30 ms was defined as borderline, while T2* less than 20 ms indicated cardiac siderosis. Severity of pituitary siderosis was determined by predefined thresholds of R2 values converted to *Z*-scores, where $Z \geq 2$ was considered clinically significant. Pituitary volume in the anterior and posterior planes was also converted to *Z*-scores, where $Z \leq -2$ was consistent with volume loss.[23,24] These pituitary parameters have been related to the risk of clinical hypogonadism in thalassemia patients.[25]

2.5 | Statistical methods

Statistical analyses were conducted in JMP Pro, Version 14.0.0 (SAS Institute Inc.) using a two-sided *p*-value of .05 for significance. Patient characteristics were reported as median (interquartile range [IQR]) for continuous variables, and as frequency (percent) for categorical variables. Continuous variables were not normally distributed, and therefore log-transformed to better fit normality for linear regression. Continuous variables were assessed against categorical variables by Wilcoxon or Kruskal–Wallis non-parametric tests. Dichotomous variables were analyzed by Pearson's χ^2 test or Fisher's exact test.

3 | RESULTS

3.1 | Characteristics of subjects

Patient characteristics are summarized in Table 1 ($n = 103$). The median age was 13.5 years (range 1–24 years) at initial evaluation for IO; 60 (58%) were male. The most frequent cancer types were high-risk

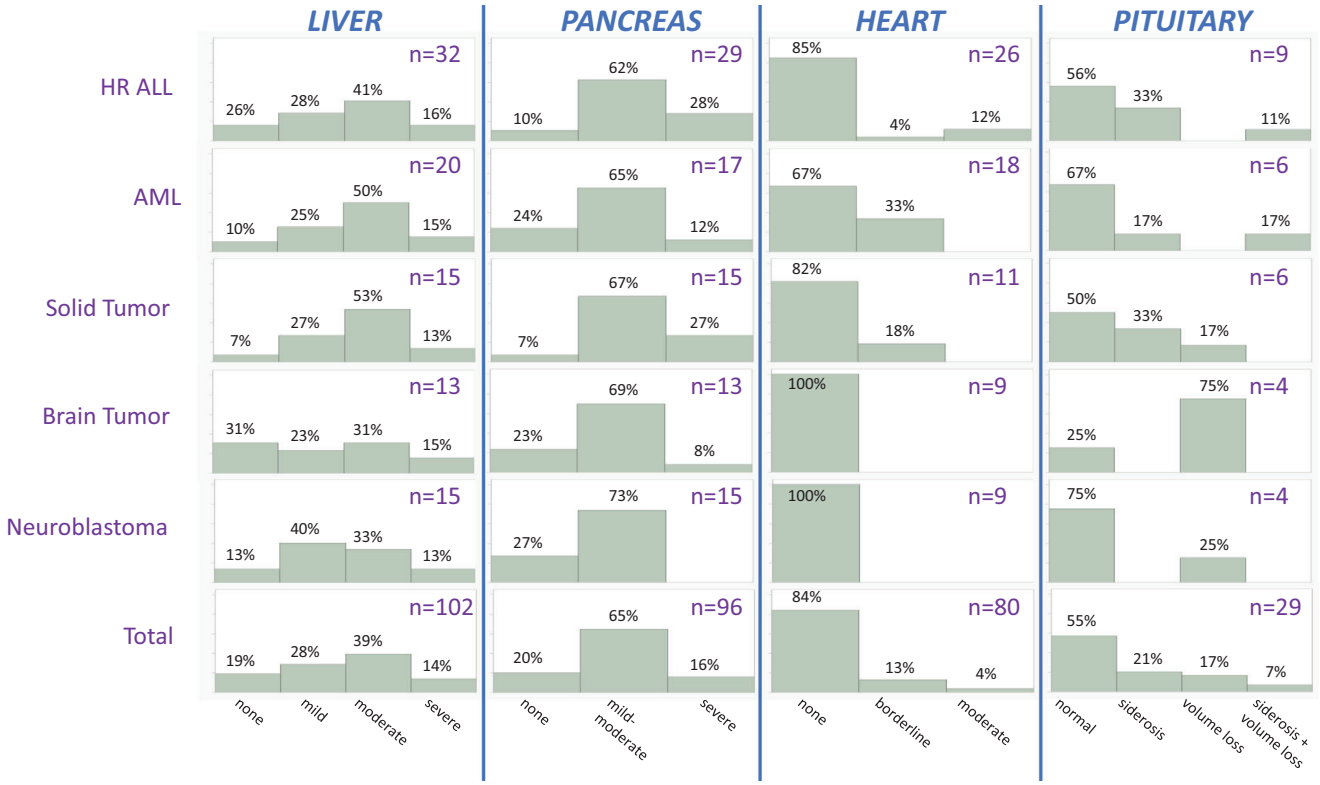

FIGURE 1 Prevalence of iron deposition by cancer diagnosis and organ. Histograms with frequencies represented as percentage of sample (*n*) for each diagnosis and organ. Liver iron severity defined by liver iron concentration (LIC) as none ($x < 3$), mild ($3 \leq x < 7$), moderate ($7 \leq x < 15$), or severe ($x \geq 15$). Pancreatic iron defined by pancreas R2* as none ($x < 28$), mild–moderate ($28 \leq x < 100$), or severe ($x \geq 100$). Cardiac iron defined by cardiac T2* as none ($x \geq 30$), borderline ($20 \leq x < 30$), or moderate ($x < 20$). Pituitary siderosis defined by pituitary R2 *Z*-score ≥2 in coronal and sagittal planes, while pituitary volume loss defined by pituitary total volume *Z*-score ≤−2.

B-cell precursor acute lymphoblastic leukemia (HR-ALL; $n = 28$, 27%), followed by acute myelogenous leukemia (AML; $n = 20$, 19%). Sixty subjects received HSCT, including autologous transplant for solid tumors ($n = 22$). At the time of assessment, 73 were post completion of treatment. Subjects received a median of 20 transfusions (173 mL/kg based on weight at the time of MRI).

3.2 | Severity and distributions of iron overload

All subjects had an abdominal MRI to assess LIC, except one who had a cardiac MRI only ($n = 102$, 99%). MRI iron evaluation was completed at a mean of 3.2 years (2 months to 12.5 years) after initial cancer diagnosis, which was after the end of therapy in 73 of 103 subjects (71%) at a mean 1.9 (range: 0.01–9.1) years. Of the 30 subjects who had IO evaluation prior to end of therapy, 17 (57%) were assessed in anticipation of HSCT, while the remaining 13 subjects were assessed due to various clinical concerns.

Figure 1 demonstrates the distribution of subjects by severity of siderosis in each organ assessed. Of the 102 subjects with LIC evaluated, 53% had moderate or greater IO (LIC ≥7 mg/g dry liver weight [DLW]). Of the 96 subjects with pancreatic iron quantification, 80% (77/96) had evidence of pancreatic siderosis (pancreatic R2* >27), and 16% (15/96) had severe siderosis (R2* >100). Borderline cardiac siderosis (T2* <30) was found in 12.5% (10/80) of subjects, and true cardiac siderosis (T2* <20) in 4% (3/80). Of the 29 patients assessed for pituitary involvement, six patients had pituitary siderosis, five had pituitary volume loss, and two patients had both.

Hepatic siderosis was associated with pancreatic siderosis ($p < .0001$), but not cardiac or pituitary siderosis, while pancreatic iron was predictive of both cardiac ($p = .0043$) and pituitary siderosis ($p = .0101$). All patients with pituitary siderosis had pancreatic siderosis as well, and only patients with severe pancreatic siderosis (>100 Hz) had moderate or greater cardiac siderosis. Neither hepatic nor pancreatic siderosis in patients with HSCT differed from non-transplanted subjects (7.9 vs. 7.7 mg/g [$p = .4378$], 39 vs. 42 Hz [$p = .7275$], respectively), even when limited to allogeneic transplant ($p = .6435$, .3798). There was no association between IO and cancer type. The distribution based on the treatment rating scale (ITR-3) is given in Table 1, and there was no association detected between the ITR-3 score and IO ($p = .1870$).

Hepatic siderosis was associated with transfusion history, whether calculated by number of transfusions ($p < .0001$, $R^2 = .30$) or total volume by weight ($p < .0001$, $R^2 = .39$) (Table 2). Pancreatic siderosis was also associated with transfusions, although with poor linear fit, for both transfusion number ($p = .0021$, $R^2 = .11$) and volume ($p = .0009$,

TABLE 2 Univariate analyses of iron markers versus characteristics.

Characteristics	Ferritin (ng/mL), $n = 85$	Iron saturation (%), $n = 71$	R2* LIC (mg/g DLW), $n = 102$	Pancreas R2* (Hz), $n = 96$	Cardiac T2* (ms), $n = 80$
Age at cancer diagnosis (years)	**1.69 (0.0007)**	**0.15 (0.0285)**	0.13 (0.1616)	**0.25 (0.0153)**	−0.05 (0.1679)
Age at MRI (years)	**1.05 (0.0438)**	0.14 (0.1850)	0.08 (0.5770)	0.27 (0.0916)	−0.08 (0.1252)
Time from cancer diagnosis (years)	**−0.80 (0.0086)**	**−0.17 (0.0097)**	−0.06 (0.4998)	−0.11 (0.3332)	−0.003 (0.9278)
Oncologic diagnosis	**(0.0016)**	**(0.0015)**	(0.4450)	(0.2204)	(0.4342)
Leukemia/lymphoma	2550	77.5	7.6	44	33.7
HR-ALL	3120	85	7.8	62	33.2
SR-ALL	617	58	1.3	27	33.2
AML	2535	82	8.6	49	34.1
JMML	919	45	6.2	29	46.7
CML	7610	55	3.0	23	32.6
Lymphoma	1840	66.5	2.4	42.5	40.5
Solid tumor[a]	1130	47	11.6	42	34.2
Brain tumor[b]	1835	46	5.1	39	35.5
Neuroblastoma	813	44	6.9	30	34.4
Treated with HSCT[c]	2010 (0.9711)	58.5 (0.7090)	7.9 (0.4509)	39 (0.7176)	34.3 (0.1172)
Autologous	**1630 (0.0206)**	**44 (0.0019)**	8.1 (0.6203)	36 (0.3222)	35.1 (0.4296)
Allogeneic	2430	73	7.6	46	34.2
Completed cancer therapy	**(0.0008)**	**(0.0006)**	(0.9350)	**(0.0353)**	**(0.0036)**
Yes	1400	51	7.2	37	35.1
No	3120	86	8.5	62	31.3
Cancer treatment intensity	(0.0935)	(0.6659)	(0.0577)	(0.4098)	**(0.0330)**
2	446	49.5	1.2	31	29.7
3	1920	65.5	7.9	42.5	31.9
4	1950	61	7.8	39	35
RBC transfusion history					
# Transfusions	**1.05 (0.0023)**	0.09 (0.2819)	**0.56 (<0.0001)**	**0.4 (0.0021)**	−0.3 (0.4931)
Total volume (mL/kg)	**0.85 (0.0043)**	0.08 (0.2837)	**0.55 (<0.0001)**	**0.37 (0.0009)**	−0.03 (0.4555)

Note: Univariate associations of iron biomarkers with subject characteristics (total $n = 103$). Association with continuous variables reported as parameter estimate (p-value) for linear regression, while association with categorical variables reported as group median and (p-value) for non-parametric tests. Bold indicates p-values significant at $\alpha = .05$.

[a]Non-neuroblastoma, including embryonal and alveolar rhabdomyosarcoma and sarcoma NOS.

Abbreviations: AML, acute myeloid leukemia; CML, Chronic myeloid leukemia; DLW, dry liver weight; HR-ALL, high-risk acute lymphoblastic leukemia; HSCT, hematopoietic stem cell transplant; JMML, juvenile myelomonocytic leukemia; LIC, liver iron concentration; MRI, magnetic resonance imaging; RBC, red blood cell; SR-ALL, standard-risk acute lymphoblastic leukemia.

[b]Including central nervous system (CNS) tumor NOS, primitive neuroectodermal tumor (PNET).

[c]p-Values reported for history of HSCT vs. none, and for history of autologous vs. allogeneic HSCT.

$R^2 = .12$). Cardiac siderosis was not associated with transfusion history (see Table 2). Based on receiver operating characteristic (ROC), using mild or greater IO as the outcome, transfusion history had greatest sensitivity and specificity at 16 transfusions ($p = .0003$, area under the ROC curve (AROC = .8447) or 118 mL/kg ($p = .0001$, AROC = .8914).

Comparing on-therapy versus off-therapy patients, both ferritin (3120 vs. 1400 ng/mL, $p = .0008$) and iron saturation (86% vs. 51%, $p = .0006$) were significantly higher in patients on therapy. However, LIC and pancreatic iron were not different between these groups (LIC 8.5 vs. 7.2 mg/g, $p = .9350$, pancreas R2* 62 vs. 37 Hz, $p = .35$). Cardiac T2* was slightly lower in those still on therapy (31.3 vs. 35.1 ms, $p < .01$), but not at a clinically relevant level.

3.3 | Treatment and follow-up of iron overload in pediatric cancer patients

Fifty-eight subjects (56%) were treated for IO. Of these, 29% (17/58) were treated with phlebotomy, 60% (35/58) with chelation, and

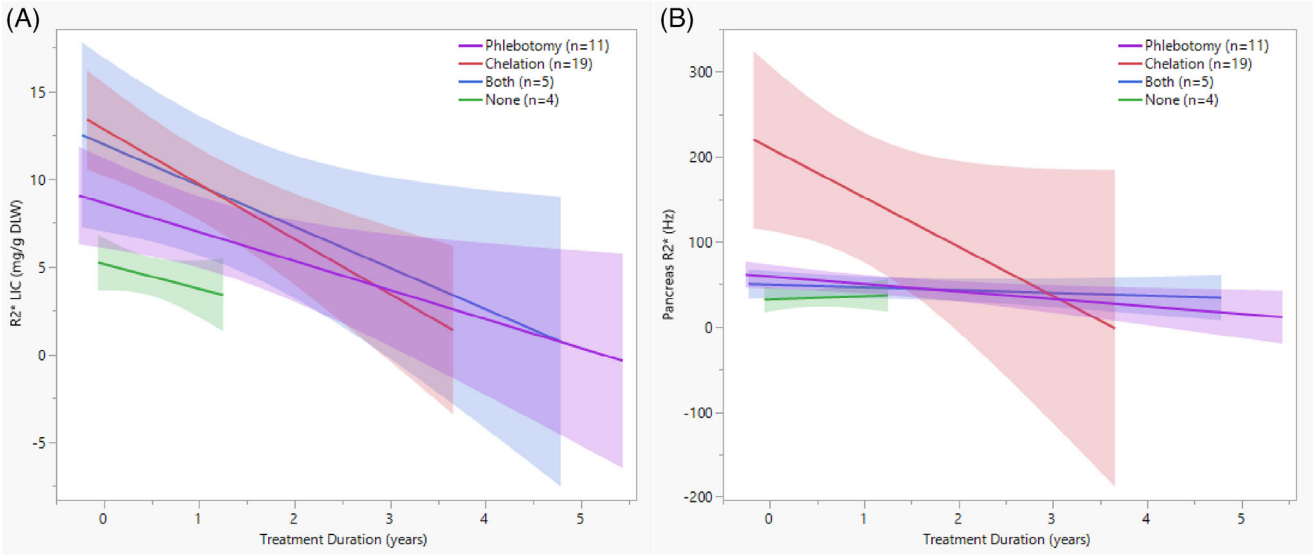

FIGURE 2 Hepatic and pancreatic iron over time by method of iron-removing therapy. Multilinear regression of (A) hepatic iron by R2* liver iron concentration (LIC) and (B) pancreatic iron by R2* from baseline to last follow-up in subgroup of subjects who had multiple magnetic resonance imaging (MRI) assessments. Subjects grouped by method of iron-removing therapy with line of fit and shaded 95% confidence interval.

10% (6/58) with both therapies. Treatment duration was a mean of 1.1 years for either phlebotomy or chelation, while subjects treated with both were on iron reducing therapy for a mean of 3.2 years. While treatment was not started until after completing cancer therapy in the majority of subjects, eight started chelation (four with deferasirox, four with deferoxamine) while receiving cancer therapy and experienced no side effects. One subject tolerated phlebotomy during maintenance chemotherapy after first relapse of B-ALL and had no issues. Follow-up MRI was available in 60% of treated subjects, and all but five demonstrated a reduction in LIC. The rate of reduction was greater with chelation than with phlebotomy, and greater still with both modalities, although these differences were not statistically significant (Figure 2A). Pancreatic iron was generally higher in subjects treated with chelation, leading to a much greater rate of reduction than with other iron-removing therapies (Figure 2B).

3.4 | Correlation between RBC transfusions, ferritin, and liver iron concentration on MRI

In 85 patients, a corresponding ferritin measurement was done in conjunction with the MRI completed for IO. Ferritin was associated with LIC, although with poor linear fit ($p = .0054$, $R^2 = .09$) and ferritin was not associated with IO by logistic regression ($p = .3698$). Multivariate analysis showed that RBC transfusion volume and ferritin were independently associated with LIC ($p < .0001$ and .0031, respectively). Ferritin remained associated with LIC ($p = .0016$, $R^2 = .17$) in only the patients who had completed cancer therapy, while ferritin was not associated with LIC ($p = .3575$, $R^2 = .03$) in patients who had not yet completed cancer therapy.

4 | DISCUSSION

The primary conclusion of this work in which we quantified liver, pancreatic, cardiac, and pituitary IO in a cohort of pediatric cancer patients evaluated for transfusional IO as part of their clinical care is that there is significant partitioning of iron into extrahepatic sites in a large percentage of pediatric patients after aggressive treatment for cancer. At least 80% had evidence of extrahepatic iron loading (pancreas, pituitary, heart). This extrahepatic loading only occurs with significant exposure to the highly reactive and toxic ferrous form of iron (labile plasma iron [LPI]), which is related to decreased marrow erythroid activity in the face of multiple transfusions.[4,14]

In addition to iron loading through transferrin receptor-mediated processes and pathological loading via ion channels,[5,14] the liver loads iron by reticuloendothelial ingestion of erythrocytes. Thus, increase in LIC is linearly related to the number of transfusions,[21] and can be considered a surrogate for transfusion volume in the absence of chelation therapy or significant bleeding, which could include iatrogenic blood loss secondary to frequent blood draws. In contrast, pathological extrahepatic iron loading occurs only when circulating reactive ferrous (Fe^{++}) iron enters via divalent ion transporters (Zn^{++} and Ca^{++}), which are not downregulated by intracellular iron. The reactive ferrous subspecies of non-transferrin-bound iron (NTBI) is referred to as LPI.[4,5,14,26]

The relationship between erythropoiesis and patterns of IO has been described in numerous studies of individuals with chronic transfusion-dependent anemia associated with normal, ineffective, and no erythropoiesis.[5,27] The liver loads first and independently of marrow activity, presumably because of the large reticuloendothelial space and direct ingestion of RBC.[27] In the presence of high circulating NTBI/LPI, the pancreas loads sooner than the heart.[22] Thus,

erythropoietic activity is a major regulator of ferrous iron levels, iron toxicity, and distribution of iron into endocrine organs and heart. Patients treated for high-risk malignancies have often been exposed to intensive chemotherapy that can transiently shut off erythropoiesis,[28] leading to prolonged exposure to elevated levels of NTBI/LPI due to the absence of erythropoiesis, with NTBI/LPI levels subsequently decreasing with marrow recovery as has been shown during HSCT conditioning.[6,29] Transferrin saturation, an indirect measure of LPI,[5] often increases to greater than 80% in HSCT,[6,29] and is inversely related to reticulocyte count.[28,30] Although other factors may differ between pediatric cancer patients and those with primary hematologic conditions, based on these studies, the assumption remains that prolonged periods of suppression of erythropoiesis with increased exposure to elevated NTBI/LPI would likely increase one's potential risk of extrahepatic iron deposition. In our study, both LIC and pancreas R2* were associated with RBC transfusion history in this cohort, but the lower R^2 for pancreas R2* (.12 vs. .39) highlights the point that factors other than transfusion increase the risk of extrahepatic iron.

Most subjects tested had some level of pancreatic IO with a small percentage demonstrating levels (>100 Hz) in the range that has been associated with glucose intolerance and diabetes and potential risk for cardiac iron.[22,31] Consistent with prior studies, the relatively low rate of true cardiac IO in our study is likely due to the fact that the majority of patients in this cohort had pancreatic iron levels less than 100 Hz, which has been shown to be a strong negative predictor for cardiac iron.[22]

Of those evaluated for pituitary IO, 28% had siderosis and pancreatic siderosis was evident in all those evaluated, consistent with prior studies of pituitary iron loading in transfusion-dependent anemia.[25] Pituitary volume loss has been associated with pituitary dysfunction in transfused populations even in the absence of pituitary siderosis,[25] suggesting that LPI may cause damage even before there is enough loading to be detected by MRI. In this cohort, cranial radiation and chemotherapy in addition to RBC transfusion may affect pituitary volume and siderosis.[32,33] These pituitary iron and volume changes are particularly thought provoking, considering that 62% of at-risk survivors of childhood cancer develop endocrine disorders.[34] Further study is needed to determine the extent to which iron deposition and volume loss contribute to endocrine dysfunction.

Not surprisingly, there was no relation between treatment intensity (ITR-3)[16] and magnitude of IO or distribution of iron loading in this cohort. This was likely due to insufficient variability among the subjects, as all were in the highest levels of the intensity scale; further, this scale does not specifically consider magnitude and duration of erythroid suppression. HSCT also did not influence iron loading or distribution in this cohort, again likely due to the overall higher intensity of treatment received by this cohort. Prior studies indicate that higher intensity therapy, particularly HSCT, is associated with higher transfusion burden and higher levels of NTBI, thereby increasing risk of IO in both hepatic and extrahepatic sites.[35–37]

While ferritin levels were obtained in this study and were often used by the referring oncologists to screen for iron loading, the diagnosis of IO was based on MRI determination of LIC. Ferritin levels do correlate with LIC in large populations, but the variability is very large, making single measurements not useful, and even trends can be misleading with ferritin trending upward when LIC is stable or dropping.[38] We used ferritin trends to optimize timing of confirmatory MRI for assessment of tissue iron, as this is the "gold standard," although ferritin can be used to infer iron burden in resource-restricted settings. Ferritin level greater than 1000 ng/dL has been used based on data from large populations of transfusion-dependent anemia patients,[38–41] and confirmation with repeated measurement should prompt MRI for verification of IO to more accurately assess if iron removing therapy is needed. Transfusion history was found to be associated with diagnosis of IO by logistic regression, and although this is not a case–control study, we found that 16 transfusions or 118 mL/kg were the most sensitive and specific thresholds. Ferritin was not associated with IO by logistic regression in this study. Transferrin saturation is also highly variable, although an elevated iron saturation, greater than 60% on several measures, indicates higher likelihood of exposure to LPI, which increases risk of endocrine and cardiac iron deposition, and thus utilization of chelation instead of or in addition to phlebotomy should be considered.

The main strength of this study is the measurement of pancreatic, pituitary, and cardiac iron loading as a surrogate for exposure to the toxic forms of iron and demonstration of significant extrahepatic loading in a diverse population of pediatric oncology patients. Inclusion of all referred patients, consistency in diagnostic approach, and assessment of available follow-up MRI in some patients are additional strengths. Limitations include that this was an at-risk cohort, specifically evaluated due to concern for possible IO. The high occurrence and magnitude of extrahepatic iron, compared to a previous cohort randomly sampled across diagnoses,[1] reflect that this is a heavily treated cohort in which IO was assessed due to clinical concern. Thus, although these results may not be generalizable to all pediatric cancer patients, the results are valid for children treated with higher intensity cancer therapy and PRBC transfusions.

The data in this study raise many interesting questions regarding IO and its incidence, prevalence, and relation to specific therapy in malignancy. Critically, we need to know how much iron is in the body after chemotherapy, and whether extrahepatic iron distribution is present. This information can be obtained by a single abdominal MRI with pancreas in the field. If the pancreas is positive, the heart and pituitary should be assessed. Long-term outcomes of pediatric cancer survivors with respect to IO are not known, but we believe pathological iron should be addressed before the patient transitions from the pediatric cancer treatment center. Future work should also evaluate whether iron chelation during chemotherapy and stem cell transplant even at a low dose may limit iron deposition in extrahepatic sites and improve long-term outcomes.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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