Transfusional Hemosiderosis in Childhood Cancer Patients and Survivors

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Abstract

Background: Children treated for cancer are at risk of developing iron toxicity due to receiving red cell transfusions and myelosuppressive chemotherapy. Transfusions administered during prolonged episodes of marrow suppression may increase exposure to toxic, reactive forms of iron and thereby increase risk of extrahepatic iron accumulation and long-term organ damage. **Objective:** This study aimed to evaluate the severity and organ distribution of clinically significant iron overload through measurement of hepatic, cardiac, pancreatic, and pituitary iron deposition in an at-risk cohort of children and young adults treated for cancer. **Methods:** This retrospective study evaluated patients treated for any type of cancer who underwent an MRI due to clinical concern for evaluation of iron overload (n=103, 73 post-treatment). Data regarding cancer type and treatment, MRI and laboratory results, and treatment for iron overload were analyzed. **Results:** Over half (53%) of this sample had moderate or greater hepatic siderosis, 80% had pancreatic siderosis, and nearly half (45%) had pituitary siderosis and/or volume loss. Pancreatic iron was associated with both cardiac (p=0.0043) and pituitary iron (p=0.0101). Patients treated for acute myeloid leukemia or high-risk acute lymphoblastic leukemia had higher liver iron concentration (LIC) compared to other cancer types (median LIC 8.5 vs. 2.9 mg/g DLW, p=0.0011). **Conclusion:** Pediatric cancer patients are at risk for transfusional iron overload, with significant exposure to toxic forms of iron indicated by extrahepatic iron deposition (pancreas, heart and pituitary). Further studies should examine the effect of exposure to reactive iron on long-term outcomes and develop management recommendations.

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Abbreviations	
IO	Iron Overload
LIC	Liver Iron Concentration
RBC	Red Blood Cell
TIBC	Total Iron Binding Capacity
HSCT	Hematopoietic Stem Cell Transplant
HR-ALL	High Risk Acute Lymphoblastic Leukemia
AML	Acute Myeloid Leukemia
NTBI	Non-Transferrin Bound Iron
LPI	Labile Plasma Iron

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Over half (53%) of this sample had moderate or greater hepatic siderosis, 80% had pancreatic siderosis, and nearly half (45%) had pituitary siderosis and/or volume loss. Pancreatic iron was associated with both cardiac (p=0.0043) and pituitary iron (p=0.0101). Patients treated for acute myeloid leukemia or high-risk acute lymphoblastic leukemia had higher liver iron concentration (LIC) compared to other cancer types (median LIC 8.5 vs. 2.9 mg/g DLW, p=0.0011).

Conclusion:

Pediatric cancer patients are at risk for transfusional iron overload, with significant exposure to toxic forms of iron indicated by extrahepatic iron deposition (pancreas, heart and pituitary). Further studies should examine the effect of exposure to reactive iron on long-term outcomes and develop management recommendations.

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 is unknown.[1,2] 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 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. 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.[3] 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.

Toxicities such as endocrine failure and malignant transformation are related to iron overload (IO)[4,5] and overlap with treatment-induced late effects observed in cancer survivors.[6-8] Considering the existing data that these are reversible or even preventable by treatment of IO in transfusion dependent anemia patients,[9,10] 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.[11] Due to expected long-term survival of most pediatric cancer patients, mitigation of complications is of increased importance. Since these iron-related complications may take decades to become apparent, appropriately-designed prospective studies to prove causality are not feasible. Based on the known risks of long-term exposure to excess iron from other disorders[11] and the current ability to easily monitor and safely remove excess iron[12], 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 iron overload syndromes, started an oncology Iron Overload Clinic in 2016. The diagnostic and treatment approaches are based on our understanding of principles of iron biology and understanding of the current 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.

Methods

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.

Study objectives

The primary objective of this study 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 iron overload. Liver iron concentration (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 a surrogate marker for labile iron exposure. Secondary objectives were to (1) assess associations between cancer type and the level and distribution of IO; (2) assess associations between hepatic and/or pancreatic IO with pituitary and cardiac iron levels; (3) assess treatment and follow up in patients diagnosed with IO; and (4) assess the correlation between serum markers of IO and MRI-confirmed organ siderosis.

Study population

Eligible subjects were currently aged 1 to 24 years old, were 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 judgement. Since 2015, all patients seen in our cancer survivorship clinic are screened for IO with a serum ferritin level and referred to IO Clinic for confirmed values > 1000. To establish the cohort, all patients with a primary oncologic diagnosis that 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 1 MRI completed for IO. Patients were included regardless of disease status or treatment type.

Study procedures

An institutional clinical database was utilized to capture study data and included evaluations for transfusional hemosiderosis with initial MRI and contemporaneous measurements of serum iron, total iron binding concentration (TIBC), transferrin saturation, and ferritin. 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, 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).[13]

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 liver iron concentration (LIC) using the calibration by Wood et al.[14] Severity of LIC, pancreatic R2*, and cardiac T2* were determined by predefined clinically important thresholds.[15-17] LIC by MRI[18] was defined as mild (LIC 3 to <7 mg/g), moderate (7 to <15 mg/g), and severe (15 mg/g or greater). Pancreatic R2* from 27 to <100 Hz was considered mild to moderate and 100 Hz or greater indicated severe pancreatic siderosis based on association of R2* > 100 with cardiac siderosis and abnormal glucose metabolism.[19] Cardiac T2* 20-30 ms was defined as borderline, while T2*$<$ 20 ms indicated cardiac siderosis. Severity of pituitary siderosis was determined by predefined thresholds of R2 values converted to Z-scores, where Z [?] 2 was considered clinically significant. Pituitary volume in the anterior and posterior planes was also converted to Z-scores,

where Z [?] -2 was consistent with volume loss.[20,21] These pituitary parameters have been related to the risk of clinical hypogonadism in thalassemia patients.[22]

Statistical methods

Statistical analyses were conducted in JMP® Pro, Version 14.0.0 (SAS Institute Inc., Cary, NC, 2018) using a two-sided p-value of 0.05 for significance. Patient characteristics were reported as mean [range] for continuous variables and as frequency (percent) for categorical variables. Univariate analyses used Student's t test or Wilcoxon non-parametric test for continuous variables, and Pearson's χ^2 test or Fisher's exact test for dichotomous variables.

Results

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 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 time of assessment, 73 were post-completion of treatment.

Prevalence and Severity of Iron Overload

All subjects had an abdominal MRI (n=102, 99%) to assess LIC except one who had a cardiac MRI only. MRI iron evaluation was completed at a mean of 3.2 years (2 months to 12.5 yrs) after initial cancer diagnosis, which was after end of therapy in 73 of 103 subjects (71%) at a mean 1.9 (range 0.01 to 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, 6 patients had pituitary siderosis, 5 had pituitary volume loss and 2 patients had both.

Hepatic siderosis was associated with pancreatic siderosis (p <0.0001) but not cardiac or pituitary siderosis, while pancreatic iron was predictive of both cardiac (p=0.0043) and pituitary iron (p=0.0101). All patients that had evidence of pituitary siderosis had evidence of pancreatic iron deposition as well, and only patients with severe pancreatic iron overload (>100Hz) were noted to have moderate or greater cardiac siderosis. Neither hepatic nor pancreatic siderosis in patients with stem cell transplant differed from non-transplanted subjects (p=0.4378, p=0.7275, respectively), even when limited to allogeneic transplant (p=0.6435, p=0.3798). There was no association between IO and cancer type. Among subjects treated for leukemia/lymphoma, higher LIC was observed in subjects treated for AML or high-risk ALL compared to other cancer types (median LIC 8.5 vs. 2.9 mg/g DLW, p=0.0011), regardless of whether they had received HSCT. Based on the Treatment Rating Scale (ITR-3), the majority of subjects (70/103) received the most intensive treatments, while 30 subjects received very intensive and two subjects received moderately intensive treatments. There was no association between the ITR-3 score and IO (p=0.1870).

Treatment and follow up of iron overload in pediatric cancer patients

Presence of hepatic siderosis was associated with utilization of iron-reducing therapy (p <0.0001), and the type of therapy (phlebotomy, chelation, or both) appeared to correlate with LIC (Figure 2).

Seventeen percent of subjects were treated with phlebotomy, 34% with chelation, and 6% with both therapies. Subjects who received chelation had higher LIC on average than subjects who received phlebotomy (p=0.0356), but the sample size was not large enough to detect a difference between those who received both

chelation and phlebotomy versus those who received either phlebotomy or chelation. Treatment duration was a mean of 1.1 years for either phlebotomy or chelation, while subjects treated with both were on therapy for a mean of 3.2 years. 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 3A). 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 3B).

Correlation between ferritin and liver iron concentration on MRI

In 85 patients, a corresponding ferritin was done in conjunction with the MRI completed for IO. Ferritin was linearly associated with LIC (p=0.0054, R^2=0.09). Based on receiver operating characteristic analyses, ferritin >800 was 80% predictive of mild or greater hepatic siderosis by MRI.

Discussion

In this novel study we analyzed the impact of pancreatic, cardiac, and pituitary IO quantified in a cohort of pediatric cancer patients evaluated for transfusional IO as part of their clinical care. The results highlight the significant level of IO in some pediatric cancer patients with the majority of patients in this cohort showing at least moderate levels of IO (LIC > 7 mg/g). Importantly, at least 80% had evidence of extrahepatic iron loading (pancreas, pituitary, heart) which implies significant exposure to the toxic, reactive ferrous form of iron.[11,23] The high occurrence and magnitude of extrahepatic iron compared to a previous cohort randomly sampled across diagnoses[1] reflects sample bias as subjects entered this cohort because their oncologists had enough clinical concern to obtain iron studies. Hence, the diagnoses in Table 1 are routinely treated with intense transfusion and chemotherapy.

In addition to iron loading through transferrin receptor-mediated processes and pathological loading via ion channels[11], the liver loads iron by reticuloendothelial ingestion of erythrocytes. Thus, increase in LIC is linearly related to the number of transfusions and can be considered a surrogate for transfusion volume in the absence of chelation therapy or significant bleeding. 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 down-regulated by intracellular iron.[11,24] The reactive ferrous sub species of non-transferrin-bound iron (NTBI) is referred to as labile plasma iron (LPI).

Normally, iron from senescent autologous or transfused RBC is recycled and used to make new RBC. However, when erythropoiesis is suppressed after cytotoxic chemotherapy, the iron binding ability of transferrin is exceeded and levels of the NTBI and ferrous iron become very elevated leading to unregulated transport of reactive iron into extrahepatic sites.[3,25,26] Thus, erythropoietic activity is a major regulator of ferrous iron levels, iron toxicity, and distribution of iron into endocrine organs and heart. Iron is only detectable by MRI after it is converted to non-reactive ferric iron and stored as aggregates of ferritin.[27-29] Organ dysfunction does correlate with the amount of extrahepatic iron detected by MRI.[11,22,30,31]

This physiology is supported by numerous studies of iron loading, extrahepatic iron distribution, end organ function and response to chelation in individuals with chronic transfusion dependent anemia associated with normal, ineffective, and no erythropoiesis.[32,33] The liver loads first and independently of marrow activity, presumably because of the large reticuloendothelial space and direct ingestion of RBC.[32] In the presence of high circulating NTBI/LPI, the pancreas loads sooner than the heart.[19] Presumably the relative loading rates of the extrahepatic sites depends on differing kinetics of ferrous iron transport in various divalent iron transporters in different organs.

Patients treated for high-risk malignancies have often been exposed to intensive chemotherapy that can transiently shut off erythropoiesis, 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 clearly shown during HSCT conditioning[3,26]. Transferrin saturation, an indirect measure of LPI,[33] often increases to >80% in HSCT even when the patient is not iron loaded at the outset[3,26] and is inversely

related to reticulocyte count.[25,34]

Most subjects (80%) had some level of pancreatic IO, and 16% had levels in range that has been associated with glucose intolerance and diabetes.[35] Twenty-eight percent (8/29) of the pituitary MRIs had siderosis and pancreatic siderosis was evident in all those evaluated, consistent with prior studies of pituitary iron loading in transfusion dependent anemia.[22] Pituitary volume loss has been associated with pituitary dysfunction in transfused populations even in the absence of pituitary siderosis,[22] suggesting that LPI may cause damage even before there is enough loading to be detected by MRI. However, in this cohort, cranial radiation or chemotherapy likely affect pituitary volume and function as well. Theoretically, oxidant damage from radiation or chemotherapy will be markedly amplified by the presence of ferrous iron. This is suggested by the 29-fold increased risk of second malignancy when radiation is delivered with myelosuppressive chemotherapy.[36] Thus, we cannot exclude a possible contribution of LPI to the volume loss seen in the brain tumor patients even though there was no detectable iron in the pituitary (Figure 1). This raises interesting questions regarding treatment timing and delivery of radiation in proximity to marrow suppression when reactive iron levels would be very high. These pituitary iron and volume changes are particularly thought provoking considering that over 75% of survivors of childhood cancer have pituitary dysfunction by 50 years of age.[37]

Not surprisingly, there was no relation between treatment intensity (ITR-3)[13] 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 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.[38-40]

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.[41] 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. We found ferritin over 800 ng/dl is 80% predictive of a LIC >3 mg/g, consistent with data from large populations of transfusion dependent anemia patients,[42-44] and confirmation with repeated measurement should prompt MRI for verification of IO to more accurately assess if iron removing therapy is needed. Transferrin saturation is also highly variable, although in large epidemiological studies, elevated transferrin alone predicts early death and higher probability of malignancy.[45] An elevated iron saturation, >60% on several measures, indicates higher likelihood of exposure to LPI, which increases risk of endocrine and cardiac iron deposition, and utilization of chelation instead of or in addition to phlebotomy should be considered. In fact, high pancreatic iron likely prompted a number of subjects in this cohort to be treated with chelation rather than phlebotomy (Figure 3B).

The primary and novel conclusion of this work is that there is significant partitioning of iron into extrahepatic sites in a large percent of pediatric patients after aggressive treatment for cancer. This extrahepatic loading only occurs with significant exposure to the highly reactive and toxic ferrous form of iron (LPI). The presence of elevated LPI is caused by decreased marrow erythroid activity in the face of multiple transfusions.

Strengths of this study include a diverse patient population with regard to age and diagnosis, and the inclusion of initial and follow up MRI evaluation of liver, pancreas, cardiac and pituitary, the last of which is novel in this setting. Inclusion of all referred patients and consistency in diagnostic approach are additional strengths. Limitations include that this was an at-risk cohort specifically evaluated over concern for possible IO. Thus, these results are generalizable to children treated with higher-intensity cancer therapy and many PRBC transfusions, but less so to childhood cancer patients as a whole. An additional limitation was the inability to include PRBC transfusion data due to variations in documentation over the study period.

The goal of this work is to protect cancer survivors from the toxicity of iron over their lifespan. It is clear from work outside the oncology realm that iron toxicity is primarily related to the amount of tissue ferrous iron and the duration of exposure.[23] This means a small amount of iron over many decades can cause damage. As an example, ineffective erythropoiesis with transfusion every three weeks and no chelation in thalassemia leads to pituitary dysfunction in about 5 years, diabetes in about 10, heart failure and death in about 15 years and increased cancer risk in the fourth and fifth decade.[35,46,47] It is also clear that reducing iron can reverse endocrine failure and reduce risk of new cancers by 30% even when iron is treated in the sixth decade.[10,48]

There are many interesting questions raised by this data 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. Since we do not know how much area-under-the-curve exposure to ferrous iron in childhood will cause issues decades later, it seems prudent to eliminate measurable pathologic iron as soon as is practical. Furthermore, measurable toxicity occurs long after loading is detected and likely after the patient leaves the pediatric environment. We think it is optimal that pathological iron be addressed before the patient transitions from the pediatric cancer treatment center. In addition to the magnitude and distribution of loading, family understanding of the reasons for iron removal, the likelihood that normalizing iron will reduce risk, reassurance of the certainty of our ability to safely remove the measurable iron, and design of a flexible approach that is acceptable to the family are all critical considerations.

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Figure Legends:

Figure 1: Prevalence of iron deposition by cancer diagnosis and organ

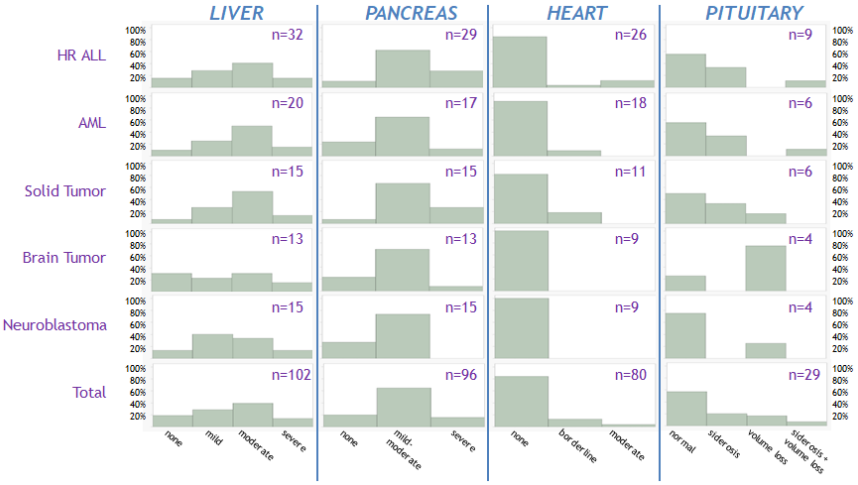
Histograms with frequencies represented as percentage of sample (n) for each diagnosis and organ. Liver iron severity defined by liver iron concentration (LIC) as none (x<3), mild (3[?]x<7), moderate (7[?]x<15), or severe (x[?]15). Pancreatic iron defined by pancreas R2* as none (x<28), mild-moderate (28[?]x<100), or severe (x[?]100). Cardiac iron defined by cardiac T2* as none (x[?]30), borderline (20[?]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.

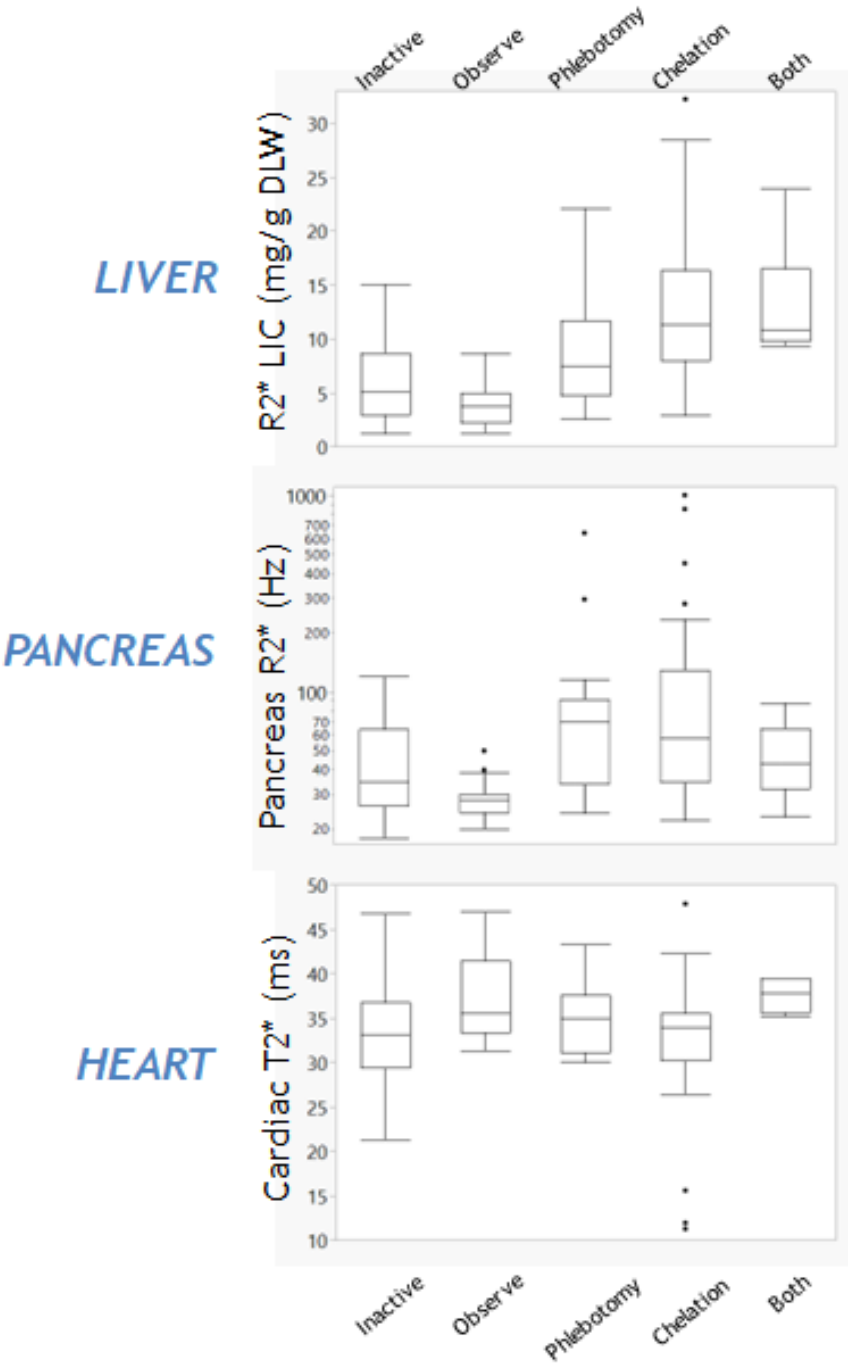
Figure 2. Iron deposition by organ and prescribed therapy

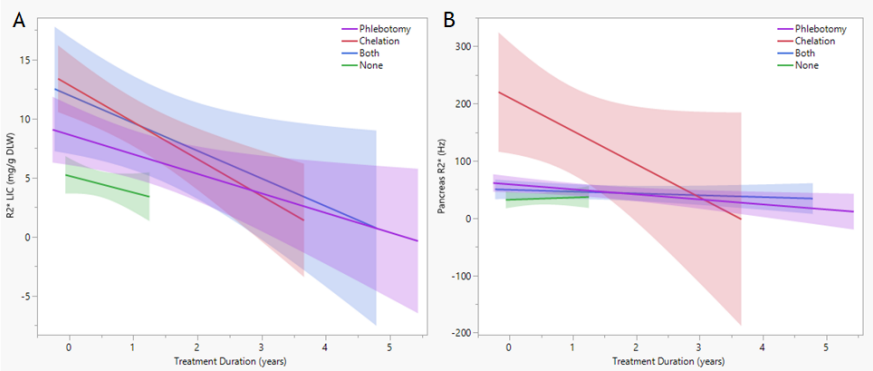
Stem and leaf plots of iron deposition by organ with respect to management. Liver iron concentration (LIC) and pancreatic iron measurements by R2* were associated with intervention (p<0.0001), as was cardiac iron by T2* (p=0.028). Pancreatic iron is expressed on logarithmic scale to accommodate outliers.

Figure 3: 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 MRI assessments. Subjects grouped by method of iron removing therapy with line of fit and shaded 95% confidence interval.







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