MAJOR ARTICLE

Increased Serum Iron Levels and Infectious Complications after Liver Transplantation

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Background. Elevated serum iron levels have been associated with infectious outcomes in various patient populations but, to our knowledge, have never been studied after liver transplantation.

Methods. The relationship between serum iron levels and infectious outcomes after liver transplantation was evaluated in a nested case-control study using prospectively collected data and serum samples. Unadjusted and adjusted hazard ratios were calculated for each iron marker predictor variable (iron level, unsaturated iron-binding capacity, total iron-binding capacity, transferrin saturation, and ferritin level) and time to development of each of 6 outcomes (cytomegalovirus [CMV] disease, invasive fungal infection, bacteremia, invasive fungal infection or bacteremia, any infection, and 1-year mortality rate).

Results. Serum measurements ($n = 109$) corresponding to increased levels of serum iron were independently associated with an increased risk of any infection and death. After adjusting for the number of red blood cell transfusions, donor CMV-seropositive status, and fungal colonization, ferritin level was independently associated with the development of any infection (hazard ratio, 1.09; 95% confidence interval, 1.04–1.14). After adjusting for the number of red blood cell transfusions, development of CMV disease, and administration of intravenous steroids for treatment of rejection, ferritin level was also was independently associated with death (hazard ratio, 1.11; 95% confidence interval, 1.04–1.18). Similar results were found for unsaturated iron binding capacity for the same 2 outcomes.

Conclusions. A better understanding of iron metabolism and its relationship to infection could help guide future infection prognosis, prevention, and management efforts in this high-risk population.

Infections following orthotopic liver transplantation (OLT) are an important cause of morbidity and mortality, despite improvements in surgical techniques, post-transplantation immunosuppressant treatment, and use of antimicrobial agents for infection prophylaxis. With a better understanding of risk factors for infections among OLT recipients, efforts can be directed towards preventing such complications. Previous studies have identified several risk factors for bacterial, viral, and fungal infections after OLT [1–7]. Of particular note, the number of cellular blood products, including red blood cells (RBCs) transfused intraoperatively, has been identified as an independent risk factor for various types of infections, such as surgical site infection and intraabdominal abscesses, in several studies of OLT recipients [3, 8–11]. This increased risk of bacterial and fungal infection associated with RBC transfusions also occurs in other surgical populations in a dose-dependent fashion [12–18].

A potential hypothesis to explain the increased risk of infection associated with RBC transfusions is the increased availability of iron, a known vital growth factor for most bacteria and fungi, in the transfused recipient's blood. Although viruses do not require iron, infected host cells need this element to synthesize viral particles. In vitro studies support the role of iron in the pathogenesis of human viral infections, such as cytomegalovirus (CMV), hepatitis C, herpes simplex virus, and human immunodeficiency virus infection [19–22]. Multiple in vitro studies and animal models have demonstrated that iron, including iron from heme sources [23], promotes increased virulence of bacterial and fungal infections by counteracting the presence of

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unsaturated iron-binding proteins and other innate antimicrobial effects of host plasma [24–26]. Readily available serum iron is metabolized by pathogen iron and heme enzyme systems, facilitating increased bacterial and fungal growth that may overwhelm other host defenses and result in clinical infection. Host iron sequestration, which occurs with "stress hypoferremia" in response to acute infection or injury [27, 28], reverses this pathogenic effect and restores the antimicrobial properties of host serum [29, 30]. This decrease in iron availability, or "iron-withholding," may serve as a defense mechanism after infection or other stressful events [31].

Although the role of iron in the pathogenesis of infection has been well documented, its exact role in the clinical setting of infection remains unclear. Approximately a dozen studies have explored the relationship between the host's iron level and clinical outcome, including bacterial and fungal infections; however, these studies were not controlled for many common risk factors for infection [5, 32–43]. In addition, to our knowledge, serum markers of iron metabolism have never been studied in the clinical setting of infection after OLT. The objective of this study was to examine whether serum iron markers (iron level, ferritin level, total iron-binding capacity [TIBC], unsaturated iron-binding capacity [UIBC], and transferrin saturation) that correlate with increased levels of serum iron are associated with an increased risk for bacterial, viral, or fungal infection after OLT in a well-defined cohort of patients.

MATERIALS AND METHODS

Patients and serum samples. We conducted a retrospective analysis of a nested cohort study of data and serum samples collected prospectively during a randomized, double-blinded, placebo-controlled trial that evaluated the effect of CMV immunoglobulin prophylaxis on the prevention of CMV disease and its complications in patients who underwent OLT [44]. The original trial included 146 children and adults who underwent liver transplantation during the period from December 1987 through June 1990 at 4 university hospitals in Boston, Massachusetts. All liver transplantation candidates and all liver donors were screened for CMV antibody. Patients were enrolled at the time of transplantation and observed clinically for up to 1 year. Subjects in the original study were randomized to receive either intravenous CMV immunoglobulin or placebo (1% serum albumin). CMV immunoglobulin was assayed for iron, and none was detected. Patients were given immunosuppressive therapy standard for the time during which the study was conducted; therapy generally included cyclosporine, azathioprine, corticosteroids, and murine monoclonal antibody to T3 antigen (OKT3). In the original study, 35% of patients received induction OKT3. Acute rejection was treated primarily with intravenous bolus steroid infusion. Refractory or recurrent rejection was treated with OKT3. Details regarding the timing of the intervention, serum sampling, and follow-up are described elsewhere [44].

Definitions of infection. CMV disease was defined as a clinical indication of organ dysfunction with biopsy-proven CMV infection in the affected organ documented either by virus isolation or histopathologic evidence [44]. Bacteremia was defined as isolation of a bacterial organism from blood samples obtained from patients with symptoms and/or signs of infection. Isolation of a gram-negative organisms, *Staphylococcus aureus, Streptococcus* species, *Listeria monocytogenes,* or *Clostridium* species from a single blood specimen was sufficient to qualify for a diagnosis of true bacteremia. Bacteremia due to other gram-positive organisms was considered significant and not due to contamination if the organism was isolated from at least 2 blood specimens obtained from different sites simultaneously or at different times but ⩽7 days from one another [2, 45]. Invasive fungal infection (IFI) was defined as the identification of fungal or yeast species by culture or histological examination of a sample from a normally sterile site. Fungal colonization was defined as isolation of a fungus or yeast species by culture or histological examination of a sample from a nonsterile site (ie, urine, throat, sputum, or skin) [8]. Decisions to obtain specimens for bacterial, fungal, or viral culture or histological examination were made by individual transplantation teams on the basis of clinical judgment. Details regarding surveillance cultures for CMV and serological studies for CMV are reported elsewhere [44].

Measurement of serum iron markers. A subset of serum samples collected from patients at least 1 week after OLT but before any infectious events of CMV disease, bacteremia, or IFI with sufficient volume remaining were thawed and analyzed for this study. This single time point selected for serum iron measurements is similar to that in previous studies that examined iron levels at a single time point and clinical outcomes [33, 35, 38, 39, 42]. The serum specimens had been stored at −20°C. Serum iron level, UIBC, and ferritin level were assessed at the Tufts Medical Center clinical laboratory (Boston, MA) using standard Clinical Laboratory Improvement Amendments–approved methods by individuals blinded to patients' clinical characteristics. TIBC was calculated as iron level plus UIBC, and transferrin saturation level was calculated as iron level divided by TIBC.

Covariates. Demographic, pretransplantation, intra-operative, and posttransplantation variables were analyzed for association with 6 different outcomes: CMV disease, bacteremia, IFI, bacteremia or IFI, any infection, and death. Pretransplantation variables included age, sex, race, CMV donor and recipient serostatus, primary liver disease leading to transplantation, and creatinine clearance. Intraoperative variables included type and number of blood products units transfused (RBCs, platelets, and fresh frozen plasma) and transplantation surgical time. Post-

transplantation variables included immunosuppressive medications received for induction and rejection, major intraabdominal operation, liver retransplantation, vascular and/or biliary complications, rejection, and receipt of CMV immune globulin for CMV infection prophylaxis.

Statistical analysis. All analyses were performed using SAS software, version 9.1 (SAS Institute). The mean values for each iron marker (iron level, transferrin saturation, TIBC, UIBC, and ferritin level) were compared for subjects with versus subjects without each outcome (CMV disease, IFI, bacteremia, bacteremia or IFI, any infection, and 1-year mortality rate) using the Student *t* test. Serum samples were selected at the time at least 1 week after transplantation and before the development of an outcome of interest, where applicable. Unadjusted hazard ratios (HRs) were calculated for each iron marker predictor variable and for time to development of each of the 6 outcomes by use of Cox proportional hazard survival models. Multivariate adjusted HRs for the 2 most predictive iron markers, UIBC and ferritin level, were calculated after building models using covariates that were found to be significant at $P<.10$ by univariate analysis for any infection and 1-year mortality rate. Because the number of perioperative RBCs transfused was considered to be an important a priori confounder, this variable was forced into each multivariate survival model. The proportional hazards assumption was tested in all final multivariable models by examining the correlation of Schoenfeld residuals with rank to time.

RESULTS

Of the 146 patients in the original clinical trial, specimens from 37 patients were either not available or in poor condition. The 37 patients with missing samples ranged in age from 1 month to 65 years (median age, 42 years) and included 11 of the 13 pediatric subjects enrolled in the original study. Among the 26 adult patients with missing samples, many had infectious events, including bacteremia (10 patients [39%]), CMV disease (8 patients [31%]), IFI (10 patients [39%]), and any infection (15 patients [58%]). Twelve of these 26 adult patients with missing serum samples died, for a 1-year mortality rate of 46%.

This left 109 patients (75%) for whom serum iron markers could be measured in samples taken at a median interval of 10 days after OLT (interquartile range, 9–14 days) and a median interval of 22 days before the infectious event (interquartile range, 13–36 days). Select cohort characteristics and outcomes are summarized in Table 1. Approximately one-third of the subjects ($n = 14$) who had any infection also died. Among the entire cohort of subjects, one-tenth experienced both outcomes of any infection and death. Among those who died, most (62%) had received transplants from CMV-seropositive donors, one-half (53%) had experienced 1–2 episodes of rejection, and the majority (71%) had been treated with solumedrol.

Serum iron markers were most closely associated with the outcomes of any infection (CMV, bacterial, or fungal) and 1-year mortality rate. The mean values for the 5 different iron measurements, by the outcomes of any infection and 1-year mortality, are shown in Table 2. Markers that corresponded to higher levels of serum iron (iron level, transferrin saturation, and ferritin level) were higher in the groups with any infection or death. The measurements of TIBC and UIBC are inversely related to iron level and were lower in the groups with any infection or death.

Unadjusted HRs for the outcomes of time to any infection and time to death as a function of iron marker measurements are summarized in Table 3. Because TIBC and UIBC are inversely related to iron, 1/HR for TIBC and UIBC are reported. Higher measurements of transferrin saturation and ferritin level and lower measurements of UIBC, which all correspond to higher levels of serum iron, were associated with a statistically significant increased risk of any infection. Likewise, higher measurements of transferrin saturation and ferritin and lower measurements of TIBC and UIBC were associated with an increased risk of 1-year mortality. On univariate analysis, transferrin saturation and ferritin level had the strongest associations with the 2 outcomes time to any infection and death. Similar univariate relationships were found between the iron markers of UIBC and ferritin level and the outcomes of CMV disease and bacteremia (data not shown). No statistical differences in iron markers were found between patients with and patients without IFI. Of note, the majority of IFIs were *Candida* infections, and none were *Mucor* infections.

The adjusted HRs for the outcomes of time to any infection and time to death as a function of iron marker measurements are also summarized in Table 3. Because the measurements of TIBC, UIBC, and transferrin saturation are interrelated, we are only reporting the multivariate HR for UIBC, which demonstrated the most statistically significant relationship to the outcomes. We found that decreasing levels of UIBC and increasing levels of ferritin were both independently associated with an increased risk of any infection after adjusting for number of RBC transfusions, donor CMV-seropositive status, and fungal colonization. In addition, decreasing levels of UIBC and increasing levels of ferritin were independently associated with an increased risk of death after adjusting for number of RBC transfusions, development of CMV disease, and administration of intravenous steroids for treatment of rejection.

Given the large percentage (32%) of patients who received OKT3 for treatment of rejection, we performed a subgroup analysis comparing patients who did with those who did not receive OKT3 (data not shown). The association between in-

Table 1. Patient Characteristics and Outcomes in Liver Transplant Recipients

Characteristic	All patients (n = 109)
Age, mean years ± SD	41 ± 16
Male sex	64 (59)
Nonwhite race	10 (9)
CMV serostatus	
CMV-seropositive donor and recipient	16 (15)
CMV-seropositive donor and CMV-seronegative recipient	28 (26)
CMV-seronegative donor and CMV-seropositive recipient	26 (24)
CMV-seronegative donor and recipient	39 (36)
Underlying diagnosis	
Sclerosing cholangitis	20 (18)
Alcoholic liver disease	19 (17)
Primary biliary cirrhosis	18 (16)
Non A/B hepatitis	13 (12)
Other (no hemochromatosis)	39 (37)
Preoperative creatinine clearance, median mL/min (IQR)	96 (65–130)
Transplantation time, mean h ± SD	8.8 ± 2.5
Median no. of units of RBCs given (IQR)	18 (11–35)
Receipt of CMV immunoglobulin	60 (55)
Rejection	83 (76)
1 rejection episode	46 (42)
2 rejection episodes	22 (20)
⩾3 rejection episodes	14 (14)
Receipt of OKT3 treatment for rejection	35 (32)
Receipt of intravenous steroid treatment for rejection	91 (83)
Major intraabdominal reoperation	27 (25)
Retransplantation	11 (10)
Bacterial infection	20 (18)
Median day of onset of bacteremia (range)	40 (12–196)
IFI	9 (8)
Median day of onset of IFI (range)	53 (10–118)
CMV disease	26 (24)
Median day of onset of CMV disease (range)	27 (12–315)
Any infection	40 (37)
Death	21 (19)
Any infection and death	14 (13)

NOTE. CMV, cytomegalovirus; IFI, invasive fungal infection; IQR, interquartile range; OKT3, murine monoclonal antibody to T3 antigen; RBC, red blood cell; SD, standard deviation.

creased iron markers and risk of infection or death did not change in this subgroup analysis.

DISCUSSION

We show an independent association between serum iron markers measured at least 1 week after OLT and the outcomes of infectious complications and 1-year mortality after OLT. Specifically, high transferrin saturation, high ferritin level, and low UIBC, which all correspond to increased levels of tissue iron stores, are associated with an increased risk of post-OLT CMV disease, bacteremia, any infection (CMV, bacterial infection, or IFI) and death. No independent associations were found between any of the serum iron markers and IFI. However, the number of subjects with this outcome was small (9 patients); therefore, the power to detect a difference was limited.

UIBC and ferritin level were the strongest predictors, with the most statistically significant associations found between these covariates and the outcomes of any infection and death. Even after adjusting for other risk factors for infection previously described in the literature [1–5, 9, 10, 46], including treatment with OKT3, CMV donor and recipient status, and number of perioperative RBC transfusions, these associations

Table 2. Serum Iron Markers in Patients by Outcome of Any Infection and Death (1-Year Mortality)

	Infection			Death		
	Mean ± SD			Mean ± SD		
Iron marker	Any infection (n = 40)	No infection (n = 69)	P	Death (n = 21)	No death (n = 88)	P
Iron level, μg/dL	150 ± 100	136 ± 92	.47	162 ± 83	136 ± 97	.12
Transferrin saturation, %	54 ± 30	47 ± 28	.20	67 ± 29	45 ± 28	**.002**
TIBC, μg/dL	276 ± 90	302 ± 96	.12	238 ± 55	305 ± 96	**.001**
UIBC, μg/dL	126 ± 100	165 ± 110	**.07**	75 ± 72	169 ± 107	**<.001**
Ferritin level, ng/mL	1597 ± 847	1076 ± 667	**.002**	1767 ± 798	1148 ± 726	**.003**

NOTE. P values denoting statistical significance are presented in boldface font. TIBC, total iron binding capacity; UIBC, unbound iron binding capacity.

remained. Because RBCs contain iron in the form of hemoglobin and exposure to intraoperative RBC transfusions has been found to be an independent risk factor for postoperative bacterial infection in patients undergoing OLT [3, 8–11], we adjusted all of our multivariate models to include this important confounding factor. In the analysis of the original trial, RBC transfusions were independently associated with the development of severe post-OLT CMV-associated disease [44].

It is not surprising that serum iron level itself was not independently associated with an increased risk of infection. Serum iron has considerable hour-to-hour physiologic variability in normal individuals. In addition, low serum iron levels do not necessarily reflect low iron stores. The more informative measurement is transferrin saturation, which is calculated from serum iron level and UIBC. Transferrin is the major extracellular transport protein and is normally only 30%–40% saturated. Increases and decreases in tissue iron stores correspond to increases and decreases in transferrin saturation, respectively. The intracellular correlate of transferrin is ferritin, which is present in virtually all cells, including hepatocytes. Plasma levels of ferritin also correlate closely with tissue concentrations of iron. Bone marrow and liver biopsies and/or hepatic magnetic resonance imaging are more specific ways to measure tissue iron stores; however, serum iron indices are less invasive, less expensive, and clinically available.

Our findings are supported by other studies that have explored the relationship between the host's iron level and adverse clinical outcomes, including bacterial and fungal infections [5, 32–43]. Among hematopoietic stem cell transplant recipients, increased iron stores measured in various forms (eg, by serum

Table 3. Univariate and Multivariate Relationships between Serum Iron Markers and the Outcomes of Any Infection and 1-Year Mortality Rate

Iron marker	Unadjusted HR[a] (95% CI)	P	Adjusted HR[a] (95% CI)	P
Any infection				
Iron level, μg/dL	1.02 (0.99–1.05)	.23	…	
Transferrin saturation, %	1.14 (1.02–1.27)	**.05**	…	
TIBC, μg/dL	1.03 (0.99–1.06)	.21	…	
UIBC, μg/dL	1.05 (1.01–1.09)	**.03**	1.05[b] (1.01–1.09)	**.01**
Ferritin level, ng/mL	1.10 (1.06–1.15)	**<.001**	1.09[b] (1.04–1.14)	**<.001**
1-Year mortality rate				
Iron level, μg/dL	1.02 (0.99–1.06)	.25	…	
Transferrin saturation, %	1.27 (1.10–1.48)	**.001**	…	
TIBC, μg/dL	1.10 (1.03–1.16)	**.002**	…	
UIBC, μg/dL	1.11 (1.04–1.16)	**<.001**	1.12[c] (1.05–1.21)	**<.001**
Ferritin level, ng/mL	1.11 (1.05–1.17)	**<.001**	1.11[c] (1.04–1.18)	**<.001**

NOTE. P values denoting statistical significance are presented in boldface font. CI, confidence interval; HR, hazard ratio; TIBC, total iron binding capacity; UIBC, unbound iron binding capacity.

[a] HR is per 10 units of iron, transferrin saturation, TIBC, and UIBC and is per 100 units of ferritin. 1/HR is reported for TIBC and UIBC.

[b] Adjusted for number of red blood cell units, donor cytomegalovirus-seropositive status, and fungal colonization.

[c] Adjusted for number of red blood cell units, cytomegalovirus disease, and intravenous steroids given for rejection.

iron markers, quantitative hepatic iron content, and qualitative bone marrow iron content) have been associated with increased rates of invasive fungal infection [34, 39, 41]. Among OLT recipients, quantitative hepatic iron content of the explanted livers has been associated with increased rates of fungal and bacterial infection [32, 47]. Most of these studies, however, had small sample sizes, and because these studies collected data retrospectively, they were unable to adjust for other known infectious risk factors (eg, prior antibiotic exposure or the presence of central venous catheters) or potential confounders (eg, receipt of RBC transfusions or immunosuppression).

The strengths of this analysis included the use of a well-characterized, prospectively followed cohort with well-defined clinical events. Data on a number of known infectious risk factors, including administration of RBC transfusions, were carefully collected. Because data were collected prospectively, we were able to use time-dependent analyses to enhance the power of our study.

There are a few limitations to this analysis. First, some subjects had missing blood samples, many of whom experienced an infectious outcome. The 26 missing adult blood samples can be attributed to their being used in previous studies. Because the pediatric subjects had smaller volumes of blood taken for the study, most of them had missing blood samples. Therefore, our results may not be applicable to pediatric OLT recipients. We also were not able to account for the time of day during which the blood samples were drawn. Because iron measurements normally exhibit diurnal variation, this could have introduced a measurement bias to our results.

Although we selected a single time point for measuring serum iron level, all previous studies described in the literature also used a single time point [5, 32–42]. This single measurement and our retrospective analysis limit the study results as hypothesis-generating and exploratory. A prospective, longitudinal study with serial iron measurements is currently underway to further explore the association we found between increased serum iron markers and infection and death after liver transplantation. Prospectively measuring levels of the iron regulatory hormone hepcidin concurrently with serum iron levels will provide more insight into potential cause-and-effect relationships between iron levels and clinical outcomes. Hepcidin is a small, acute-phase peptide predominantly produced by the liver and is thought to be the single, central regulator hormone of extracellular iron homeostasis [48]. Hepcidin synthesis is induced by infection and inflammation, [49–51] and also by iron loading or iron stores by yet unknown, complex mechanisms [51–53]. Conversely, hepcidin production is suppressed by anemia, hypoxia [54], and erythropoiesis [55, 56]. Hepcidin acts by decreasing iron influx into plasma from tissues engaged in iron transport and storage [51]. Without hepcidin measurements, one cannot determine whether serum iron measurements are increased as a result of iron overload or in reaction to early infection and/or inflammation.

In terms of generalizability, this is an older OLT cohort that received more blood transfusions, less effective antimicrobial prophylaxis (ie, non–systemically absorbed antifungals such as nystatin), and more intense immunosuppressive medications for both prophylaxis and treatment of rejection, all of which differ from current OLT surgical and medical management practices. These older clinical practices likely contributed to higher rates of infectious complications, although the types of infections have remained the same over time. However, we statistically adjusted our multivariate model to account for all of these differences, and we were still able to demonstrate an independent and statistically significant relationship between serum iron measurements and infection or mortality.

Our study found that increased serum iron markers were an independent risk factor for infectious complications and 1-year mortality rate in OLT recipients. If a better understanding of iron metabolism and its relationship to infection in OLT recipients is elucidated in future studies, this could potentially help guide infection prognosis, prevention, and management efforts in this high-risk population.

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