Published in final edited form as:

Dig Dis Sci. 2016 December ; 61(12): 3609–3620. doi:10.1007/s10620-016-4316-x.

Siglec-7 as a novel biomarker to predict mortality in decompensated cirrhosis and acute kidney injury

Andrew S. Allegretti, MD MSc¹, Guillermo Ortiz, MD², Sahir Kalim, MD¹, Joshua Wibecan, BA¹, Dongsheng Zhang, MD PhD³, Hui Yi Shan, MD⁴, Dihua Xu, PhD¹, Raymond T. Chung, MD⁵, S. Ananth Karumanchi, MD³, and Ravi I. Thadhani, MD MPH¹

Andrew S. Allegretti: aallegretti@partners.org; Guillermo Ortiz: ortiz.sanjuan@gmail.com; Sahir Kalim: skalim@partners.org; Joshua Wibecan: j.wibecan@gmail.com; Dongsheng Zhang: dzhang4@bidmc.harvard.edu; Hui Yi Shan: shanh01@yahoo.com; Dihua Xu: DXU3@mgh.harvard.edu; Raymond T. Chung: chung.raymond@mgh.harvard.edu; S. Ananth Karumanchi: sananth@bidmc.harvard.edu; Ravi I. Thadhani: RTHADHANI@mgh.harvard.edu

¹Division of Nephrology, Department of Medicine, Massachusetts General Hospital, Boston, MA, USA

²Department of Medicine, St. Elizabeth's Hospital, Boston, MA, USA

³Division of Nephrology, Department of Medicine, Beth Israel Deaconess Medical Center, Boston, MA, USA

⁴Division of Nephrology and Hypertension, Department of Medicine, Keck School of Medicine of USC, Los Angeles, CA, USA

⁵Liver Center and Gastrointestinal Division, Department of Medicine, Massachusetts General Hospital, Boston, MA, USA

Abstract

Background—Patients with decompensated cirrhosis have high morbidity and are commonly hospitalized with acute kidney injury.

Aims—We examined serum levels of Siglec-7, a transmembrane receptor that regulates immune activity, as a biomarker for mortality in patients with cirrhosis and acute kidney injury.

Methods—Serum Siglec-7 was measured in hospitalized patients with cirrhosis and acute kidney injury, as well as in reference groups with acute liver injury/acute kidney injury, cirrhosis without acute kidney injury, and sepsis without liver disease. Clinical characteristics and subsequent outcomes were examined using univariate and multivariable analyses according to initial Siglec-7 levels. Primary outcome was death by 90 days.

Results—One hundred twenty-eight subjects were included, 92 of which had cirrhosis and acute kidney injury and were used in the primary analysis. Average Model for End Stage Liver Disease (MELD) score was 24 [95% CI 23, 26] and serum creatinine was 2.5 [2.2, 2.8] mg/dL at the time Siglec-7 was measured. After adjusting for age and MELD score, high serum Siglec-7 level

Corresponding Author: Andrew Allegretti, aallegretti@partners.org. 55 Fruit Street, GRB 1008, Boston, MA, USA 02114. Tel: 617-724-8018. Fax: 617-724-1122.

Conflict of Interest: The authors declare that they have no conflict of interest.

predicted mortality with a hazard ratio of 1.96 [1.04, 3.69; $p = 0.04$]. There was no difference in Siglec-7 levels by etiology of AKI ($p = 0.24$). Addition of serum Siglec-7 to MELD score improved discrimination for 90-day mortality (category-free net reclassification index = 0.38 ($p = 0.04$); integrated discrimination increment = 0.043 ($p = 0.04$)).

Conclusion—Serum Siglec-7 was associated with increased mortality among hospitalized patients with cirrhosis and acute kidney injury. Addition of Siglec-7 to MELD score may increase discrimination to predict 90-day mortality.

Keywords

cirrhosis; hepatitis C; liver immunology; acute kidney injury; hepatorenal syndrome

Introduction

Decompensated cirrhosis is a common and morbid condition. (1, 2) Despite recent improvements in therapy, only modest reductions in cirrhosis-related mortality have been observed. (3) Many patients with cirrhosis require hospitalization for acute decompensation, such as from acute kidney injury (AKI). (1, 4, 5) It is estimated that up to 50% of those with decompensated cirrhosis will suffer from AKI, including 20% diagnosed with hepatorenal syndrome (HRS). (6, 7) Commonly used risk prediction tools, such as the Model for End Stage Liver Disease (MELD) score, may not fully capture the added mortality risk that stems from complications like AKI. (8–10) Increasingly, we have recognized the role of immune dysregulation and local hepatic and endothelial inflammation in the pathogenesis of complications of advanced liver disease. A serum biomarker that helps quantify such inflammation could identify those at high risk for poor outcomes and refine our ability to estimate survival in this population.

Sialic-acid-binding immunoglobulin-like lectin-7, or Siglec-7, is a member of a family of transmembrane receptor proteins that may hold potential as an important biomarker in decompensated liver disease. Siglecs are expressed on cells of the innate immune system and regulate inflammation. (11, 12) Siglec-7 is found predominantly on natural killer cells, as well as monocytes, some T cells, and cells in the intestine and liver. (13–17) Siglec-7 is a 51 kDa inhibitory receptor that down-regulates innate immunity when activated. (18–20) This mechanism is manipulated by several pathogens and tumors in order to subvert host immune response, including HIV and hepatitis C infections. (21–30) In addition, a recent publication showed that in chronic hepatitis C infection, known markers of liver inflammation and advanced fibrosis correlated with increasing soluble serum Siglec-7 levels, but no study has yet investigated the association of Siglec-7 and concrete clinical outcomes in liver disease, such as survival. (14)

Given this recent publication showing relationship between Siglec-7 and biochemical liver injury, we sought to evaluate serum Siglec-7 as a biomarker for mortality in decompensated liver disease with an exploratory study design. We analyzed serum Siglec-7 in a hospitalized cohort with cirrhosis and AKI in order to determine the relationship between Siglec-7 and survival. Sub-analysis was performed to determine if the etiology of AKI (see “Definitions”) impacted Siglec-7 levels. We hypothesized that increased serum Siglec-7 levels would be

associated with higher mortality. We also hypothesized that Siglec-7 would be preferentially elevated in HRS compared to other types of AKI, as hepatic vascular inflammation and vasodilation is thought to play a role in the pathogenesis of HRS. (31–33)

Methods

Study Population and Setting

Between January 2013 and April 2014, adults (age >18 years) who were hospitalized at Massachusetts General Hospital and meeting entry criteria were enrolled and followed prospectively for 90-days from hospital admission. Subjects were included if they had decompensated cirrhosis and AKI. Additional reference groups were also included to compare Siglec-7 levels in those with (a) acute liver injury and AKI, (b) decompensated cirrhosis without AKI, and (c) sepsis without liver disease. Potential subjects were excluded if they were pregnant or nursing.

Definitions of Diagnoses

Diagnoses of decompensated cirrhosis, acute liver injury, and etiology of cirrhosis were based on clinical evaluation by the treating hepatologist. A member of the research team performed an in-depth review of medical record to ensure accuracy. Etiology of cirrhosis was listed as multifactorial if there were 2 causes felt to be contributing to cirrhosis. Subjects were classified as having decompensated cirrhosis if they were diagnosed with cirrhosis prior to or during the index study admission. The Acute Kidney Injury Network (AKIN) criteria were used to diagnose AKI. (34) Acute kidney injury was classified into one of four subgroups by etiology: (1) prerenal kidney injury (resolution of AKI within 48 hours after volume resuscitation), (2) acute tubular necrosis (clinical history consistent with ischemic or nephrotoxic AKI and failure to respond to volume administration), (3) HRS (exclusion of other potential causes of AKI based on the 2007 Ascites Club Criteria), (35) or (4) other AKI (evidence of another process on serology or renal biopsy, such as glomerulonephritis). Sepsis was defined per the Surviving Sepsis Campaign. (36)

Data Collection and Outcomes

All data except for Siglec-7 measurements was registered as part of routine clinical care and taken from review of the medical record. Model for End Stage Liver Disease (MELD) score was calculated at the time of admission. (8, 37) Participants provided serum, plasma, and urine samples on enrollment and day 5 and 30 after enrollment (as available) for longitudinal analysis of Siglec-7 and future evaluation of novel biomarkers of kidney and liver injury. Members of the study team did not participate or intervene in the clinical care of the subjects. The primary outcome for this study was all-cause mortality at 90 days. The primary outcome was modeled only for those with cirrhosis and AKI.

Measurement of Serum Siglec-7

Serum Siglec-7 levels were measured using a commercially available quantitative ELISA assay (Catalog number DY1138, R & D Systems, MN). This assay is specific for Siglec-7 and is not reported to cross-react to Siglecs 2, 3, 5, 9 and 10. The coefficient of variation for

this assay is <10%. Given this assay is not yet FDA-approved for clinical use, results of Siglec-7 testing were not made available to the clinical care team.

Statistical Analysis

Subjects were divided into two equally sized groups (“high” versus “low” serum Siglec-7 levels) by dividing at the median value for the primary analysis group (4241 pg/mL). Differences in the primary outcome of death by 90 days by high/low Siglec-7 level were visualized using a Kaplan-Meier curve and compared using a log-rank test. A pre-specified Cox proportional hazard model was selected for analysis of mortality by high/low Siglec-7 level after adjusting for age and MELD score. Five sensitivity analyses of the primary outcome were performed: (1) including all 118 subjects with liver disease (irrespective of AKI status), (2) using the optimally derived cut-point for Siglec-7 by receiver operating characteristic (ROC) curve analysis, (3) excluding subjects with either prior liver transplant, HIV positive status, or hepatocellular carcinoma, (4) using a composite outcome of liver transplantation or death by 90 days, and (5) stratified analysis by hepatitis C infection status. The assumption of proportional hazards was assessed for all models using Martingale residuals. No evidence of non-proportional hazards was detected. Results of Cox proportional hazard models are summarized with hazard ratios and Wald asymptotic 95% confidence intervals. All model assumptions were checked and given a normal distribution of deviance residuals, parametric testing was used for all univariate and multivariable analysis and continuous variables were presented as means and 95% confidence intervals. Descriptive tables and figures were generated using Student’s t-tests, paired t-tests, Chi square tests (or Fisher’s exact test when cells contained <5 observations), Pearson correlation, and analysis of variance (ANOVA), as appropriate. For all subjects with liver disease where the calculation of a MELD score was appropriate, the incremental improvement of adding Siglec-7 to models containing (1) MELD score, MELD-Na score, (2) MELD score, age, and presence of cirrhosis/acute liver injury/AKI was measured by c statistic, category-free net reclassification index (NRI) and integrated discrimination index (IDI). SAS version 9.4 (Gary, NC) and R version 3.2.2 were used for analysis. Two-tailed p values <0.05 were considered statistically significant. Given the exploratory design of this study and lack of prior data of serum Siglec-7 in decompensated liver disease, no formal power calculation was performed.

Ethics Statement

This study was approved by the site’s institutional review board and abides by the guidelines set forth by the Declaration of Helsinki. No donor organs were obtained from executed prisoners or other institutionalized persons at this liver transplant center. All participants (or their health care designee) provided written informed consent.

Results

General Demographics

Serum Siglec-7 levels were analyzed in 128 hospitalized subjects: 92 with cirrhosis and AKI (primary analysis group), 9 with acute liver injury and AKI, 17 with decompensated cirrhosis and no AKI, and 10 with sepsis and no liver disease. In the primary cohort of those

with cirrhosis and AKI, average age was 57 [55, 60] years, 72% were male, 90% were of White race, and 88% were of non-Hispanic ethnicity. Average length of inpatient stay was 18 [15, 22] days. Mean time from admission to initial Siglec-7 measurement was 5.7 [4.6, 6.9] days. Mean admission MELD score was 24 [23, 26]. Serum creatinine was 2.5 [2.2, 2.8] mg/dL at the time Siglec-7 was measured. One subject (1%) had HIV. Twenty-nine subjects (32%) had hepatitis C. Of the 15 subjects with “other etiologies” of cirrhosis, 4 had cardiac cirrhosis, 3 had hepatitis B, and 3 cholestatic disease. Thirteen subjects (15%) subsequently received a liver transplant after enrollment. Twenty-eight subjects (31%) with cirrhosis and AKI required renal replacement therapy during their admission. See Table 1 for a comparison of clinical characteristics by serum Siglec-7 level among the primary group with cirrhosis and AKI.

Serum Siglec-7 levels

Serum siglec-7 levels differed by liver/kidney disease status (Figure 1). Siglec-7 levels were 4537 [4089, 4987] pg/mL in cirrhosis and AKI, 4283 [3097, 5469] pg/mL in acute liver injury and AKI, 4627 [3320, 5934] pg/mL in cirrhosis and no AKI, and 2718 [1660, 3777] pg/mL in sepsis ($p = 0.09$). Siglec-7 values were higher in subjects with cirrhosis or acute liver injury compared to subjects with sepsis/no liver disease ($p = 0.01$). There was no difference in Siglec-7 levels by etiology of cirrhosis ($p = 0.65$). See Table 2 for a comparison of clinical characteristics by disease group.

In the primary analysis group, subjects with high Siglec-7 levels had significantly higher MELD scores (28 [25, 30] vs. 21 [19, 23]; $p < 0.001$) compared to those with low Siglec-7 levels. Pearson correlation between continuous serum Siglec-7 levels and several clinical variables are reported in Figure 2, including MELD score (and its components), AST, ALT, and AST to platelet ratio index (APRI). Only 6 subjects had a liver biopsy available, thus correlation between Siglec-7 and fibrosis stage was not performed.

Among those with cirrhosis and AKI ($n=92$), 33 (36%) had prerenal AKI, 25 (27%) had HRS, 26 (28%) had acute tubular necrosis, and the remaining 8 (9%) had other AKI. There was no difference in Siglec-7 levels by etiology of AKI (4707 [3583, 5562] pg/mL for prerenal AKI, 4226 [3562, 4889] pg/mL for HRS, 5056 [4122, 5990] pg/mL for acute tubular necrosis, and 3380 [1852, 4908] pg/mL for other AKI; $p = 0.24$).

Forty-six subjects had Siglec-7 measured at study enrollment then 5 days thereafter. Siglec-7 levels decreased from 4247 [3709, 4786] pg/mL to 3691 [3196, 4186] pg/mL ($p = 0.03$) after five days. Among these 46 subjects, 21 were alive at 90 days and had an interval decrease in Siglec-7 (782 [16, 1548] pg/mL; $p = 0.05$). There was no statistically significant interval decrease in Siglec-7 among the 25 remaining subjects who died by 90 days (366 [−299, 1032] pg/mL; $p = 0.27$).

Siglec-7 and 90-day mortality

Fifty-five out of 128 hospitalized subjects (43%) died within 90-days follow-up (47/92 [51%] with cirrhosis and AKI, 4/9 [44%] with acute liver injury and AKI, 3/17 [18%] with cirrhosis and no AKI, and 1/10 [10%] with sepsis; $p = 0.007$). Among 92 subjects with cirrhosis and AKI, initial serum Siglec-7 levels were 5092 [4393, 5789] pg/mL in those who

died and 3960 [3427, 4492] pg/mL in those who survived 90 days ($p = 0.01$). A Kaplan Meier curve depicts survival over 90 days by high versus low serum Siglec-7 level (Figure 3; $p = 0.02$). For those with liver disease, the unadjusted hazard ratio of death for high Siglec-7 level was 2.01 [1.12, 3.64]; $p = 0.02$. After adjusting for age and MELD score, high serum Siglec-7 level predicted mortality with a hazard ratio of 1.96 [1.04, 3.69]; $p = 0.04$.

Several sensitivity analyses were performed. When dividing “high” versus “low” Siglec-7 subgroups at the optimal cutoff by ROC curve analysis (4300 pg/mL), hazard ratios of death were similar to the primary model in multivariable analysis. When excluding a total of 15 subjects with prior liver transplant, hepatocellular carcinoma, or HIV positive status, the hazard ratios of death for high Siglec-7 were similar to the primary analysis (unadjusted HR 2.21 [1.12, 4.37]; $p = 0.02$ and adjusted HR 2.02 [0.98, 4.19]; $p = 0.057$). When assessing for the composite outcome of death or liver transplant, the hazard ratios for high Siglec-7 were similar to the primary analysis (unadjusted HR 2.55 [1.46, 4.47]; $p = 0.001$ and adjusted HR 2.36 [1.30, 4.27]; $p = 0.005$). Among 63 subjects without hepatitis C infection, the unadjusted hazard ratio of death for high Siglec-7 was 2.32 [1.16, 4.65]; $p = 0.02$. Among 29 subjects with hepatitis C infection, the unadjusted hazard ratio of death for high Siglec-7 was non-significant (1.52 [0.50, 4.64]; $p = 0.47$). Adjusted analysis was not performed for these subgroups due small sample sizes.

Added Usefulness of Siglec-7 to Predict Mortality

Among all subjects with liver disease ($n = 118$), MELD score on hospital admission predicted 90-day mortality with a c statistic of 0.64. As a continuous measure, serum Siglec-7 predicted 90-mortality with a c statistic of 0.66. Addition of serum Siglec-7 to MELD score improved the c statistic to 0.68, with a category free NRI of 0.38 ($p = 0.04$), and an IDI of 0.043 ($p = 0.03$). Results were similar when serum Siglec-7 was added to MELD-Na score.

In a multivariable model to predict 90-day mortality containing age, MELD score, and presence of cirrhosis/acute liver injury/AKI, the c statistic was 0.76. Addition of serum Siglec-7 to this model improved the c statistic to 0.78, with a category free NRI of 0.41 ($p = 0.03$) and an IDI of 0.052 ($p = 0.02$; see Table 3).

Siglec-7 before and after liver transplant

Nine subjects had a Siglec-7 levels measured 30 days after their initial sample (Figure 4). Five of those subjects underwent liver transplantation between the two measurements. Siglec-7 levels decreased significantly after liver transplantation by 3809 [1944, 5674] pg/mL ($p = 0.005$). There was no decrease in Siglec-7 levels among the four subjects who did not undergo liver transplantation (-97 [$-1951, 2144$] pg/mL; $p = 0.89$).

Discussion

Currently, there are several tools at physicians’ disposal to help predict mortality in those with advanced liver disease. These include clinical prediction models, like the MELD score, as well as markers of advanced fibrosis, such as liver biopsy, APRI and ultrasound elastography. (1, 42) However, these models and markers may be limited at capturing the

relative increased mortality associated with acute decompensation of cirrhosis, especially in those who are hospitalized with AKI. (9, 10) Markers of immune function and regulation of inflammation, like Siglec-7, may provide insight into pathways that are affected by these clinical insults. In our cohort of subjects hospitalized with advanced liver disease, including decompensated cirrhosis, acute liver injury, and acute kidney injury, serum Siglec-7 was associated with increased mortality in adjusted and unadjusted analysis. Addition of Siglec-7 to clinical prognostic scores resulted in improvement in predictive ability for 90-day mortality, suggesting that this biomarker may provide added value to existing clinical prognostic tools.

An elegant recent publication by Varchetta *et al.* highlighted the potential pathobiologic connection between Siglec-7, immune dysregulation, and severe liver disease. (14) In a large cross-sectional study, these investigators demonstrated an increase in soluble serum Siglec-7 in a population with hepatitis C infection, and a correlation between Siglec-7 and levels of other clinical biomarkers of liver disease. This suggests that higher soluble Siglec-7 may denote a high inflammatory state in the liver and may be mechanistically important in this disease process. In Figure 2, we show similar correlation coefficients between Siglec-7 and these serologies as Varchetta *et al.* did, highlighting the reproducibility of the Siglec-7 assay in a distinct population. (14) We are able to build on these findings in our prospective study by extending that serum Siglec-7 levels were associated with increased mortality in an independent cohort. This helps confirm an important connection between liver inflammation, immune dysregulation, and clinical outcomes in cirrhosis. We showed these results in a more heterogeneous population of liver disease, expanding the application of Siglec-7 beyond those with only viral hepatitis.

Several of our results support serum Siglec-7 may be a specific clinical marker in liver disease. First, Siglec-7 levels in the three sub-groups with liver disease were higher than those hospitalized without liver disease. Second, Siglec-7 levels were similar between those with AKI and those without AKI, suggesting that levels were not affected by renal dysfunction. We originally hypothesized that Siglec-7 would differ across subtypes of AKI due to varying levels of inflammatory involvement depending on the type of AKI. Although this hypothesis was not confirmed, it is reassuring that Siglec-7 measurements correlated best with overall survival rather than a specific etiology of AKI. This may speak to the complicated pathophysiology of processes like hepatorenal syndrome, which is highlighted in the updated diagnostic criteria set forth by the International Ascites Club. (43)

In our cohort, the presence of AKI itself predicted higher mortality. Even though MELD score captures a higher risk of death in these subjects (given that higher serum creatinine translates into more MELD points), serum Siglec-7 still added incremental value in predicting mortality when added to the MELD score (as well as to a multivariable model that included MELD score). In fact, the c statistic of serum Siglec-7 alone was similar to that of MELD score. It is biologically interesting that a single lab test – serum Siglec-7 – had a similar predictive value to the reference standard of MELD score, which requires three clinical lab components for calculation. We observed only a weak statistical correlation between Siglec-7 and MELD score, suggesting that the biologic pathways these predictors measure may be independent. Regardless of its pathway, there may be tremendous value in a

single, clinically available biomarker like serum Siglec-7 that has such strong predictive power for mortality.

Additionally, we noted that serum Siglec-7 decreased dramatically following liver transplantation, suggesting its production is influenced by injured hepatocytes. There may be some additional effect from transplant immunosuppressive medications as well, though this is hard to quantify. Because our sample size for this cohort was small, additional study of this subgroup is needed to draw firm conclusions. Further study is also needed to determine the longitudinal effect of other supportive treatments in this population, as Siglec-7 levels decreased among those with repeated values who were alive at 90 days and did not change among those who had died by 90 days. It may be that trends of repeated measurements of Siglec-7 may hold prognostic value over time.

It is possible that Siglec-7 is not just a biomarker of disease activity, but a damage associated molecule as well. Prior data suggests that natural killer cells, which express Siglec-7, are important in controlling liver fibrosis. (44, 45) The absence of Siglec-7 expression on natural killer cells and the increase in soluble serum levels suggest that these immune cells are dysfunctional in advanced liver disease. (14) Future studies may be able to better elicit whether modulation of the Siglec-7 pathway is a viable target of therapy. There are monoclonal antibodies that target Siglec-2 and Siglec-3 receptors and are treatments for hematologic malignancies. (46) Further investigation is required to clarify the effect of systemic inhibition of Siglec-7 receptors.

This study should be interpreted within the context of its limitations. This was a single-center study, which may limit generalizability of our results to other populations. To this point, our MELD score had relatively low c statistic for 90-day mortality compared to some other studies. (47, 48) External validation of our results in other cohorts is required, since they are exploratory in nature and thus should be considered hypothesis generating. We used the same commercial assay as Varchetta *et al.* for measuring serum Siglec-7; however, differences in lab processing may limit comparability of absolute values between our results. Because of sample size, we could not adjust for etiology of liver disease or fibrosis stage in our analysis. We were not powered to perform a sub-group analysis in subjects with Hepatitis C, though we show similar correlations between Siglec-7 and markers of liver fibrosis across a broader range of liver disease as previously shown in subjects with Hepatitis C. Given this study included only hospitalized patients, primarily with AKI, our findings are best applied to this population. Despite these limitations, we believe our results provide important validation and extension of prior work in Siglec-7 and make an important contribution to the literature.

Conclusion

Serum siglec-7 levels were associated with increased mortality in a cohort of hospitalized patients with advanced liver disease and acute kidney injury. Addition of Siglec-7 to existing risk models such as MELD score increased discrimination to predict mortality at 90-days. There was no difference in Siglec-7 levels across subgroups of acute kidney injury. Further study of the association of serum Siglec-7 and mortality should be performed in broader

populations of cirrhosis to better delineate its role as a diagnostic marker and potential therapeutic target.

Acknowledgments

Financial Support: ASA is supported by NIH Grant 5T32DK007540-30. RTC is supported by NIH Grant K24DK078772. RIT is supported by NIH grants R01DK094486 and K24DK094872. SAK was supported by funds from the Howard Hughes Medical Institute.

List of Abbreviations

MELD	Model for End Stage Liver Disease
AKI	acute kidney injury
HRS	hepatorenal syndrome
AKIN	Acute Kidney Injury Network
ROC	receiver operating characteristic
ANOVA	analysis of variance
NRI	net reclassification index
IDI	integrated discrimination index
APRI	AST to platelet ratio index

References

1. Garcia-Tsao G, Lim JK, Members of Veterans Affairs Hepatitis C Resource Center Program. Management and treatment of patients with cirrhosis and portal hypertension: recommendations from the Department of Veterans Affairs Hepatitis C Resource Center Program and the National Hepatitis C Program. *Am J Gastroenterol.* 2009; 104:1802–29. [PubMed: 19455106]
2. Murray CJ, Atkinson C, Bhalla K, et al. The state of US health, 1990–2010: burden of diseases, injuries, and risk factors. *JAMA.* 2013; 310:591–608. [PubMed: 23842577]
3. Asrani SK, Larson JJ, Yawn B, et al. Underestimation of liver-related mortality in the United States. *Gastroenterology.* 2013; 145:375, 82.e1–2. [PubMed: 23583430]
4. Martin-Llahi M, Guevara M, Torre A, et al. Prognostic importance of the cause of renal failure in patients with cirrhosis. *Gastroenterology.* 2011; 140:488–496.e4. [PubMed: 20682324]
5. Allegretti, A.; Ortiz, G.; Cui, J., et al. Renal Outcomes following Transjugular Intrahepatic Portosystemic Shunt Placement for Refractory Ascites: A Large Case Series. *ASN Kidney Week 2015 Annual Meeting*; San Diego, CA. 2015;November 6; Presentation
6. Garcia-Tsao G, Parikh CR, Viola A. Acute kidney injury in cirrhosis. *Hepatology.* 2008; 48:2064–77. [PubMed: 19003880]
7. Tsien CD, Rabie R, Wong F. Acute kidney injury in decompensated cirrhosis. *Gut.* 2013; 62:131–7. [PubMed: 22637695]
8. Wiesner R, Edwards E, Freeman R, et al. Model for end-stage liver disease (MELD) and allocation of donor livers. *Gastroenterology.* 2003; 124:91–6. [PubMed: 12512033]
9. Bernardi M, Gitto S, Biselli M. The MELD score in patients awaiting liver transplant: strengths and weaknesses. *J Hepatol.* 2011; 54:1297–306. [PubMed: 21145851]

10. Gotthardt D, Weiss KH, Baumgartner M, et al. Limitations of the MELD score in predicting mortality or need for removal from waiting list in patients awaiting liver transplantation. *BMC Gastroenterol.* 2009; 9:72. 230X-9-72. [PubMed: 19778459]
11. Crocker PR, Paulson JC, Varki A. Siglecs and their roles in the immune system. *Nat Rev Immunol.* 2007; 7:255–66. [PubMed: 17380156]
12. Pillai S, Netravali IA, Cariappa A, et al. Siglecs and immune regulation. *Annu Rev Immunol.* 2012; 30:357–92. [PubMed: 22224769]
13. Nicoll G, Ni J, Liu D, et al. Identification and characterization of a novel siglec, siglec-7, expressed by human natural killer cells and monocytes. *J Biol Chem.* 1999; 274:34089–95. [PubMed: 10567377]
14. Varchetta S, Mele D, Lombardi A, et al. Lack of Siglec-7 expression identifies a dysfunctional natural killer cell subset associated with liver inflammation and fibrosis in chronic HCV infection. *Gut.* 2015
15. Miyazaki K, Sakuma K, Kawamura YI, et al. Colonic epithelial cells express specific ligands for mucosal macrophage immunosuppressive receptors siglec-7 and -9. *J Immunol.* 2012; 188:4690–700. [PubMed: 22467657]
16. Varchetta S, Brunetta E, Roberto A, et al. Engagement of Siglec-7 receptor induces a proinflammatory response selectively in monocytes. *PLoS One.* 2012; 7:e45821. [PubMed: 23029261]
17. Mitsuki M, Nara K, Yamaji T, et al. Siglec-7 mediates nonapoptotic cell death independently of its immunoreceptor tyrosine-based inhibitory motifs in monocytic cell line U937. *Glycobiology.* 2010; 20:395–402. [PubMed: 20032046]
18. Falco M, Biassoni R, Bottino C, et al. Identification and molecular cloning of p75/AIRM1, a novel member of the sialoadhesin family that functions as an inhibitory receptor in human natural killer cells. *J Exp Med.* 1999; 190:793–802. [PubMed: 10499918]
19. Yamaji T, Teranishi T, Alpey MS, et al. A small region of the natural killer cell receptor, Siglec-7, is responsible for its preferred binding to alpha 2,8-disialyl and branched alpha 2,6-sialyl residues. A comparison with Siglec-9. *J Biol Chem.* 2002; 277:6324–32. [PubMed: 11741958]
20. Varki A. Since there are PAMPs and DAMPs, there must be SAMPs? Glycan “self-associated molecular patterns” dampen innate immunity, but pathogens can mimic them. *Glycobiology.* 2011; 21:1121–4. [PubMed: 21932452]
21. Delputte PL, Nauwynck HJ. Porcine arterivirus infection of alveolar macrophages is mediated by sialic acid on the virus. *J Virol.* 2004; 78:8094–101. [PubMed: 15254181]
22. Rempel H, Calosing C, Sun B, et al. Sialoadhesin expressed on IFN-induced monocytes binds HIV-1 and enhances infectivity. *PLoS One.* 2008; 3:e1967. [PubMed: 18414664]
23. Zou Z, Chastain A, Moir S, et al. Siglecs facilitate HIV-1 infection of macrophages through adhesion with viral sialic acids. *PLoS One.* 2011; 6:e24559. [PubMed: 21931755]
24. Varchetta S, Lusso P, Hudspeth K, et al. Sialic acid-binding Ig-like lectin-7 interacts with HIV-1 gp120 and facilitates infection of CD4pos T cells and macrophages. *Retrovirology.* 2013; 10:154. 4690-10-154. [PubMed: 24330394]
25. Brunetta E, Fogli M, Varchetta S, et al. The decreased expression of Siglec-7 represents an early marker of dysfunctional natural killer-cell subsets associated with high levels of HIV-1 viremia. *Blood.* 2009; 114:3822–30. [PubMed: 19710502]
26. Hudak JE, Canham SM, Bertozzi CR. Glycocalyx engineering reveals a Siglec-based mechanism for NK cell immunoevasion. *Nat Chem Biol.* 2014; 10:69–75. [PubMed: 24292068]
27. Jandus C, Boligan KF, Chijioke O, et al. Interactions between Siglec-7/9 receptors and ligands influence NK cell-dependent tumor immunosurveillance. *J Clin Invest.* 2014; 124:1810–20. [PubMed: 24569453]
28. Miyazaki K, Ohmori K, Izawa M, et al. Loss of disialyl Lewis(a), the ligand for lymphocyte inhibitory receptor sialic acid-binding immunoglobulin-like lectin-7 (Siglec-7) associated with increased sialyl Lewis(a) expression on human colon cancers. *Cancer Res.* 2004; 64:4498–505. [PubMed: 15231659]

29. Bax M, Kuijff ML, Heikema AP, et al. Campylobacter jejuni lipooligosaccharides modulate dendritic cell-mediated T cell polarization in a sialic acid linkage-dependent manner. *Infect Immun*. 2011; 79:2681–9. [PubMed: 21502591]
30. Kannagi R. Carbohydrate antigen sialyl Lewis a—its pathophysiological significance and induction mechanism in cancer progression. *Chang Gung Med J*. 2007; 30:189–209. [PubMed: 17760270]
31. Gines P, Schrier RW. Renal failure in cirrhosis. *N Engl J Med*. 2009; 361:1279–90. [PubMed: 19776409]
32. Wadei HM, Mai ML, Ahsan N, et al. Hepatorenal syndrome: pathophysiology and management. *Clin J Am Soc Nephrol*. 2006; 1:1066–79. [PubMed: 17699328]
33. Adebayo D, Morabito V, Davenport A, et al. Renal dysfunction in cirrhosis is not just a vasomotor nephropathy. *Kidney Int*. 2015; 87:509–15. [PubMed: 25296092]
34. Anonymous Section 2: AKI Definition. *Kidney Int Suppl* (2011). 2012; 2:19–36. [PubMed: 25018918]
35. Salerno F, Gerbes A, Gines P, et al. Diagnosis, prevention and treatment of hepatorenal syndrome in cirrhosis. *Gut*. 2007; 56:1310–8. [PubMed: 17389705]
36. Dellinger RP, Levy MM, Rhodes A, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. *Crit Care Med*. 2013; 41:580–637. [PubMed: 23353941]
37. Moreau R, Jalan R, Gines P, et al. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology*. 2013; 144:1426–37. 1437.e1–9. [PubMed: 23474284]
38. Hajian-Tilaki K. Receiver Operating Characteristic (ROC) Curve Analysis for Medical Diagnostic Test Evaluation. *Caspian J Intern Med*. 2013; 4:627–35. [PubMed: 24009950]
39. Kim WR, Biggins SW, Kremers WK, et al. Hyponatremia and mortality among patients on the liver-transplant waiting list. *N Engl J Med*. 2008; 359:1018–26. [PubMed: 18768945]
40. Pencina MJ, D’Agostino RB, Vasan RS. Statistical methods for assessment of added usefulness of new biomarkers. *Clin Chem Lab Med*. 2010; 48:1703–11. [PubMed: 20716010]
41. R Core Team. R: A Language and Environment for Statistical Computing. 2015
42. Poynard T, Munteanu M, Luckina E, et al. Liver fibrosis evaluation using real-time shear wave elastography: applicability and diagnostic performance using methods without a gold standard. *J Hepatol*. 2013; 58:928–35. [PubMed: 23321316]
43. Angeli P, Gines P, Wong F, et al. Diagnosis and management of acute kidney injury in patients with cirrhosis: revised consensus recommendations of the International Club of Ascites. *Gut*. 2015; 64:531–7. [PubMed: 25631669]
44. Radaeva S, Sun R, Jaruga B, et al. Natural killer cells ameliorate liver fibrosis by killing activated stellate cells in NKG2D-dependent and tumor necrosis factor-related apoptosis-inducing ligand-dependent manners. *Gastroenterology*. 2006; 130:435–52. [PubMed: 16472598]
45. Melhem A, Muhanna N, Bishara A, et al. Anti-fibrotic activity of NK cells in experimental liver injury through killing of activated HSC. *J Hepatol*. 2006; 45:60–71. [PubMed: 16515819]
46. O’Reilly MK, Paulson JC. Siglecs as targets for therapy in immune-cell-mediated disease. *Trends Pharmacol Sci*. 2009; 30:240–8. [PubMed: 19359050]
47. Silva PE, Fayad L, Lazzarotto C, et al. Single-centre validation of the EASL-CLIF consortium definition of acute-on-chronic liver failure and CLIF-SOFA for prediction of mortality in cirrhosis. *Liver Int*. 2015; 35:1516–23. [PubMed: 24840673]
48. Lee M, Lee JH, Oh S, et al. CLIF-SOFA scoring system accurately predicts short-term mortality in acutely decompensated patients with alcoholic cirrhosis: a retrospective analysis. *Liver Int*. 2015; 35:46–57. [PubMed: 25203221]

Figure 1. Serum Siglec-7 levels by disease group

P = 0.09 for overall comparison. P = 0.01 for between group comparisons between the three groups with cirrhosis/acute liver injury (in blue) compared with those with sepsis and no liver disease (in black).

Key: ALI (acute liver injury) AKI (acute kidney injury).

Figure 2. Correlation between serum Siglec-7 and clinical variables

Key: MELD (Model for End Stage Liver Disease), INR (international normalized ratio), AST (aspartate aminotransferase), ALT (alanine aminotransferase), APRI (AST to platelet ratio index). Total bilirubin, INR, creatinine, AST, ALT, and APRI underwent log transformation due to skew of data.

Figure 3. Ninety-day probability of survival by high or low serum Siglec-7 level
Siglec-7 dichotomized at the median for all hospitalized subjects (4241 pg/mL)

Figure 4. Change in serum Siglec-7 levels among subjects with 30-day follow-up measurements
Solid blue line represents mean Siglec-7 levels for each panel. Left: Subjects who received liver transplant between measurements. Right: subjects who did not receive liver transplant

Table 1

Demographics and clinical characteristics by initial serum Siglec-7 level

	Low Siglec-7 <4134 pg/mL (n = 46)	High Siglec-7 4134 pg/mL (n = 46)	P value
Age (years)	59 [55, 63]	56 [53, 58]	0.22
Male sex (%)	35 (76%)	31 (68%)	0.35
White race (%)	37 (80%)	44 (96%)	0.05
Non-Hispanic ethnicity (%)	53 (83%)	61 (95%)	0.02
Body mass index (kg/m²)	30.0 [27.8, 32.1]	30.1 [27.7, 32.4]	0.95
Co-morbidities (%)			
Diabetes mellitus	14 (30%)	14 (30%)	0.99
Chronic kidney disease	16 (36%)	14 (31%)	0.65
Cardiovascular disease	14 (30%)	8 (18%)	0.14
Hypertension	18 (41%)	13 (28%)	0.21
HIV positive	0 (0%)	1 (2%)	0.99
Reason for admission (%)			0.004
Complications of cirrhosis/acute liver injury	25 (54%)	32 (70%)	
Acute kidney injury	2 (4%)	7 (15%)	
Infection	5 (11%)	5 (11%)	
Other	14 (30%)	2 (4%)	
Etiology of Cirrhosis (%)			0.22
Hepatitis C	7 (15%)	9 (20%)	
Alcohol	17 (37%)	13 (28%)	
Non-alcoholic steatohepatitis	1 (2%)	7 (15%)	
Multifactorial	13 (28%)	10 (22%)	
Other	8 (17%)	13 (15%)	
Prior complications of liver disease (%)			
Ascites requiring prior paracentesis	19 (41%)	28 (61%)	0.06
Encephalopathy	8 (17%)	20 (43%)	0.01
Gastrointestinal bleeding	4 (9%)	6 (13%)	0.50
Spontaneous bacterial peritonitis	3 (7%)	3 (7%)	0.99
Portosystemic shunt	2 (4%)	7 (16%)	0.07
Hepatocellular carcinoma	6 (11%)	7 (11%)	0.92
Prior liver transplantation (%)	2 (4%)	1 (2%)	0.99
MELD score	21 [19, 23]	28 [25, 30]	<0.001
APRI	2.4 [1.3, 3.5]	8.7 [1.4, 16.0]	0.09
Mean arterial pressure (mmHg)	78 [75, 81]	75 [72, 78]	0.13
Laboratory values			
Sodium (mEq/L)	133 [132, 135]	134 [132, 136]	0.52
Creatinine (mg/dL)	2.5 [2.0, 3.0]	2.6 [2.2, 3.0]	0.77

	Low Siglec-7 <4134 pg/mL (n = 46)	High Siglec-7 4134 pg/mL (n = 46)	P value
White blood count (K/uL)	10.0 [7.8, 12.3]	9.5 [7.6, 11.5]	0.75
Hemoglobin (g/dL)	9.5 [8.9, 10.1]	9.2 [8.7, 9.6]	0.38
Platelets (K/uL)	109 [92, 126]	69 [58, 80]	<0.001
Albumin (g/dL)	3.1 [2.9, 3.3]	3.1 [2.9, 3.3]	0.99
International normalized ratio (INR)	1.6 [1.5, 1.7]	2.0 [1.8, 2.2]	<0.001
Total bilirubin (mg/dL)	3.9 [2.3, 5.5]	15.6 [11.5, 19.7]	<0.001
Aspartate aminotransferase (U/L)	74 [36, 112]	216 [42, 211]	0.11
Alanine aminotransferase (U/L)	45 [9, 80]	122 [33, 251]	0.05
Alkaline phosphatase (U/L)	163 [118, 208]	146 [114, 177]	0.53

Key: MELD (Model for End Stage Liver Disease), APRI (aspartate aminotransferase to platelet ratio index)

Table 2

Demographics and clinical characteristics by disease group

	Cirrhosis and AKI (n = 92)	Acute Liver Injury and AKI (n = 9)	Cirrhosis, no AKI (n = 17)	Sepsis (n = 10)	P value
Age (years)	57 [55, 60]	46 [34, 57]	55 [47, 62]	72 [63, 82]	<0.001
Male sex (%)	66 (72%)	8 (89%)	14 (82%)	10 (100%)	0.14
White race (%)	83 (90%)	8 (89%)	14 (83%)	9 (90%)	0.74
Non-Hispanic ethnicity (%)	81 (88%)	8 (89%)	15 (88%)	10 (100%)	0.67
Body mass index (kg/m ²)	30.0 [28.5, 31.6]	29.4 [24.0, 34.9]	28.0 [23.6, 32.5]	24.2 [20.1, 28.3]	0.11
Co-morbidities (%)					
Diabetes mellitus	28 (31%)	0 (0%)	4 (25%)	3 (30%)	0.25
Chronic kidney disease	30 (33%)	0 (0%)	3 (18%)	2 (20%)	0.11
Cardiovascular disease	22 (24%)	0 (0%)	1 (6%)	2 (20%)	0.15
Hypertension	31 (34%)	2 (22%)	6 (38%)	6 (60%)	0.34
HIV positive	1 (1%)	0 (0%)	1 (7%)	0 (0%)	0.47
Reason for admission (%)					<0.001
Complications of cirrhosis/acute liver injury	57 (62%)	7 (78%)	12 (71%)	0 (0%)	
Acute kidney injury	9 (16%)	0 (0%)	0 (0%)	0 (0%)	
Infection	10 (11%)	0 (0%)	4 (23%)	10 (100%)	
Other	16 (18%)	2 (22%)	1 (6%)	0 (0%)	
Etiology of Cirrhosis/Liver Injury (%)					0.30
Hepatitis C	16 (17%)	0 (0%)	4 (23%)	–	
Alcohol	30 (33%)	4 (44%)	5 (29%)	–	
Non-alcoholic steatohepatitis	8 (9%)	0 (0%)	0 (0%)	–	
Multifactorial	23 (25%)	1 (11%)	3 (18%)	–	
Other	15 (16%)	4 (44%)	5 (29%)	–	
Prior complications of liver disease (%)					
Ascites requiring prior paracentesis	47 (51%)	0 (0%)	9 (53%)	–	0.01
Encephalopathy	28 (30%)	0 (0%)	3 (18%)	–	0.10
Gastrointestinal bleeding	10 (11%)	0 (0%)	4 (29%)	–	0.17

	Cirrhosis and AKI (n = 92)	Acute Liver Injury and AKI (n = 9)	Cirrhosis, no AKI (n = 17)	Sepsis (n = 10)	P value
Spontaneous bacterial peritonitis	6 (7%)	0 (0%)	3 (18%)	–	0.19
Portosystemic shunt	9 (10%)	0 (0%)	2 (12%)	–	0.58
Hepatocellular carcinoma	11 (12%)	0 (0%)	2 (12%)	–	0.55
Prior liver transplantation (%)	3 (3%)	0 (0%)	1 (6%)	–	0.69
MELD score	24 [23, 26]	30 [22, 39]	17 [15, 21]	–	<0.001
APRI	5.6 [1.9, 9.3]	–	2.7 [1.9, 3.5]	–	0.19
Mean arterial pressure (mmHg)	77 [75, 79]	87 [74, 101]	81 [75, 88]	77 [67, 87]	0.03
Laboratory values					
Sodium (mEq/L)	134 [132, 135]	132 [127, 138]	133 [130, 135]	138 [135, 141]	0.07
Creatinine (mg/dL)	2.5 [2.2, 2.8]	5.4 [3.1, 7.7]	0.8 [0.7, 1.0]	1.1 [0.5, 1.7]	<0.001
White blood count (K/uL)	9.8 [8.3, 11.2]	14.0 [9.0, 18.9]	7.3 [4.2, 10.4]	10.5 [7.5, 13.5]	0.12
Hemoglobin (g/dL)	9.3 [9.0, 9.7]	10.0 [8.3, 11.8]	10.0 [8.8, 11.2]	9.8 [8.3, 11.4]	0.35
Platelets (K/uL)	89 [78, 100]	171 [87, 256]	84 [47, 120]	196 [92, 300]	<0.001
Albumin (g/dL)	3.1 [3.0, 3.2]	2.6 [2.3, 3.0]	2.6 [2.3, 2.8]	2.4 [2.0, 2.7]	<0.001
International normalized ratio (INR)	1.8 [1.7, 1.9]	1.7 [1.4, 2.0]	1.6 [1.4, 1.8]	1.2 [1.1, 1.4]	0.006
Total bilirubin (mg/dL)	9.8 [7.3, 12.3]	15.6 [6.4, 24.8]	8.3 [–0.4, 16.9]	1.1 [0.4, 1.7]	0.11
Aspartate aminotransferase (U/L)	146 [56, 236]	531 [98, 964]	76 [41, 111]	548 [–628, 1723]	0.04
Alanine aminotransferase (U/L)	84 [36, 132]	455 [26, 884]	35 [24, 45]	195 [–172, 563]	0.001
Alkaline phosphatase (U/L)	154 [127, 181]	140 [97, 182]	184 [96, 272]	140 [46, 235]	0.80
Serum Siglec-7 (pg/mL)	4537 [4089, 4987]	4283 [3097, 5469]	4627 [3320, 5934]	2718 [1660, 3777]	0.09

Key: MELD (Model for End Stage Liver Disease), APRI (aspartate aminotransferase to platelet ratio index)

Table 3

Added predictive ability for 90-day mortality with serum Siglec-7 among 118 subjects with acute or chronic liver disease

	MELD	MELD-Na	Siglec-7	MELD + Siglec-7	MELD-Na + Siglec-7	Multivariable Model	Multivariable Model + Siglec-7
C statistic	0.64	0.62	0.66	0.68	0.67	0.76	0.78
Category-free net reclassification index							
P-value				0.04	0.04		0.03
Integrated discrimination increment				0.043	0.051		0.052
P-value				0.03	0.01		0.02

Key: MELD (Model for End Stage Liver Disease), MELD-Na (MELD score with sodium). Multivariable model included three predictors: age, MELD score, and presence of cirrhosis/acute kidney injury.