Bleeding Complications in Acute Liver Failure

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Abbreviations.

ALF, acute liver failure
APAP, acetaminophen
ICP, intracranial pressure
ICU, intensive care unit
INR, International Normalized Ratio of the prothrombin time
LT, liver transplantation
NAC, *N*-acetylcysteine
RBC, red blood cell
rFVIIa, recombinant Factor VIIa
RRT, renal replacement therapy
SIRS, systemic inflammatory response syndrome
SRMD, stress-related mucosal disease
TFS, transplant-free survival/survivors
UGI, upper gastrointestinal

In patients with acute liver failure (ALF), elevated prothrombin time and thrombocytopenia can fuel a perception of a bleeding tendency. However, the incidence, site, risk factors, and clinical significance of bleeding complications have not been previously quantified in a large cohort of patients with ALF. We studied 1770 adult patients enrolled in the ALF Study Group Registry between 1998 and 2016. Bleeding complications and blood component transfusions were collected for 7 days after admission. The relationship of bleeding complications to 21-day mortality was assessed. Despite a median INR of 2.7 and platelet count of 96×10^9/L on admission, bleeding complications were observed in only 187 patients (11%), including 173 spontaneous and 22 post-procedural bleeding episodes. Eighty-four percent of spontaneous bleeding episodes were from an upper gastrointestinal source and rarely resulted in red blood cell (RBC) transfusion. Twenty patients experienced an intracranial bleed, half of which occurred spontaneously and half after intracranial pressure (ICP) monitor placement, and was the proximate cause of death in 20% and 50%, respectively. Bleeders and patients who received RBC transfusions were more acutely ill from extra-hepatic organ system failure, but not from hepatocellular failure. Consistent with this observation, bleeding complications were associated with lower platelet counts, but not higher INR. Transfusion of any blood component was associated with nearly 2-fold increased death or need for liver transplantation at day 21, but bleeding complications were the proximate cause of death in only 5% of cases. *Conclusions.* Despite a perceived bleeding diathesis, clinically significant bleeding is uncommon in patients with ALF. Bleeding complications in patients with ALF are markers of severe systemic inflammation rather than of coagulopathy and so, portend a poor prognosis.

Abnormal hemostasis is integral to the definition of acute liver failure (ALF), and is characterized by an elevated International Normalized Ratio (INR) of the prothrombin time (1). Recent studies have suggested that, despite the frequently intimidating elevation of the INR and moderate thrombocytopenia, global hemostasis in most patients with ALF remains normal or "rebalanced," at least *in vitro* (2, 3). Compensatory mechanisms have been identified for each phase of hemostasis in patients with ALF such that pro-hemostatic drivers may actually overcompensate for deficient liver-derived coagulation factors, and result in a relative hypercoagulable state (4).

Whether *in vitro* rebalance equates with clinical rebalance and a low risk of bleeding complications in patients with ALF has not been determined. Early clinical series suggested that patients with ALF had a propensity to bleed, and that bleeding episodes resulted in increased morbidity and mortality (5-7). Capillary-type bleeding from mucosal erosions of the upper gastrointestinal (UGI) tract were most frequently identified as the source of bleeding, but other mucosal sites included the nasopharynx, lung, and female genitourinary tract (7). Curiously, there has been scant mention of the bleeding incidence or its contribution to the morbidity and mortality of patients with ALF in recent series, leading to a perception that bleeding complications are not an important determinant of outcome. For example, in one of the largest series of ALF patients ever reported (over 2000 cases), bleeding was not mentioned as a complication of ALF or a co-factor in poor outcome (8). The absence of a contemporary update on the incidence and significance of bleeding complications, and the recent laboratory evidence of rebalanced hemostasis may have contributed to the recommendation that "no routine correction of coagulation abnormalities" is warranted in patients with ALF (9).

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Routine assays of hemostasis performed *in vitro* may not reflect hemostasis *in vivo* accurately; all coagulation assays are deficient in certain components of the hemostatic system, for example, endothelial factors (10), anti-coagulant pathways and activated platelets. Nevertheless, clinicians continue to rely on the INR to assess bleeding risk, either from spontaneous bleeding or after an invasive procedure. In the present study, we have sought to reanalyze the risk of bleeding in patients with ALF enrolled in the US ALF Study Group Registry. The Registry has prospectively collected clinical and laboratory data on more than 2800 patients with ALF since 1998, including bleeding complications and transfusions of blood components early after admission. In the following work, we have quantified the incidence of spontaneous and post-procedural bleeding complications, estimated the severity of bleeding, explored the indications for blood component transfusion, and associated bleeding complications and receipt of transfusions to 21-day outcome of patients with ALF.

Methods.

Patients. Study patients were recently described (11), and accrual in the present study followed the algorithm in **Supplemental Figure 1**. ALF was defined by standard criteria reported by the ALF Study Group previously (12). All patients with ALF in the ALF Study Group Registry enrolled between January 1, 1998 and January 1, 2016 were eligible for inclusion. Of the 2345 patients who were screened, 575 (24%) were excluded for missing data. The 21 day outcome of the remaining 1770 patients who met entry criteria included 781 (44%) who recovered without liver transplantation (spontaneous survivors; SSs), 430 (24%) who underwent liver transplantation (LT), and 559 (32%) who died.

As the percent of excluded patients due to missing data was significant, we performed a comparison of included and excluded Registry enrollees (**Supplemental Table 1**). Excluded patients appear to have been less acutely ill than included patients, and the missing data suggest that they were less intensively followed. Specifically, excluded patients were less likely to have the systemic inflammatory response syndrome (SIRS), less likely to have high grade hepatic encephalopathy, and had higher platelet counts and lower INRs on admission, than included patients. Excluded patients were also slightly less likely to have had a bleeding complication after study admission (7.4% *vs*. 10.6%), were less likely to receive a blood component transfusion during admission, and less likely to have died at 21 days than included patients. However, bleeding complications as the cause of death were similar in the two groups.

Definitions and Data Collection. Bleeding complications were reported daily by the study site clinical investigators from admission (day 1) through day 7. No formal definition of bleeding was specified in the Registry's Manual of Operations, although sites were required to specify on each day whether bleeding had occurred, and if so, the site of origin. Bleeding was recorded as

"gastrointestinal," or "other," where the other site of bleeding was required to be entered manually. The case report forms for all bleeders was reviewed by the study principal investigator (RTS) and classified as either spontaneous (non-procedure-related) or post-procedural, and further classified according to the site of bleeding. The adjudication process was deliberately designed to be overly-inclusive without regard to the severity of bleeding or whether the bleeding episode resulted in red blood cell (RBC) transfusion. Additional bleeders were identified through review of case report forms where subjects were identified as having a hemorrhage on imaging studies, as an ICP complication, or as the cause of death. Case report forms of all patients in whom the site clinical investigator indicated that a bleeding complication contributed to a patient's death by day 21 were reviewed in order to adjudicate the role of bleeding in the death, and to classify the bleeding as spontaneous or post-procedural. Daily recording of the following was also required: transfusion of blood products (RBCs, platelets, and plasma); administration of vitamin K, recombinant activated Factor VII (rFVIIa), gastric acid suppression, vasopressors, and *N*-acetylcysteine (NAC); and management decisions including hemodialysis, renal replacement therapy (RRT), mechanical ventilation, liver biopsy, and intracranial pressure (ICP) monitoring. Subjects were not considered bleeders if the episode occurred during or after liver transplantation.

Statistical Analysis. SAS software (version 9.4; Cary, NC) was used to perform statistical analyses. Baseline variables were described using counts and percentages for categorical data, or means and standard deviations (medians and interquartile ranges) for continuous normal (skewed) data. For variables identified as clinically relevant, statistical tests were performed using Chi-square, ANOVA, or Kruskal-Wallis tests. Modeling of hemoglobin, platelet, and INR values over time was performed using a linear mixed model with unstructured covariance for

each patient to account for the within-patient correlation across measurement days, where bleeding was defined as a dichotomous indicator of bleeding at any time between admission and day 7, LT, discharge, or death. Figures illustrate mean hemoglobin, platelet, and INR estimates for each observation day adjusted for the correlation across measurement days (*ie.*, least square means). Hazard of LT or death are shown as Kaplan-Meier curve with log-rank test statistic. All statistical tests are reported as two-sided with a type I error rate of 5%.

Results.

Incidence and clinical characteristics of bleeding complications.

A total of 1770 adults with ALF entered the study, of whom 430 (24%) underwent LT and 559 (32%) died by day 21. Two hundred thirty-seven patients (13%) underwent ICP monitor placement, the procedure with highest potential morbidity and mortality from bleeding complications. One hundred ninety-five bleeding complications occurred in 187 patients with ALF (**Table 1**), for an overall incidence of 10.6% during the first 7 days of admission; 8 patients experienced more than one bleeding complication. Of all bleeding episodes, 173 were spontaneous (89%) and 22 were post-procedural (11%). The vast majority of spontaneous bleeding complications were from an upper gastrointestinal (UGI) source (163 of 173 complications; 94%), with 10 additional spontaneous bleeds occurring at an intracranial site either before placement, or in the absence, of an ICP monitor. Post-procedural bleeding from venous access sites were recorded in 8 cases, a nasopharyngeal source after naso-gastric tube placement in 2 cases, genitourinary source after urinary bladder catheterization in 2 cases, and after intracranial pressure (ICP) monitor placement in 10 cases, 5 of which resulted in death. In patients with a recorded primary cause of death (484/599), bleeding complications (spontaneous or post-procedural) were deemed the proximate cause of death in 10 patients (2.1%), 7 of whom had spontaneous or post-ICP monitor intracranial bleeding. An additional 6 patients who died between 8-21 days also had bleeding listed as a cause of death (3.3%). Bleeding episodes most frequently occurred on a single day after admission to the study site (58%) or resolved within two days (22%), and most commonly occurred on day 1 (52%; data not shown).

Comparison of patients with and without bleeding complications.

Clinical features of bleeders and non-bleeders are compared in **Table 2**. Bleeders were more often male than non-bleeders (40 *vs.* 30%, respectively; P=0.006), but were otherwise demographically similar. Bleeding complications occurred as frequently in patients with acetaminophen (APAP)-induced as non-APAP-induced etiology. The time delay of presentation for medical care (onset of ALF symptoms to study enrollment) was similar between the two groups (5 *vs.* 6 days in bleeders and non-bleeders, respectively; P=0.10). Although bleeding episodes prior to transfer to the study site were not recorded, bleeders were more likely to have received plasma transfusion prior to study admission (65 *vs.* 42%, respectively; P<0.001), and more likely to have been taking aspirin on admission (9 *vs.* 5%, respectively; P=0.045), than non-bleeders. However, there was no difference in the proportion of patients on anticoagulants at the time of study admission in the two groups.

Bleeders were generally more systemically ill than non-bleeders and had more extra-hepatic organ dysfunction (Table 2). Although the prevalence of the SIRS defined as ≥2 positive components was only modestly higher in bleeders than non-bleeders (80 *vs.* 74%, respectively; P=0.127), individual components of the SIRS reflected a greater severity of systemic inflammation in bleeders, including the two-sided temperature (<36 or >38ºC; 37 *vs.* 28%, P=0.014) and WBC criteria (<4 or >12x10^9/L; 53 *vs.* 46%, P=0.052), pulse (P<0.001), and PCO_2 (P=0.028), with a trend toward higher respiratory rate in bleeders (P=0.09). Bleeders more frequently developed high-grade (grade 3 or 4) hepatic encephalopathy (62 *vs.* 48% on admission, and 86 *vs.* 63% over days 1-7), had higher serum creatinine (2.2±2.3 *vs.* 1.6±2.1mg/dl), higher lactate (5.4±8.2 *vs.* 4.4±6.0mg/dl), lower bicarbonate (20.0±10.0 *vs.* 22.0±8.0mg/dl), and higher phosphate (3.8±2.9 *vs.* 3.1±2.3mg/dl) than non-bleeders (P<0.001,

except for lactate and bicarbonate, P=0.002). Although abnormal laboratory parameters of extra-hepatic organ dysfunction were more severe in bleeders, bleeders and non-bleeders had similar degrees of liver dysfunction as assessed by INR, bilirubin, ammonia, and albumin, which were not statistically different between the two groups.

Baseline hemoglobin on admission to the study in bleeders was lower than in non-bleeders (10.3±2.8 *vs.* 11.1±3.1g/dl, respectively; P<0.001); nadir hemoglobin over the first 7 days of admission was also lower in bleeders than non-bleeders (8.4±2.1 *vs.* 9.2±2.5g/dl; P<0.001). However, the mean percent decrease in hemoglobin from admission to nadir in bleeders and non-bleeders was similar (decrease of 15 and 14%, respectively), and the hemoglobin concentration was similar between the groups on each day after day 2 (**Figure 1A**).

Although both the admission and the nadir platelet count were lower in bleeders (96±92 and 53±45 x10^9/L) than non-bleeders (128±108 and 77±81 x10^9/L, respectively; both P<0.001), the admission and peak INR in bleeders (2.7±2.4 and 3.2±3.3) was not significantly different than non-bleeders (2.8±2.2 and 3.3±2.7, respectively). In fact, bleeders had significantly lower platelet counts than non-bleeders on each study day (P<0.001 for each day; **Figure 2A**), while INR was similar between the groups on each day (**Figure 2B**). There was no relationship of the platelet count to the INR on admission in bleeders or non-bleeders (r^2 =0.03 and 0.01, respectively; data not shown).

Bleeders also received many more therapeutic interventions than non-bleeders, reflecting the severity of their systemic illness (Table 2). The incidence of ICP monitor placement (20 *vs.* 14%), RRT (57 *vs.* 34%), vasopressor administration (65 *vs.* 34%), use of gastric acid suppressants (87 *vs.* 80%), and transfusion of plasma (64 *vs.* 34%), and platelets (78 *vs.* 54%) was higher in bleeders than non-bleeders, respectively (all P<0.001 except for ICP monitor

placement and gastric acid suppressants, P=0.019 and P=0.039, respectively). Interestingly, transfusion of RBC in bleeders was not different than in non-bleeders (16 *vs.* 17%, respectively), again suggesting that the magnitude of the bleeding complication was small. The administration of *N*-acetylcysteine (NAC), which theoretically might increase bleeding episodes by decreasing vonWillebrand Factor multimer size(13), was not associated with bleeding complications.

Comparison of patients who received and did not receive red blood cell transfusions.

The clinical characteristics of patients who received RBC transfusions differed from those of bleeders in several regards. As shown in **Table 3**, many more patients received RBC transfusions (651/1770; 37%) than experienced bleeding complications (187/1770; 11%); of the 651 patients who received RBC transfusions, only 120 (18%) experienced bleeding complications. However, bleeding complications were significantly more common in patients who received RBC transfusions than in those who did not (18 *vs.* 6%, respectively; P<0.001). Although a greater proportion of bleeders than non-bleeders were men, a greater proportion of those who received RBC transfusions than those who did not were women (73 *vs.* 67%, respectively; P=0.003), possibly due to the fact that females entered the study with a lower mean hemoglobin than did men (10.7 *vs.* 11.9 g/dl, data not shown). In addition, comparison of serum hemoglobin concentrations in RBC-transfused and non-transfused patients (**Figure 1B**) suggests that the former were transfused for anemia on admission rather than an acute drop in hemoglobin after admission. Indeed, the mean decrease in hemoglobin from admission to nadir in RBC-transfused and non-transfused patients was similar (15% and 13%, respectively), and the difference in hemoglobin in transfused and non-transfused patients (Figure 1B) was greater than

the difference between bleeders and non-bleeders (Figure 1A), both suggesting that the indication for transfusion was anemia rather than bleeding.

Otherwise, patients who received RBC transfusions resembled bleeders (Table 3): they were more acutely ill than those who were not transfused, more frequently met SIRS criteria (P=0.002), had a higher proportion of patients reaching high-grade (3 or 4) encephalopathy (P<0.001), had laboratory parameters reflecting more severe systemic illness, and they received more therapeutic interventions than those who did not receive RBC transfusions. Also similar to bleeders, receiving RBC transfusions had no consistent relationship to the severity of liver injury by INR, but was significantly related to the platelet count, a marker of systemic inflammation.

Relationship of blood component transfusion to bleeding complications.

Although patients who experienced bleeding complications were more likely to receive transfusion of platelets and/or plasma than non-bleeders (Table 2), the majority of blood components transfused did not appear to be in response to bleeding complications. As shown in **Table 4**, 37%, 56%, and 26% of study patients received RBC, plasma, and platelets, respectively, over the course of the first 7 days of admission. However, most patients who received RBC, plasma, or platelets were non-bleeders (82%, 85%, and 81%, respectively). In addition, 5-6% of patients transfused received blood components before the occurrence of their bleeding complication, indicating that bleeding was not the indication for the transfusion. Therefore, at a minimum, 87%, 91%, and 86% of patients who received RBC, plasma, and platelets, respectively, were transfused for an indication other than active bleeding (for example, for non-hemorrhagic anemia, or prophylaxis before an invasive procedure).

The practice of administering blood products (RBC, plasma, and/or platelets) during days 1-7 for any indication has decreased by 2.6%/year between 1998-2015. Nevertheless, the incidence of bleeding complications has remained stable over the same time frame (mean 10.7±3.8%/year) (**Supplementary Figure 2**). Since the majority of transfusions were given as prophylaxis, this observation suggests that withholding transfusions has not increased bleeding complications in patients with ALF.

Relationship of blood product transfusion and bleeding complications to outcome of ALF.

The incidence of bleeding complications according to 21 day outcome in spontaneous survivors, those who underwent LT, and those who died was 7%, 5%, and 18%, respectively. The data in **Table 5** depict the association of blood product transfusion, vitamin K, and rFVIIa during the first 7 days of admission to outcome at day 21. Patients who received RBC, plasma, or platelet transfusions were each nearly twice as likely to have died or undergone LT at day 21 than non-transfused patients. Of the 944 patients who died or underwent LT, RBC, plasma, or platelets were transfused in 45, 70, and 32%, respectively; of 713 spontaneous survivors, transfusions were recorded in 27, 39, and 16%, respectively (all P<0.001). Receipt of any blood product was observed in approximately 70% of patients who died or underwent LT, but only approximately 30% of spontaneous survivors (data not shown). Although relatively few patients received rFVIIa (N=37), most died or underwent LT (76%), and the prevalence of receiving rFVIIa was over 3-fold higher in this group compared to spontaneous survivors (P=0.022). Outcomes were significantly better in patients who received vitamin K than those who did not, with vitamin K received by 20% of spontaneous survivors, but by those who died or underwent LT in only 15% (P=0.006).

As a total study population, bleeding complications during the first 7 days of admission were associated with negative outcomes at day 21, with a transplant-free survival (TFS) rate among bleeders of 28% (median time to transplant/death 6 (95% CI 4-7) days), while the TFS rate among non-bleeders was 45% (median time to transplant/death 12 (95% CI 9-18) days (P<0.001; data not shown). However, as seen in the Kaplan-Meier curves in **Figure 3**, the presence of bleeding complications only affected TFS in patients with APAP-induced ALF (TFS 34% in bleeders *vs.* 63% in non-bleeders; P<0.001), but not in patients with non-APAP ALF.

Discussion.

The incidence of bleeding complications in this large, contemporary population with ALF was approximately 11%, the majority of whom had clinically insignificant bleeding. These findings from the ALF Study Group Registry contrast with older historical reports, in which many patients not only bled, but died from the bleed. In an early autopsy series of patients with ALF, Gazzard, *et al*., (6) reported that ~30% died of a bleeding complication, ~90% of whom bled from a gastrointestinal source. Other early series reported the incidence of bleeding complications ranged between 50-70%, with death reported from bleeding in ≥30% (5, 7, 14). Although the incidence and mortality of bleeding complications has decreased for critically ill patients in general (15), these data support the emerging consensus that patients with ALF are not at major risk of significant bleeding complications despite their dramatically elevated INR.

The International Society of Thrombosis and Haemostasis has defined major bleeding as causing death, occurring in a critical area or organ (including intracranial), and/or the need for RBC transfusion in the setting of a bleed associated with a ≥2g/dl drop in hemoglobin (16). The preponderance of evidence in the present series suggests that few bleeding complications in

patients with ALF would be categorized as major or even clinically significant. First, 87% of RBC-transfused patients received the transfusion for an indication other than a bleeding complication, presumably for anemia (Table 4). Second, the magnitude of decrease in hemoglobin from study admission to nadir within 7 days was nearly identical in patients who experienced bleeding complications and those who did not (decrease of 15 *vs.* 14%, respectively). Third, in patients who died with a reason for death recorded, only 10 of 1770 patients reviewed were considered to have died as a consequence of a bleeding complication, 7 of whom died of intracranial hemorrhage, and 5 of which were likely related to ICP monitor placement. Finally, differences in mortality between bleeders and non-bleeders were only observed after day 7 (Figure 3), suggesting a temporal dissociation between the bleeding episode and death. The finding that 10 patients had intracranial bleeds in the absence of an ICP monitor, presumably a complication of cerebral edema, has not been previously reported.

Although post-procedural bleeding complications remain a significant concern for clinicians caring for patients with ALF, the data suggest that they are rare. For example, 641 patients underwent central venous catheter placement for RRT, yet there were fewer than 5 instances of bleeding at the insertion site (0.8%); no other more serious bleeding complications were reported. The observation appears to support a recent series from an intensive care liver unit, in which 658 central venous cannulations in 283 patients (~35% with APAP overdose) with INR ≥1.5 and/or platelet count ≤150 x 10^9/L and without routine plasma or platelet transfusion resulted in only 1 serious bleeding complication, a hemothorax (17). Our low incidence of bleeding after RRT catheter insertion may have been influenced by blood product prophylaxis, but the above study in which plasma and platelets were only transfused in 1.8 and 4.2% of patients, respectively, also raises the question of whether such prophylaxis were necessary in the

first place. The observation that there has been a steady decline in blood component transfusion over the 17 years of data collection by the ALF Study Group without an increase in bleeding complications (Supplementary Figure 2) also raises this question. Intracranial bleeding after ICP monitor placement, however, remains a significant threat although is uncommon, occurring in 10 of 237 (4.2%) of our study patients, 5 of whom (2%) probably died of complications of the bleed. These statistics compare favorably with fatal bleeding complication rates of 1%, 5%, and 4% of patients with ALF who received ICP monitors in epidural, subdural, and intraparenchymal locations, respectively (18). These data highlight the fact that placement of an ICP carries a significant risk of intracranial bleeding and death.

Despite their generally mild severity, bleeding complications were associated with lower 21-day TFS (Figure 3), but only for patients with APAP-induced ALF. The occurrence of bleeding complications had no effect on TFS in patients with non-APAP-induced ALF. In other analyses, we have found the occurrence of bleeding complications in patients with APAP-induced ALF to be a marker of particularly severe acute illness (data not shown). As shown in Table 5, the 21-day outcome of LT or death was nearly 2-fold higher in patients who received transfusion of any blood component within the first 7 days of admission. Therefore, it is also conceivable that administering blood components early after admission has adverse effects that are particularly significant for patients with APAP-induced ALF, possibly by exacerbating a pre-existing hypercoagulable state, resulting in microvascular thrombosis within the liver and peripheral circulation, and compounding the primary liver injury (19) and poor peripheral tissue perfusion (20), respectively. Other hazards of blood product transfusion, such as volume overload, transfusion-associated lung injury, immune dysregulation, and exacerbation of intracranial hypertension, are also possible.

The data also suggest that bleeding complications and the development of anemia are primarily due to severe systemic inflammation. In Tables 2 and 3, both bleeders and those receiving RBC transfusions are characterized by clinical features and laboratories suggestive of more severe systemic injury than non-bleeders and non-transfused patients, respectively. The finding that platelet count was significantly lower in bleeders and RBC-transfused patients is consistent with this hypothesis, since we have recently shown in the same patient population that the decline in platelet count after admission is proportional to the severity of systemic complications and the SIRS (11). The fact that the INR is not different between these populations is also consistent with our previous observations that INR does not vary with the severity of systemic inflammation. Another interpretation of these data might be that lower platelet count, but not higher INR, is a more important risk factor for bleeding complications, as has been suggested by other authors (6).

For the vast majority of patients with ALF and UGI bleeding, the likely source is "stress-related mucosal disease" (SRMD), a manifestation of critical illness characterized by intense systemic inflammation (21). In a recent survey of >1000 ICU patients, clinically significant UGI bleeding occurred in only 2.6%, but risk factors included three integral features of the ALF syndrome: liver disease (OR 7.6), coagulopathy (OR 5.2), and RRT (OR 6.9) (15). The responsible lesion, sub-epithelial hemorrhage, is caused by gastric mucosal ischemia proportional to the severity of underlying illness, rather than defective hemostasis (21). Consequently, prophylaxis with acid suppression has not been universally shown to decrease its incidence (22). Although early studies in patients with ALF suggested benefit from histamine-2-receptor antagonists (23), our data suggest no apparent benefit from gastric acid inhibition. The

difference between early and contemporary series may be a recent overall decrease in SRMD as a result of improved ICU care, resulting in decreased mucosal hypoperfusion (22).

There are multiple possible explanations for the low incidence and mild severity of bleeding complications in patients with ALF. Thrombin generation in ALF is generally normal *in vitro* in the presence of thrombomodulin (24, 25). Deficiency of liver-derived procoagulant proteins is proportional to deficiency of anticoagulant proteins (2). VonWillebrand factor and Factor VIII levels are increased dramatically due to endothelial activation/injury from the SIRS (26, 27). Procoagulant microparticles are released by systemic inflammation (28). Finally, fibrinolysis is severely impaired such that clot lysis in many patients cannot be detected (24). Another obvious possibility remains unproven: administering prophylactic blood components to treat the high INR and low platelets is effective in decreasing bleeding complications.

We acknowledge limitations in our data and conclusions. The ALF Study Group Registry was not designed to link bleeding complications to specific procedures, blood product transfusions, and episodes of hemodynamic instability, and *post-hoc* analyses without pre-defined research questions increase the risk of bias. Missing data led to exclusion of 24% of Registry participants with the consequences noted in Supplemental Table 1: the apparent exclusion of less acutely ill patients from the analysis. We emphasize, however, that we aimed to describe a "worst case scenario" in order not to underemphasize the risks of bleeding in patients with ALF, and the exclusion of these patients should, therefore, not have contributed to an underestimate of the risk. Although an earlier version of the Registry Operations Protocol Manual required quantitation of administered blood products, a later version has not. The clinical indication for transfusion of a specific blood product was also not captured. Therefore, some of our conclusions were admittedly reached on circumstantial evidence.

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In conclusion, bleeding complications in patients with ALF are primarily clinically insignificant. Spontaneous bleeding complications are comprised overwhelmingly of self-limited UGI bleeding, which appears to have decreased in incidence and severity over the last 40 years. Post-procedural complications remain rare, and those linked to the death of patients with ALF were universally complications of ICP monitor placement. Transfusion of blood components is associated with increased 21-day poor outcome (LT and/or death). Future study must answer an important question raised by this report: does pro-hemostatic blood component transfusion decrease bleeding complications or adversely affect patients with ALF?

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Hepatology

Figure Legends.

Figure 1. **Blood hemoglobin concentration (g/dl) on each day after admission for ALF.** (A).
Hemoglobin according to study day in early bleeders *vs.* non-bleeders. (B). Hemoglobin
according to study day in patients who received red blood cell (RBC) transfusions *vs*. those who
did not. *P<0.05, **P<0.01, ***P<0.001.

Figure 2. **INR (A) and platelet count (B) of patients on days 1-7 after admission to the study
according to the occurrence of bleeding complications.** ***P<0.001.

Figure 3. **Kaplan-Meier curve of transplant-free survival (TFS) according to the occurrence of
bleeding complications between days 1-7 in patients with acetaminophen (APAP)- and non-APAP-
induced ALF.** Overall, the TFS among non-bleeders was 45.2%, with median time to transplant/death
occurring at 12 (95% CI 9-18) days, and TFS among bleeders was 27.6%, with median time to
transplant/death occurring at 6 (95% CI 4-7) days (P<0.001). According to etiology, this difference is due
to a difference in patients with APAP-induced ALF (P<0.001); there was no significant difference in TFS
in patients with non-APAP-induced ALF according to the occurrence of bleeding complications.

1

Cause of Bleeding	Bleeding Site	Total Occurrences* (N)	Cause of Death (N)
Spontaneous	UGI	163	3
	Intracranial	10	2
Post-Procedural	Venous access site	8	0
	Intracranial (ICP monitor)	10	5
	Nasopharyngeal	2	0
	Genitourinary	2	0
Total		195	10

Table 1. **Sites and causes of bleeding complications in patients with ALF.**

*N=2 had both spontaneous UGI and spontaneous intracranial bleeding
N=2 had both spontaneous UGI and post-procedural intracranial bleeding
N=4 had both spontaneous UGI and post-procedural bleeding at venous access site

ICP, intracranial pressure; UGI, upper gastrointestinal

2

Clinical Feature	N	Non-Bleeders (N = 1,583) N±SD (%)	Bleeders (N = 187) N±SD (%)	P
Demographics				
Age (Years)	1770	41±15	42±14	0.499
Gender (% Female)	1770	1109(70.1)	112(59.9)	0.006
Race (% Caucasian)	1770	1164(73.5)	145(77.5)	0.274
APAP Etiology of ALF	1770	710(44.9)	88(47.1)	0.62
Clinical Features on Admission				
Symptoms to enrollment (d)	1719	6±13	5±11	0.100
Plasma before admission	1720	644(41.9)	118(64.8)	< 0.001
Anticoagulants	1770	41(2.6)	5(2.7)	---
Aspirin	1770	83(5.2)	17(9.1)	0.047
SIRS (% ≥2 components)	1364	894(74.4)	130(80.2)	0.127
SIRS-Temperature (N <36 or >38°C)	1710	425(28)	66(37)	0.014
SIRS-Pulse (beats/min)	1757	97±29	105±32	<0.001
SIRS-Respiratory rate (breaths/min)	1721	19±8	20±10	0.09
SIRS-PCO_2 (mmHg)	1335	31±10	30±10	0.028
SIRS-WBC (N <4 or >12x10^9/L)	1770	723(46)	100(53)	0.052
Ammonia (μM)	1032	99.0±95.0	93.0±79.5	0.244
Encephalopathy Grade 3/4	1721	733(47.8)	115(61.5)	< 0.001
Creatinine (mg/dl)	1766	1.6±2.1	2.2±2.3	<0.001
INR	1770	2.8±2.2	2.7±2.4	0.895
Total bilirubin (mg/dl)	1752	7.6±16.4	6.8±13.1	0.226
Albumin (g/dl)	1616	2.7±0.8	2.7±0.6	0.549
Lactate (mg/dl)	971	4.4±6.0	5.4±8.2	0.002
Bicarbonate (mg/dl)	1481	22.0±8.0	20.0±10.0	0.002
Phosphate (mg/dl)	1539	3.1±2.3	3.8±2.9	<0.001
Platelet count (x10^9/L)	1770	128±108	96±92	<0.001
WBC (x10^9/L)	1770	10.1±8.1	10.6±9.7	0.239
Hemoglobin (g/dl)	1764	11.1±3.1	10.3±2.8	<0.001
Clinical Features after Admission, Days 1-7				
INR peak	1770	3.3±2.7	3.2±3.3	0.22
Platelet count nadir (x10^9/L)	1770	77±81	53±45	<0.001
Hemoglobin nadir (g/dl)	1770	9.2±2.5	8.4±2.1	<0.001
Encephalopathy Grade 3/4	1734	981(63.4)	161(86.1)	< 0.001
Infection	1770	155(9.8)	16(8.6)	0.682
Interventions after Admission, Days 1-7				
ICP Monitor Placement	1634	201(13.8)	36(20.7)	0.019
Renal Replacement Therapy	1770	534(33.7)	107(57.2)	< 0.001
Vasopressors	1770	531(33.5)	121(64.7)	< 0.001
N-acetylcysteine	1770	967(61.1)	121(64.7)	0.378
Gastric Acid Suppression	1770	1267(80.0)	162(86.6)	0.039
RBC Transfusion	1770	265(16.7)	30(16.0)	0.89
Plasma Transfusion	1770	531(33.5)	120(64.2)	< 0.001
Platelet Transfusion	1770	849(53.6)	145(77.5)	< 0.001
Outcomes at Day 21				
Transplant-free Survival	1770	723(45.7)	58(31.0)	< 0.001
Liver Transplantation	1757	407(25.9)	23(12.4)	< 0.001
Died after LT	430	39(9.6)	1(4.3)	< 0.001
Died	1770	492(31.1)	107(57.2)	< 0.001

3

Table 2. **Demographic and clinical features of patients with ALF with and without bleeding complications during days 1-7.**

APAP, acetaminophen; LT, liver transplantation; PCO_2, partial pressure of carbon dioxide; RBC, red blood cells; SIRS, systemic inflammatory response syndrome (and individual components); WBC, white blood cells.

Clinical Feature	N	No RBC Transfusions N = 1119 (Mean N, %)	RBC Transfusions N = 651 (Mean N, %)	P
Demographics				
Age (Years)	1770	42±15	41±14	0.15
Gender (% Female)	1770	744(66.5)	477(73.3)	0.003
Race (Caucasian)	1770	842(75.2)	467(71.7)	0.117
APAP Etiology of ALF	1770	523(46.7)	275(42.2)	0.075
Clinical Features on Admission				
SIRS (% ≥ 2)	1364	580(72.0)	444(79.4)	0.002
Ammonia (μM)	1032	98.0±90.0	101.0±97.0	0.826
Encephalopathy Grade (% 3/4)	1721	451(41.9)	397(61.6)	< 0.001
Creatinine (mg/dl)	1766	1.3±1.9	2.1±2.3	<0.001
INR	1770	2.9±2.2	2.8±2.2	0.129
Total bilirubin (mg/dl)	1752	7.0±15.6	8.0±17.0	<0.001
Albumin (g/dl)	1616	2.7±0.7	2.7±0.8	0.17
Lactate (mg/dl)	971	3.9±4.7	5.9±7.7	<0.001
Bicarbonate (mg/dl)	1481	22.0±8.0	20.0±8.8	<0.001
Phosphate (mg/dl)	1539	3.0±2.2	3.4±2.7	<0.001
Platelet count (x10^9/L)	1770	133.0±104.0	108.0±102.5	<0.001
WBC (x10^9/L)	1770	9.6±7.4	10.9±9.9	<0.001
Hemoglobin (g/dl)	1764	11.5±2.9	9.9±2.9	<0.001
Clinical Features after Admission, Days 1-7				
INR peak	1770	3.3±2.7	3.3±2.8	0.586
Platelet count nadir (x10^9/L)	1770	94.0±81.0	50.0±48.0	<0.001
Hemoglobin nadir (g/dl)	1770	9.8±2.5	8.2±1.6	<0.001
Encephalopathy Grade ¾	1734	613(56.3)	529(82.0)	< 0.001
Infection	1770	125(11.2)	46(7.1)	0.006
Bleeding Complication	1770	67(6.0)	120(18.4)	< 0.001
Interventions after Admission, Days 1-7				
ICP Monitor Placement	1634	99(9.4)	138(23.7)	< 0.001
Renal Replacement Therapy	1770	271(24.2)	370(56.8)	< 0.001
Vasopressors	1770	300(26.8)	352(54.1)	< 0.001
N-acetylcysteine	1770	722(64.5)	366(56.2)	< 0.001
Gastric Acid Suppression	1770	892(79.7)	537(82.5)	0.172
Plasma Transfusion	1770	210(18.8)	85(13.1)	0.002
Platelet Transfusion	1770	451(40.3)	543(83.4)	< 0.001

Table 3. **Clinical characteristics of patients with ALF who received or did not receive red blood cell transfusion during days 1-7.**

5

Blood Component	Patients Receiving Blood Component Transfusions N (%)				
	Total Transfused	Bleeders Transfused		Non-Bleeders Transfused	Total Transfused for Non-Bleeding Indication**
		Before Bleeding	At/after Bleeding*		
RBC	651 (37%)	34 (5.2%)	86 (13%)	531 (82%)	565 (87%)
Plasma	994 (56%)	60 (6.0%)	85 (8.6%)	849 (85%)	909 (91%)
Platelets	435 (26%)	22 (5.1%)	60 (14%)	353 (81%)	375 (86%)

Table 4. **Relationship of blood component administration to early bleeding complications.**
RBC, red blood cells.

*Defined as patients who received blood component transfusion on the same day as, or any day after, bleeding complication.
**Includes non-bleeders who received blood component transfusions and bleeders who received transfusions before their bleeding episodes.

Treatment During Inpatient (Day 1 -7)	Outcome at Day 21				
	Spontaneous Survivors (N=713) N (%)	Non-Spontaneous Survivors (NSS)			P*
		All NSS (N=944) N (%)	Liver Transplantation (N=430) N (%)	Death (N=599) N (%)	
Vitamin K	152(20%)	143(15%)	54(13%)	92(15%)	0.006
rVIIa	9(1.2%)	28(2.8%)	16(4%)	13(2%)	0.022
RBC Transfusion	207(27%)	444(45%)	197(46%)	278(46%)	< 0.001
Plasma Transfusion	302(39%)	692(70%)	312(73%)	415(69%)	< 0.001
Platelet Transfusion	122(16%)	313(32%)	141(33%)	192(32%)	< 0.001
*p-value compares probability of receiving the indicated treatment between spontaneous survivors and subjects who either received a liver transplant and/or died.					

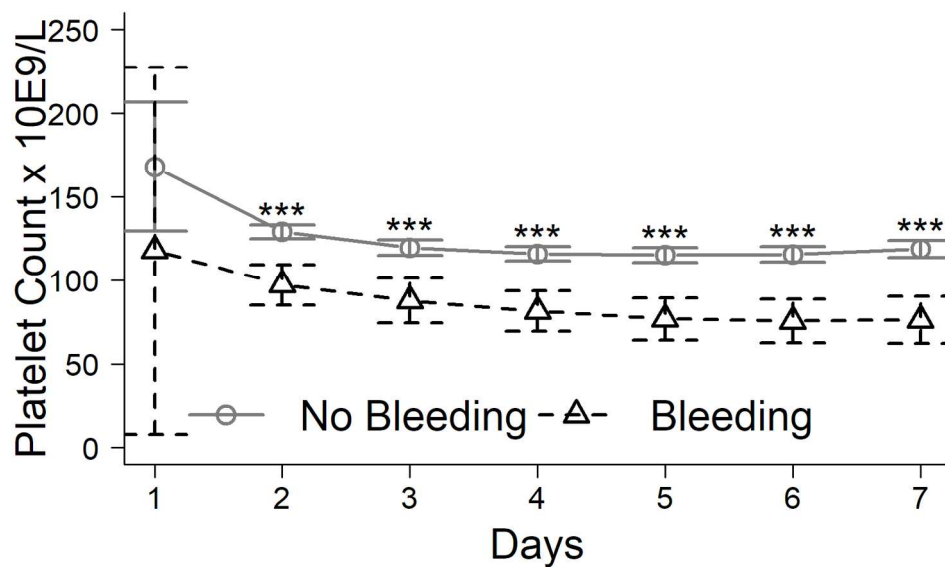Table 5. **Relationship between transfusion of RBC, plasma, or platelets between days 1-7 to outcome of ALF at day 21.**
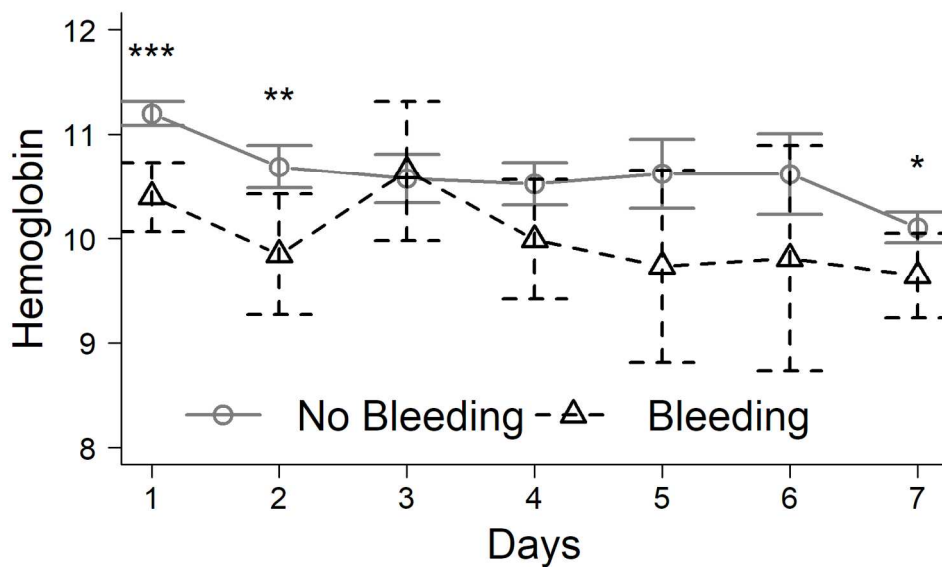
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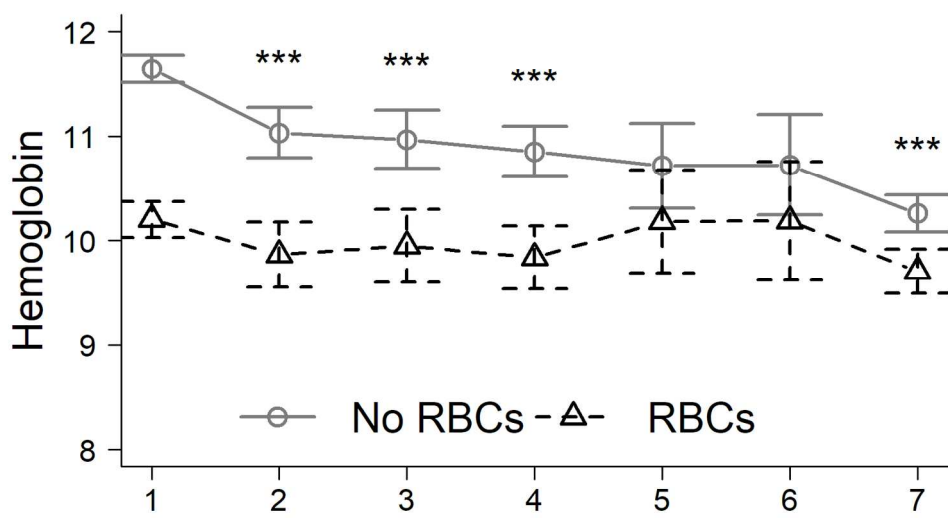
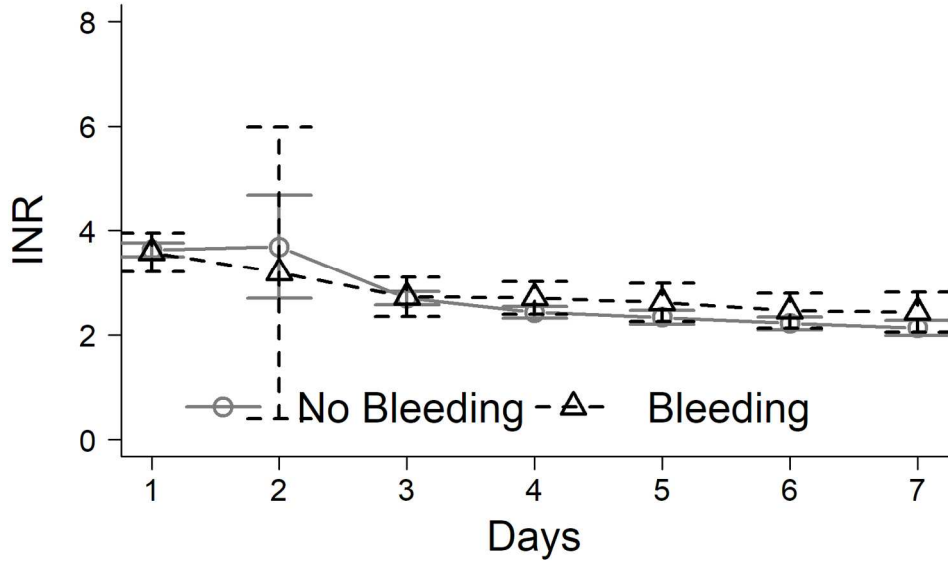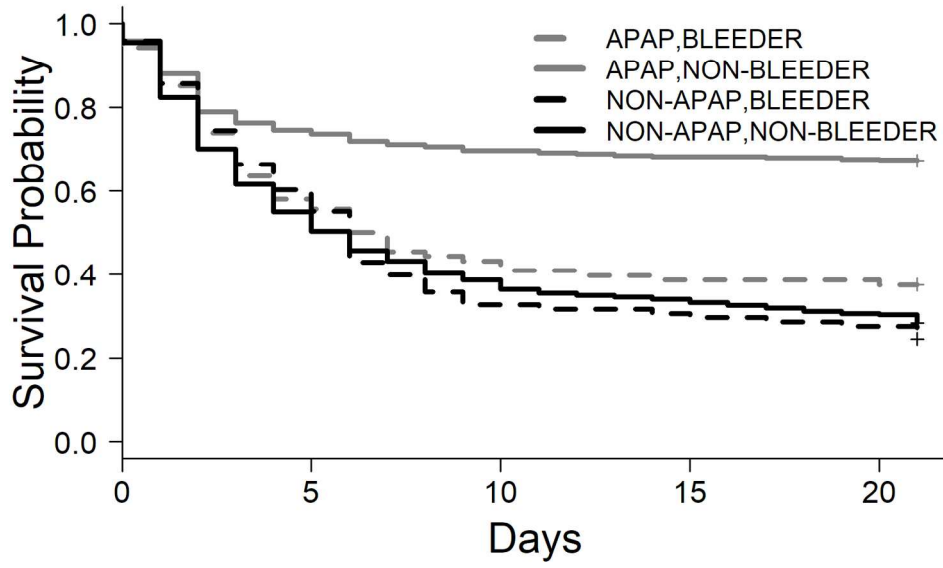152x95mm (300 x 300 DPI)

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Clinical Feature	N	Included N=1770 N±SD (%)	Excluded N=575 N±SD (%)	P
Demographics				
Age (Years)	2345	41±15	40±15	0.15
Gender (% Female)	2345	1221(69.0)	405(70.4)	0.55
APAP Etiology of ALF	2345	798(45.1)	283(49.2)	0.09
Clinical Features on Admission				
Plasma before admission	2268	762(44.3)	205(37.4)	0.005
Anticoagulants on admission	2345	46(2.6)	17(3.0)	0.756
Aspirin on admission	2345	100(5.6)	35(6.1)	0.77
SIRS (% ≥2)	1780	1024(75.1)	286(68.8)	0.013
Encephalopathy Grade 3/4	2274	848(49.3)	202(36.5)	< 0.001
INR^	2025	2.8±2.2	2.5±1.6	0.001
Platelet Count (x10^9/L)^	2012	124.0±109.0	137.0±84.0	0.002
WBC (x10^9/L)	2321	10.2±8.3	10.2±7.5	0.73
Hemoglobin (g/dl)	2318	10.9±3.1	11.1±3.3	0.14
Clinical Features and Interventions after Admission, Days 1-7				
Bleeding Complication*	2339	187(10.6)	42(7.4)	0.032
RBC Transfusion	2345	651(36.8)	148(25.7)	< 0.001
Plasma Transfusion	2345	994(56.2)	238(41.4)	< 0.001
Platelet Transfusion	2345	435(24.6)	71(12.3)	< 0.001
Outcomes at Day 21				
Liver Transplantation	2331	430(24.5)	117(20.4)	0.051
Died†	2107	599(33.8)	73(21.7)	< 0.001
COD Bleeding†	672	16(2.7)	1(1.4)	1.00

** Excludes N=6 subjects with missing bleeding data*
^ Excludes N=331 subjects with missing platelet and/or INR data
†Excludes N=238 subjects with unknown 21 day status

Supplemental Table 1. **Comparison of patients included and excluded from the study.** (COD, cause of death).
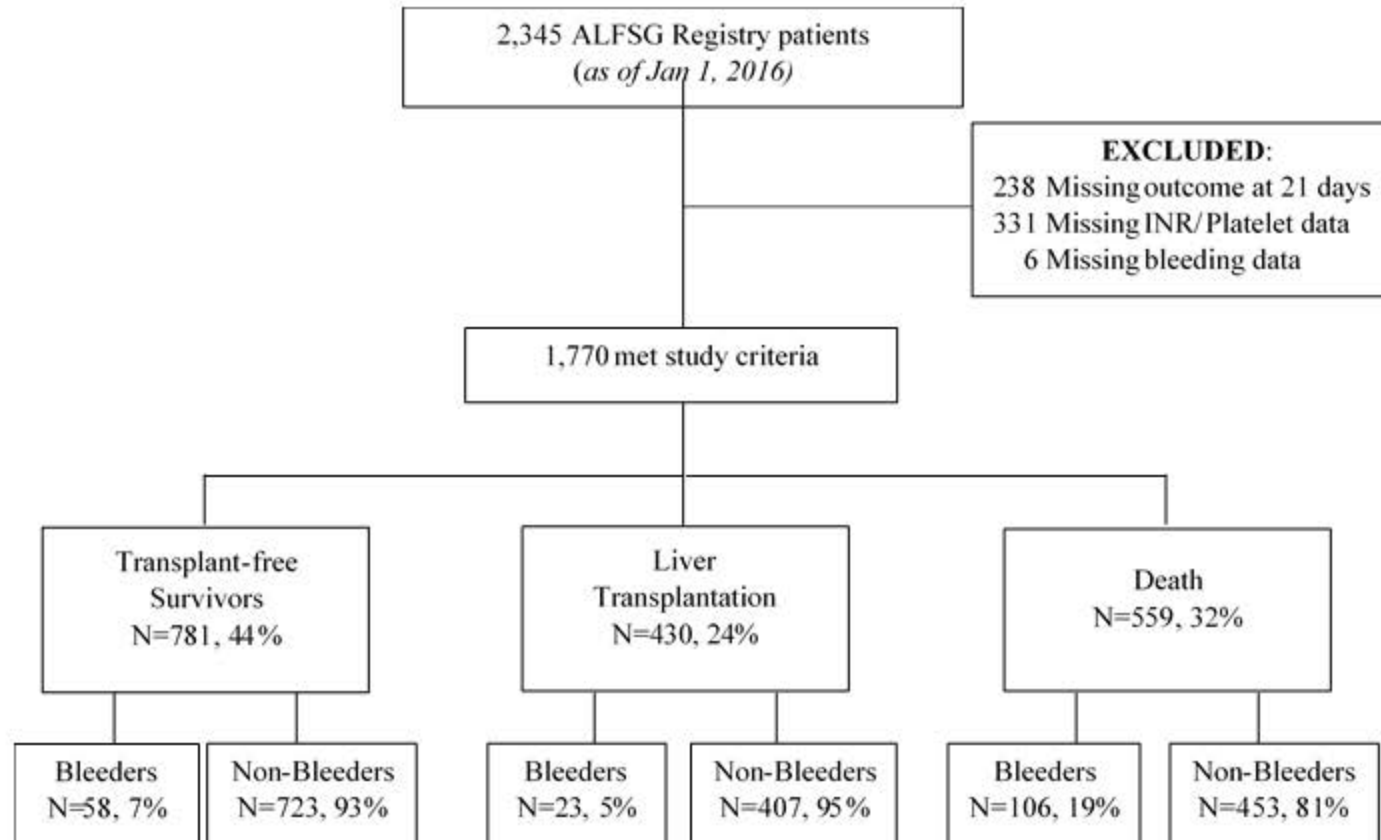
Supplemental Figure 1. **Patient accrual according to outcome at day 21, and distribution of bleeding complications.**
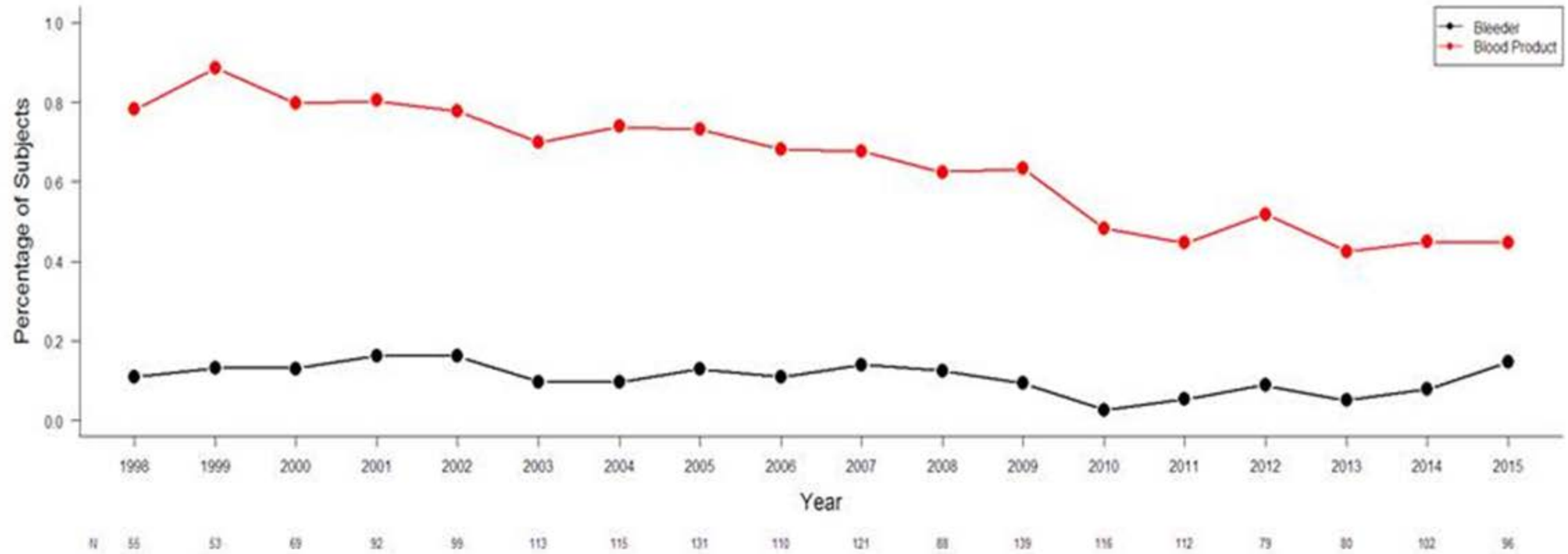
Supplemental Figure 2. Percentage of patients enrolled into the ALF Study Group Registry who received blood products (RBC, plasma, and/or platelets; upper line) and who experienced bleeding complications (lower line) between days 1-7 by year of enrollment.