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Iron-Related Markers are Associated with Infection after Liver Transplantation

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Abstract

Though serum iron has been known to be associated with an increased risk of infection, hepcidin, the major regulator of iron metabolism, has never been systematically explored in this setting. Finding early biomarkers of infection, such as hepcidin, could help identify patients in whom early empiric antimicrobial therapy would be beneficial. We prospectively enrolled consecutive patients (N=128) undergoing first time, single organ orthotopic liver transplantation (OLT) without known iron overload disorders at two academic hospitals in Boston from August 2009-November 2012. Cox regression compared the associations between different iron markers and the development of first infection at least one week after OLT. 47 (37%) patients developed a primary outcome of infection at least one week after OLT and one patient died. After adjusting for peri-operative bleeding complications, number of hospital days, and hepatic artery thrombosis, changes in iron markers were associated with the development of infection post-OLT including: increasing ferritin (hazards ratio [HR], 1.51 [95% CI, 1.12 - 2.05]), rising ferritin slope (HR, 1.10 [95% CI, 1.03 -1.17]), and increasing hepcidin (HR, 1.43 [95% CI, 1.05-1.93]). A decreasing iron (HR, 1.76 [95% CI, 1.20-2.57]) and a decreasing iron slope (HR, 4.21 [95% CI, 2.51-7.06]) were also associated with subsequent infections. Conclusion: Hepcidin and other serum iron markers and

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Potential Conflicts of Interest: MW reports being a scientific cofounder, consultant, and shareholder of Intrinsic LifeSciences. TG reports being a scientific cofounder, consultant, and shareholder of Intrinsic LifeSciences, a scientific cofounder, consultant and grant recipient for Merganser Biotech, a scientific cofounder, consultant and grant recipient for Silarus Pharma, and a consultant, grant recipient for Keryx Pharma. DS reports being on advisory boards for Merck, Takeda, Shire, Summit, Chimerix, and Seres Therapeutics and being a grant recipient from Merck, Summit, Actelion, Tetraphase and Seres Therapeutics. The remaining authors of this manuscript report no potential conflicts.

their slope patterns or their combination are associated with infection in vulnerable patient populations.

Keywords

hepcidin; ferritin; risk factor

Invasive infections are a common cause of morbidity and mortality among immunocompromised hosts including orthotopic liver transplant (OLT) recipients. New biomarkers for infection in high risk populations could facilitate targeted preventative or early treatment strategies leading to improved prognosis and outcomes. Increased levels of tissue and serum iron have been associated with invasive infections in OLT recipients (1-3). Serum iron markers could serve as early predictive biomarkers for infection in this vulnerable patient population.

Multiple *in vitro* studies and animal models have demonstrated that iron promotes increased virulence of bacterial and fungal pathogens by counteracting the innate antimicrobial effects of host plasma (4;5). Iron may become available as nutrition for microorganisms from multiple intracellular and extracellular sources (6-9). Readily available plasma iron is metabolized by pathogens, facilitating increased bacterial and fungal growth which may overwhelm other host defenses and result in clinical infection. Host iron sequestration reverses this pathogenic effect and restores the antimicrobial properties of host serum (10). Furthermore, acute events, such as infection, trauma, and surgery, lead to rapid drops in serum iron, or “stress hypoferremia”, and increased iron storage (11;12). This decrease in iron availability, or iron-withholding from host serum, may serve as a defense mechanism after infection or other stressful events and is referred to as “nutritional immunity” (13).

Hepcidin, the master regulator hormone of systemic extracellular iron homeostasis, is an acute phase peptide predominantly produced by hepatocytes (14). Hepcidin synthesis is induced by infection and inflammation, and, independently, by iron loading or iron stores (15-17). Conversely, hepcidin production is suppressed by anemia, hypoxia, and active erythropoiesis (18;19). Hepcidin acts to decrease duodenal enterocyte dietary iron absorption, decrease iron efflux from macrophages that recycle iron from senescent erythrocytes, and decrease iron efflux from hepatocytes that store iron (17). These hepcidin-mediated mechanisms of iron sequestration reduce serum iron levels thereby reducing the amount of iron available to extracellular pathogens.

Since the discovery of hepcidin, much progress has been made in understanding the molecular basis of iron metabolism, however the study of hepcidin in the clinical setting remains limited. Hepcidin has never been systematically and prospectively studied in an OLT population as a predictor for infection. Studies to date examining iron and infection in solid organ transplant recipients were retrospective and used banked specimens to quantify iron (1-3;20;21). The purpose of this hypothesis-generating study is to begin exploring the host-pathogen relationship between iron metabolism and infection in a post-OLT population.

Materials and Methods

Patient Population

Consecutive adults undergoing OLT at Tufts Medical Center and Lahey Medical Center in Boston, MA were enrolled from August 2009 until November 2012 after giving written informed consent. Exclusion criteria included dual organ transplant recipients, re-transplantation of the liver, and any known pre-existing conditions predisposing to iron overload (e.g. hemochromatosis). Patients were followed for 6 months post-OLT as inpatients and outpatients. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the institutional review boards at each institution

Clinical Data

Data collected included demographics, donor characteristics, intra and post-operative transfusions, operative complications, immunosuppressant and antimicrobial treatments, supplemental nutrition, and routine laboratory data. Patients were initially on a corticosteroid taper, mycophenolate mofetil (MMF) and tacrolimus. The immunosuppression protocols did not differ by center, donor type or hepatitis C virus (HCV) infection status. Valganciclovir was used for cytomegalovirus prophylaxis for 6 months for those at highest risk (Donor +/Recipient-) and for 3 months in all others.

Blood Sample Collection and Iron Marker Measurements

Serial blood plasma samples were prospectively collected at baseline within 72 hours after OLT, then weekly while hospitalized, and at each outpatient visit at increasing intervals post-OLT. Blood samples were also obtained within 48 hours of suspected infectious events. Triggers to collect a blood sample for suspected infection included fever >38.0 C, ordering of microbiologic cultures, or ordering of empiric antimicrobial therapy. Hepcidin was measured by a validated competitive enzyme-linked immunosorbent assay (22) and iron, ferritin, and C-reactive protein (CRP) were measured by standard clinical lab assays.

Outcome Definitions

Our primary outcome was time to first infection at least one week after OLT. Definite infection was defined as microbiologic, histologic or pathologic isolation of an organism from a normally sterile site with clinical signs and symptoms of infection (23-25). Possible infection was defined as clinical signs and symptoms of infection warranting treatment with empiric antibiotics per infectious disease physician with no evidence of a positive microbiology, histology or pathology test. All potential infections were adjudicated by a panel of three transplant infectious disease physicians who were blinded to iron measurements. Nosocomial infection were classified using Centers for Disease Control definitions (26). The date from which the positive culture or tissue pathology sample was taken or the date during which a triggered blood sample was collected for suspected infection per above criteria was defined as the date of infection.

Statistical Analyses

Baseline patient characteristics were summarized for those with definite or possible infection and those without infection. Because hepcidin is not induced in acute HCV infection, patients with HCV re-activation alone were classified as non-infected for all analyses (27). Univariate survival analysis was performed on both baseline and time-dependent covariates to determine which variables were associated with development of a first infection using Cox Proportional Hazards models.

Boxplots of iron parameters were compared between those who developed a first definite infection at least one week after OLT and those who never developed an infection. Patients with possible infections were excluded. For patients who did not develop an infection, the levels of iron parameters were matched to each infection date at the same number of days after OLT +/- 2 days and also were matched to each infection date + 30 days. The non-infected patients were re-sampled to calculate the corresponding “non-infected” iron parameter medians for each matched date. Given that longitudinal patterns of iron markers over time have not previously been characterized in relationship to infections, multiple continuous iron marker metrics were explored to develop statistically and clinically meaningful models. Examples of raw and derived iron variables for modeling are shown in Supplemental Table S1.

Multivariable Cox proportional hazards models were used to examine the relationship between iron markers and time to first infection at least one week after OLT. Covariates thought *a priori* to be of clinical importance and those with univariate statistical significance at $p<0.20$ were used as candidate variables for possible inclusion in multivariable model building. To avoid overfitting the models, they were restricted to three covariates using best subsets selection methods. The proportional hazards assumption of each model was assessed using a method based on weighted Schoenfeld residuals (28). All statistics were performed using SAS version 9.4 (Carey, NC).

Sensitivity analyses

Clinically meaningful sub-groups were selected *a priori* for comparison, including those with and without HCV infection, those with and without hepatocellular carcinoma, and those with living versus deceased liver donors. In addition, different definitions of infection were used as sensitivity analyses including: definite infections as the outcome with possible infections reclassified as not infected, or with possible infections excluded from analysis, or with possible infections censored on the date of possible infection.

We decided, *a priori*, to test clinically important interactions between transfusion products, heparin, and iron markers (29). In addition, because of the high variability seen during the initial period of post-transplant recovery, we performed another sensitivity analysis that excluded data from the first week post-OLT.

Results

Patient Population

A total of 130 patients were enrolled in the study (Figure 1). One patient died within three weeks and one patient was re-transplanted within seven days. Both were excluded due to lack of blood samples and neither had developed an infection, yielding 128 subjects who were analyzed. The majority of study subjects were white males (Table 1) and were transplanted for either HCV or alcoholic liver disease. Almost half of patients also had hepatocellular carcinoma. Of note, one third of our study subjects received organs from living donors. Most subjects received red blood cell transfusions with more transfusions given to patients in the group that developed infections. The infected group also more often underwent complex transplant operations, had more post-transplant surgical complications and had longer hospital stays. The mean tacrolimus trough did not differ significantly between those with and without infection. MMF was tapered off by 3 months post-OLT in 60% of subjects. Immunosuppression did not differ by HCV status or by donor type (data not shown).

Infectious Outcomes

There were a total of 67 patients with at least one infection (Figure 1). We classified patients who only had recurrent HCV as non-infected (N=10) (27). In order to avoid including peri-transplant infections, infectious episodes occurring within the first week after OLT were excluded, however, subsequent infections were analyzed as infectious outcomes. For our primary endpoint of time to first infection at least one week after OLT, there were a total of 47 infections that met inclusion criteria, three quarters of which were definite and one quarter of which were possible. The median time between transplant and infection was 53 days (25-75%, 16 to 69 days) for definite infections and 55 days (25-75%, 14 to 94 days) for possible infections. The most common type of infection was intra-abdominal which included cholangitis, intra-abdominal abscesses and peritonitis, and was followed by bloodstream infections. The most common organism isolated was *Enterococcus spp* (Table 2). Among analyzed subjects, one patient with infection died and none of the non-infected patients died during the study period.

Iron Markers

An average of 13 blood samples per subject were available for analysis of iron markers. When distributions of iron markers were compared, the median hepcidin was significantly higher at time of definite infection (200 ng/mL, 25-75% 113-269 ng/mL, $p<0.001$) when compared to those who did not develop infection (102 ng/mL, 25-75% 78-113 ng/mL, Figure 2A). At baseline and 30 days after infection, there was no significant difference in hepcidin between those who did and did not develop infection (Figure 2B and 2C). The median ferritin patterns were similar to those of hepcidin with a higher median ferritin at time of definite infection (518 ng/mL, 25-75% 320-824 ng/mL, $p=0.003$) when compared to those who did not develop infection (350 ng/mL, 25-75% 184-495 ng/mL), and no significant differences were found at baseline or at 30 days after infection (Supplementary Figure 1). In contrast, the median iron was lower at time of definite infection (43 mcg/dL, 25-75% 17-74 mcg/dL, $p=0.05$) when compared to those who did not develop infection (62

mcg/dL, 25-75% 51-70 mcg/dL, Supplementary Figure 2). Some general patterns in iron markers were observed. Immediately post-OLT, ferritin would usually fall faster than hepcidin while iron increased during the first 30-45 days post-OLT before all iron markers reached a relatively steady state (Figure 3, Panel A). In general, before an infection was diagnosed, hepcidin would rise first, followed by a rise in ferritin and a decrease in iron (Figure 3, Panel B).

Univariate Associations of Non-Iron Covariates with Infection

The associations between clinically important, non-iron marker risk factors and infectious outcomes using univariate survival analysis are shown in Table 2. Comparing types of underlying liver disease, HCV was associated with a decreased risk of infection after OLT. When examining time-dependent variables, hospital length of stay, the number of days receiving red blood cell transfusions or tube feeds, and number of hospital re-admissions were associated with an increased risk for infection. Several surgical complications were also associated with increased infections including two-staged liver transplant procedures, bile leaks, bleeding, and hepatic artery thrombosis. In terms of laboratory values, decreasing albumin, increasing white blood count (WBC), and increasing CRP were associated with increased infections. Donor type, baseline iron markers and baseline CRP were not associated with infectious risk. Transferrin saturation and total iron binding capacity (did not demonstrate as strong a relationship with infection (data not shown) compared to iron, ferritin and hepcidin. In addition, no relationship was found between liver explant qualitative iron content and subsequent infectious events (HR 1.51, 95% CI 0.83-2.73; p=0.18).

Multivariable Iron Models

Many different iron metrics were found to be associated with infection on multivariable analyses, however, the focus here will be on the most easily clinically interpretable, statistically significant metrics (Table 3). To facilitate interpretation of the hazards ratios, iron variables were modeled to be associated with an increased risk of infection (Supplementary Table S2). An increase in the ferritin slope and in ferritin were independently associated with an increased risk of infection after OLT after adjusting for hospital length of stay, bleeding complications and hepatic artery thrombosis. Increases in hepcidin also were associated with increased infections. In contrast, decreases in iron slope and in iron were associated with an increased risk of infection. CRP, an acute phase reactant, and iron markers were correlated with each other (correlation coefficients ranged between 0.1-0.5) and with the infection outcomes. They were not independent predictors of infection when modeled together (data not shown). HCV status did not have an independent effect on iron markers. WBC and albumin (as a crude marker for liver synthetic function) also did not change the results of the multivariable models (Supplement Table S3).

Subgroup and Sensitivity Analyses

The multivariate models for the different iron parameters were analyzed using sub-groups including those with and without HCV, those with and without hepatocellular carcinoma and those with living versus deceased liver donors. Results were similar for all iron markers and for all subgroups (Supplement Table S3). Sensitivity analyses were also performed using different definitions of infectious outcomes and yielded similar results (Supplement Table

S3). We also tested for clinically important interactions between iron markers and donor type, iron markers and different types of transfusions, and iron markers and heparin; however no statistically significant interactions were found (data not shown).

Discussion

This is the first prospective longitudinal study of iron parameters, including hepcidin, and their association with infection in OLT. We found several different serum iron marker patterns to be associated with infection in OLT recipients. Increases in hepcidin and ferritin and decreases in iron were associated with an increased risk of infection after adjustment for other known risk factors for infection. The models accounted for the complexity of the post-OLT course by analyzing recognized risk factors for infection, such as surgical complications and blood product transfusions. Furthermore, the relationship between iron markers and infection remained similar in all of our sensitivity, subgroup, and interaction analyses. Our findings confirm retrospective studies where iron and infection relationships were previously found in immunocompromised patient populations (1-3;21;30;31) and support the hypothesis that iron markers could serve as predictors for infection. The biology of iron metabolism provides a plausible pathophysiologic basis for a clinically meaningful relationship between iron and infection. By beginning to explore how iron markers behave in the setting of clinical infection, this sets the stage for future investigations of iron markers measured at standardized time points to determine diagnostic cutoffs, such as positive predictive and negative predictive values.

Changes in serum hepcidin corresponded to opposite changes in serum iron, supporting the interpretation that hepcidin was driving the changes in serum iron and not vice versa (17). Thus, increases in hepcidin coincided with decreases in serum iron, and both of these patterns were associated with an increased risk of infection. With resolving infection, serum hepcidin and ferritin decreased and the serum iron increased. Lowering extracellular iron is hypothesized to be a general defense mechanism against infection by withholding iron from various pathogens according to the concept of “nutritional immunity” (13). In an animal model, the protective effect of hypoferremia was shown when hepcidin-deficient mice infected with *Vibrio vulnificus* became septic and could be saved from sepsis by administration of a hepcidin agonist (32). When hepcidin and iron inversely correlate, hepcidin synthesis is being primarily driven by infection and inflammation leading to hypoferremia. This decrease in iron feeds back to decrease hepcidin levels, thus blunting the hepcidin signal over time. This negative feedback hepcidin regulation loop could explain why hepcidin was not as strong a predictor for infection when compared to iron and ferritin (33).

While our study suggests that iron markers and their slope patterns or their combination could serve as potential early biomarkers for infection, they may not be accurate predictors of acute or chronic HCV infections. Armitage *et al.* found no correlation between hepcidin or iron and acute hepatitis B or acute HCV infection (initial viremic phase) but did find an increase in hepcidin and a decrease in iron with acute Human Immunodeficiency Virus infection. Our findings found a similar lack of relationship between HCV status and iron markers. Patients with recurrent HCV infection alone were therefore classified in our study

as not infected (27). In addition, hepcidin synthesis is suppressed in patients which chronic HCV infection (34;35).

Limitations to our study include the small numbers of viral or fungal infections preventing our ability to draw any specific conclusions about these types of infections. Our cohort also had a larger percentage of live liver donors compared to most other liver transplant centers, which could limit generalizability of our results. There were also small numbers of those with hepatic artery thrombosis, which also could limit generalizability; however, we felt it was important to control for this covariate in the final multivariable models based on clinical considerations. There was only one death observed during the study period in a patient with infection, and perhaps our results would have been more dramatic in a sicker patient population. Also, due to limited blood sampling, we cannot accurately determine the exact hourly or daily time-course of rise in hepcidin and ferritin and decrease in iron in all cases. We also did not collect the pre-OLT baseline iron markers in serum or in native livers and could not confirm the association between baseline iron status and infections found in previous studies (1;2;21). Finally, there could have been some imprecision in the “day of diagnosis” of an infectious episode, because it was based on clinical factors, however this reflects the reality of clinical practice.

Future studies to determine the numeric parameters at which iron markers best serve as early predictors for infection could inform when cultures should be obtained or empiric antimicrobial therapy should be started. Also, better definition of which patient populations would most benefit from using iron-related biomarkers for earlier detection or prediction of infection would be helpful. With further study, hepcidin agonists could also potentially be administered to humans with infections for therapeutic purposes. In experimental animal models of infection with *Klebsiella pneumonia*, iron-loaded hepcidin knock-out mice were highly susceptible to infection and had higher mortality compared to wild-type mice. When treated with hepcidin analogs, which lowered serum iron concentrations, mortality was prevented or decreased amongst infected iron-loaded hepcidin knock-out mice. The protective effect of hepcidin was caused by the restriction of available serum iron. These animal models support that hepcidin plays a role in innate immunity (36;37). Further exploration of hepcidin-mediated iron sequestration in human host defense could lead to using hepcidin as an anti-infective therapeutic to supplement antimicrobials in immunosuppressed hosts.

In conclusion, in this prospective longitudinal study, we observed a relationship between serum iron markers and the subsequent development of infection in a cohort of liver transplant recipients after adjusting for potential confounders and known risk factors of infection. Time dependent associations showed that increasing ferritin, ferritin slope, and hepcidin, as well as decreasing iron and iron slope were associated with the development of infection post-OLT. The recognized risk factors for infection of length of hospital stay, bleeding complications and hepatic artery thrombosis were verified (38-44). Our study also supports the hypothesis that acute hypoferremia is a nearly universal host defense mechanism activated across the spectrum of infections, including in immunocompromised hosts. Our results make a case for further exploration of the use of hepcidin and other iron-related parameters as potential early biomarkers of infections in vulnerable patient

populations. We hope this study generates ideas for future applications where manipulation of the iron metabolism pathway could be a potential therapeutic target for infection intervention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CRP	C reactive protein
HCV	hepatitis C virus
MMF	mycophenolate mofetil
OLT	orthotopic liver transplantation
WBC	white blood count

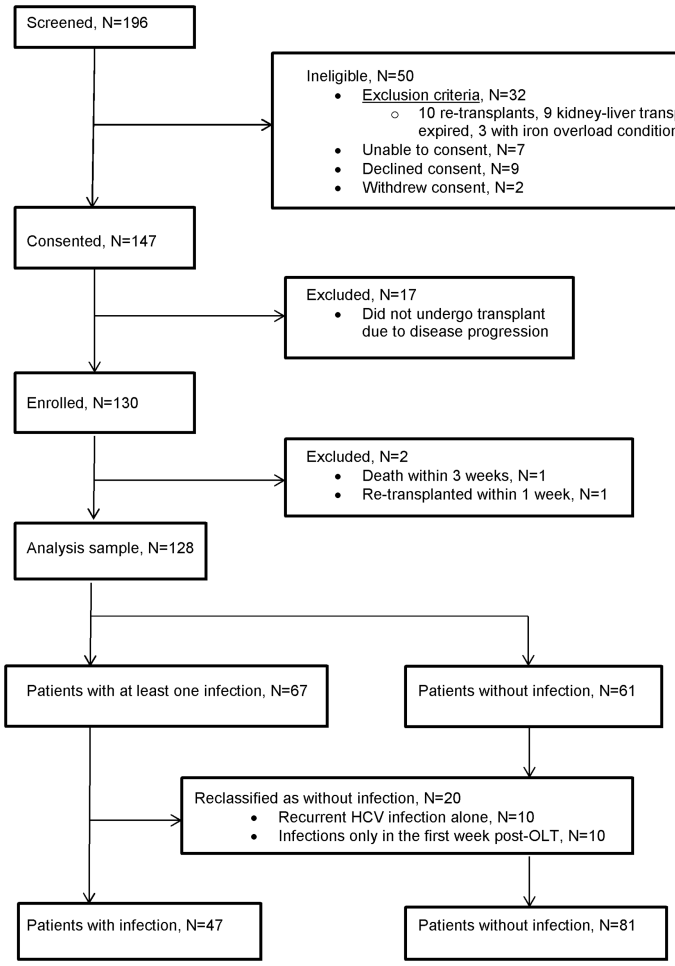

Figure 1. Flow Diagram of Study Recruitment and Infectious Events

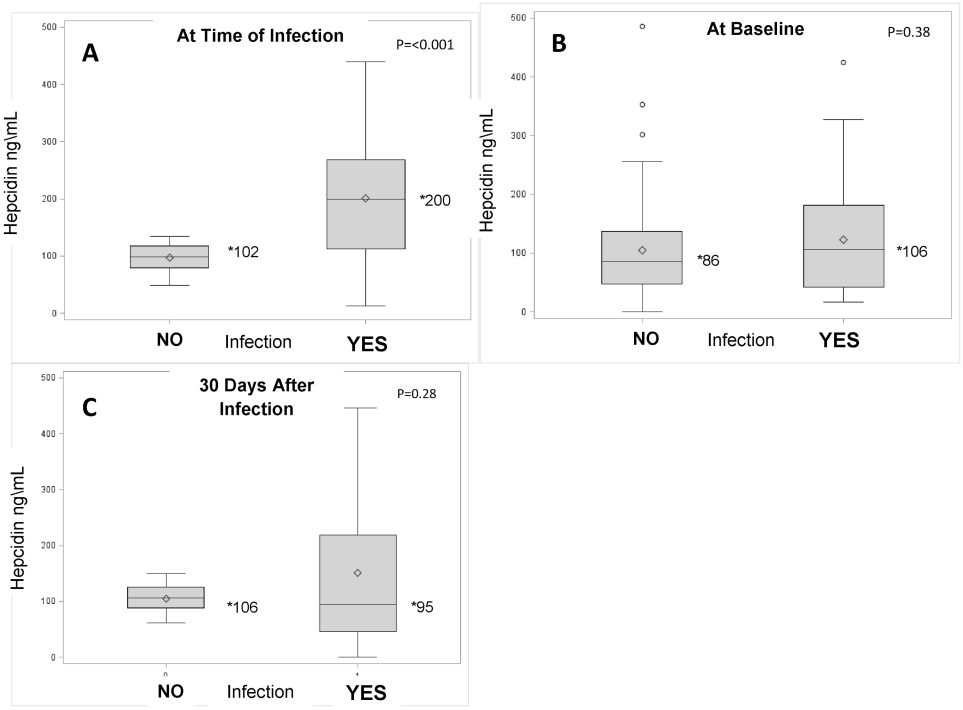

Figure 2. Comparisons of Median Hepcidin Measurements in Patients who did and did not Develop Infections

Panel A: The *median levels of hepcidin in those who developed definite infections (N=35) were taken at time of infection. For those who did not develop infection (N=81), the levels of hepcidin were matched to each infection date at the same number of days after OLT +/- 2 days. The median value was then used as the non-infected value for that corresponding date.

Panel B: The *median levels of hepcidin at baseline (within 48 hours after OLT) in those who did (N=35) and who did not (N=81) develop definite infections.

Panel C: The *median levels of hepcidin in those who developed definite infection (N=35) were taken 30 days after the infection. For those who did not develop infection (N=81), the levels of hepcidin were matched to each infection date + 30 days. The median value was then used as the non-infected value for that corresponding date.

◊ Represents mean values.

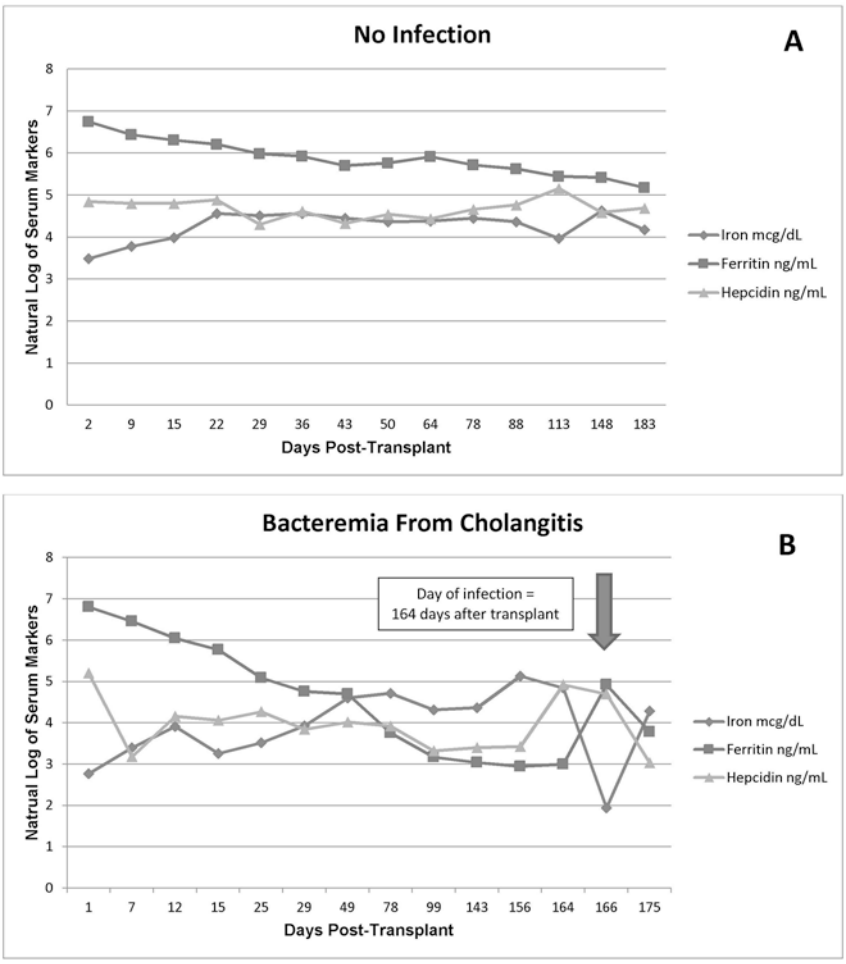

Figure 3. Iron Markers Post-Liver Transplant in a Patient who did not Develop Infection and in a Patient who Developed Bacteremia

Table 1

Baseline Characteristics of Patients who did and did not Develop Infection.

Characteristic	Total Cohort, % (N=128)	Definite or Possible Infection, % (N=47)	No Infection, % (N=81)	P value, Infection vs. No Infection
Age in years; median (25-75%)	56 (49 - 60)	57 (47- 60)	56 (49 - 61)	NS
Ethnicity				
Hispanic	7.0 (9)	6.4 (3)	7.4 (6)	NS
Non-Hispanic	93.0 (119)	93.6 (44)	92.6 (75)	
Gender				
Male	76.6 (98)	70.2 (33)	80.2 (65)	NS
Female	23.4 (30)	29.8 (14)	19.8 (16)	
Race				
White	85.2 (109)	89.4 (42)	82.7 (67)	NS
Non-White	14.8 (19)	10.6 (5)	17.3 (14)	
Etiology of Liver Disease[a]				
Hepatocellular Carcinoma	42.2 (54)	34.0 (16)	46.9 (38)	NS
Alcoholic Liver Disease	32.0 (41)	31.9 (15)	32.1 (26)	NS
NASH	11.7 (15)	12.8 (6)	11.1 (9)	NS
Autoimmune Liver Disease[b]	17 (22)	21 (10)	15 (12)	NS
Hepatitis B	3.1 (4)	2.1 (1)	3.7 (3)	NS
Hepatitis C	46.1 (59)	34.0 (16)	53.1 (43)	0.04
Comorbidities				
Ulcerative Colitis	7.8 (10)	12.8 (6)	4.9 (4)	NS
Crohn's Disease	1.6 (2)	0 (0)	2.5 (2)	NS
Cardiovascular Disease	3.9 (5)	4.3 (2)	3.7 (3)	NS
Diabetes	19.5 (25)	25.5 (12)	16.0 (13)	NS
MELDScore[c]; mean (stddev)	23.1 (8.3)	24.4 (9.5)	22.3 (7.5)	NS
CMV Serostatus				
D+/R-	25.0 (32)	23.4 (11)	25.9 (21)	NS
D+/R+	21.1 (27)	23.4 (11)	19.8 (16)	NS
D-/R+	23.4 (30)	25.5 (12)	22.2 (18)	NS
D-/R-	29.7 (38)	27.7 (13)	30.9 (25)	NS
Donor Type				
Live	32.0 (41)	38.3 (18)	28.4 (23)	NS
Deceased	68.0 (87)	61.7 (29)	71.6 (58)	
Donor Age in years; mean(stddev)	43.7 (17.0)	46.7 (17.5)	42.0 (16.6)	NS
Organ Location				
Local	27.3 (35)	27.7 (13)	27.2 (22)	NS
Regional	20.3 (26)	19.1 (9)	21.0 (17)	NS

Characteristic	Total Cohort, % (N=128)	Definite or Possible Infection, % (N=47)	No Infection, % (N=81)	P value, Infection vs. No Infection
National	20.3 (26)	14.9 (7)	23.5 (19)	NS
Anastamosis				
End-to-End	85.2 (109)	80.9 (38)	87.7 (71)	NS
Roux-en-Y	10.9 (14)	12.8 (6)	9.9 (8)	NS
Both	3.9 (5)	6.4 (3)	2.5 (2)	NS
Operative Time in hours;median(25-75%)	4.75 (4 – 5.5)	5 (4.5 – 6)	4.5 (4 – 5.5)	NS
Warm Ischemic Time in hours; median (25-75%)	0.43 (0.37- 0.50)	0.42 (0.35 – 0.48)	0.45 (0.38 – 0.50)	NS
Cold Ischemic Time in hours; median(25-75%)	5.13 (0.98 – 6.94)	4.52 (0.90– 6.40)	5.37 (1.73 – 7.15)	NS
Any heparin	89 (114)	91.5 (43)	50.6 (71)	NS
Cumulative heparin dose in units; median (25-75%)	775 (400 - 25625)	1000 (400 - 15000)	750 (400 - 25750)	NS
Any RBC	93 (119)	98 (46)	90 (73)	NS
Cumulative RBC days; median (25-75%)	2 (1- 3.5)	3 (1 - 4)	1 (1 - 3)	0.02
Cumulative RBC units; median (25-75%)	12 (6 - 20)	13 (8 - 24)	9 (4 - 17)	0.04
Any platelets	75 (96)	85 (40)	69 (56)	NS
Any FFP	95 (121)	98 (46)	93 (75)	NS
Cumulative FFP units; median (25-75%)	13.5 (6 – 22.5)	16 (7 - 23)	12 (5 - 20)	NS
Any cryoprecipitate	32 (41)	38 (18)	28 (23)	NS
Cumulative number of hospital days; median (25-75%)	12.5 (9.5 - 17)	14 (10 - 17)	12 (8 - 16)	NS
# of patients with repeat hospitalizations	52.3 (67)	63.8 (30)	45.7 (37)	NS
# of patients with post-OLT exploratory laparotomies	21.1 (27)	2.1 (9)	22.2 (18)	NS
# of patients with bile leaks	8.6 (11)	14.9 (7)	4.9 (4)	0.05
# of patients with bleeding	28.9 (37)	38.3 (18)	23.8 (19)	NS
# of patients with two-staged OLT	18.8 (24)	31.9 (15)	11.1 (9)	0.004
# of patients with hepatic artery thrombosis	4.7 (6)	11 (5)	1.2 (1)	0.04
# of patients with rejection	7.0 (9)	14.9 (7)	2.5 (2)	<0.001
Baseline Iron Measurements[d]; median (25-75%)				
Iron, mcg/dL	66 (39 - 131)	61 (37 - 121)	64 (36 - 112)	NS
Ferritin, ng/mL	691 (330 - 1194)	436 (296 - 1076)	792 (378 - 1452)	NS
Hepcidin, ng/mL	91.6 (48.6 - 148.5)	103.4 (45.6 - 181.6)	85.7 (47 - 137.4)	NS
Baseline Laboratory Measurements[c]; mean (stddev)				
WBC, 10^3/mcL	6.3 (4.1)	8.4 (5.7)	5.1 (2.0)	<0.001
Albumin, g/dL	3.7 (0.8)	2.9 (0.7)	4.1 (0.5)	<0.001
Creatinine, mg/dL	1.2 (0.4)	1.2 (0.6)	1.1 (0.3)	NS
eGFR[e],mL/min	62.2 (25.6)	63.6 (31.9)	61.3 (21.3)	NS

Characteristic	Total Cohort, % (N=128)	Definite or Possible Infection, % (N=47)	No Infection, % (N=81)	P value, Infection vs. No Infection
CRP,mg/L	2.25 (4.26)	5.17 (5.8)	0.55 (1.22)	<0.001
Tacrolimus trough, ng/mL	8.5 (1.6)	8.3 (1.8)	8.6 (1.4)	NS

Abbreviations: NS: non-significant, NASH: non-alcoholic steatohepatitis, MELD: Model for end-stage liver disease, stddev: standard deviation, CMV: cytomegalovirus, D: donor, R: recipient, RBC: red blood cells, FFP: fresh frozen plasma, OLT: orthotopic liver transplant, WBC: white blood cells, eGFR: estimated glomerular filtration rate, CRP: C-reactive protein.

[a] Patients may have more than one etiology of liver disease and the % do not add to 100%.

[b] Primary biliary cirrhosis, primary sclerosing cholangitis or autoimmune hepatitis.

[c] At time of transplant.

[d] Within 72 hours after transplant.

[e] CKD-EPI estimated glomerular filtration rate. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-12.

Table 2
Description of Primary Outcome of Definite and Possible Infections

Definite Infections, N=35	**Possible Infections, N=12**
Intra-abdominal infection: cholangitis, abscess or peritonitis, N=13 • Enterococcusspecies, N=6 • CoNS, N=1 • Polymicrobial, N=3 • Unknown, N=3	Intra-abdominal infection, N=6
Bloodstream infection, N=11 • *Enterococcusspecies*, N=4 • CoNS, N=4 • Gram negative rod, N=3	Pneumonia, N=2
Clostridum difficile colitis, N=3	Surgical site infection, N=2
Skin and soft tissue infection, N=3 • Polymicrobial, N=1 • Unknown, N=2	Other • Herpes Zoster, N=1 • Tracheobronchitis, N=1
Urinary tract infection, N=2 • ESBL gram negative rod, N=2	
CMV infection, N=1	
Candida esophagitis, N=1	
Surgical site infection • Unknown, N=1	

Abbreviations: CoNS=coagulase-negative staphylococcus, ESBL=extended-spectrum beta-lactamase, CMV=*Cytomegalovirus*,

Table 3

Unadjusted and Adjusted Hazards Ratio Estimates between Covariates and Iron Markers and the Development of First Definite or Possible Infection

Baseline Covariates	Unadjusted HR	95% CI	p-value	Adjusted HR[a]	95% CI	p-value
Gender (Male)	0.68	0.36 - 1.26	0.22			
UNOS Status (Home vs. Hospital)	0.57	0.25 - 1.27	0.17			
Donor Type (Living vs. Deceased)	1.39	0.77 - 2.50	0.28			
Hepatitis C	0.55	0.30 - 1.00	**0.05**			
Hepatocellular Carcinoma	0.65	0.36 - 1.20	0.17			
Autoimmune Liver Disease[b]	1.40	0.70 - 2.81	0.34			
Ulcerative Colitis	2.11	0.89 - 4.96	0.09			
Diabetes	1.54	0.78 - 2.97	0.20			
Baseline Hepcidin (Per 100 mcg/mL)	1.26	0.98 - 1.64	0.08			
Baseline Ferritin (Per 100 mcg/mL)	0.997	0.98 - 1.01	0.68			
Baseline Iron (Per 10 mcg/dL)	1.002	0.96 - 1.05	0.92			
Baseline CRP (Per mg/L)	1.02	0.95 - 1.10	0.55			
Time-dependent covariates						
# of RBC transfusion days	1.19	1.04 - 1.38	**0.01**			
# of RBC transfusion units	1.01	0.996 - 1.33	0.12			
# of tube feed days	1.02	1.01 - 1.04	**0.001**			
# of total hospital days including OLT stay	1.10	1.07 - 1.12	**<0.001**			
# of hospital re-admissions after OLT stay	3.04	2.15 - 4.29	**<0.001**			
Two-staged OLT	2.84	1.58 - 5.11	**<0.001**			
Bile leaks	2.74	1.23 - 6.12	**0.01**			
Hepatic artery thrombosis	2.87	1.02 - 8.02	**0.05**			
Bleeding	1.85	1.13 - 3.03	**0.02**			
Increase in albumin (per g/dL)	0.39	0.23 - 0.68	**<0.001**			
Increase in CRP[c] (per mg/L)	2.02	1.59 - 2.58	**<0.001**			
Increase in CRP Slope (per mg/L per day)	2.38	1.62 - 3.48	**<0.001**			

Baseline Covariates	Unadjusted HR	95% CI	p-value	Adjusted HR[a]	95% CI	p-value
Increase in WBC (per 10^3/mcL)	1.08	1.02 - 1.13	**0.004**			
Increase in eGFR[d] (per mL/min/1.73 m^2)	1.00	0.99 - 1.01	0.83			
Iron Markers						
Decrease in Iron Slope (per 10 mcg/dL per day)	4.53	2.71 - 7.57	**<0.001**	4.21	2.51 - 7.06	**<0.001**
[c]Decrease in Iron (mcg/dL)	2.24	1.60 - 3.14	**<0.001**	1.76	1.20 - 2.57	**0.004**
Increase in Ferritin Slope (per 50 ng/mL per day)	1.12	1.05 – 1.19	**<0.001**	1.10	1.03 - 1.17	**0.003**
[c]Increase in Ferritin (ng/mL)	1.89	1.39 - 2.56	**<0.0001**	1.51	1.12 - 2.05	**0.008**
[c]Increase in Hepcidin(ng/mL)	1.58	1.16 - 2.16	**0.004**	1.43	1.05 - 1.93	**0.02**

Abbreviations: HR: hazard ratio, CI: confidence interval, UNOS: united network for organ sharing, CRP: C-reactive protein, RBC: red blood cell, OLT: orthotopic liver transplant, WBC: white blood cells, eGFR: estimated glomerular filtration rate.

[a]Adjusted for number of hospital days during OLT stay, bleeding complications and hepatic artery thrombosis.

[b]Autoimmune hepatitis, primary biliary cirrhosis or primary sclerosing cholangitis.

[c]Natural log

[d]CKD-EPI estimated glomerular filtration rate. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-12.