Acute Liver Failure: Biomarkers Evaluated by the Acute Liver Failure Study Group

Jorge L. Rakela, MD[1], Constantine J. Karvellas, MD, SM, FRCPC[2], David G. Koch, MD, MSCR[3], Suneela Vegunta, MD[4] and William M. Lee, MD[5]

There has been a growing interest in identifying prognostic biomarkers that alone or with available prognostic models (King's College Criteria, KCC; MELD and ALFSG Prognostic Index) would improve prognosis in acute liver failure (ALF) patients being assessed for liver transplantation. The Acute Liver Failure Study Group (ALFSG) has evaluated 15 potential prognostic biomarkers: serum AFP; apoptosis-associated proteins; serum actin-free Gc-globulin; serum glycodeoxycholic acid; sRAGE/RAGE ligands; plasma osteopontin; circulating MBL, M-, L-, H-ficolin and CL-1; plasma galectin-9; serum FABP1; serum Lct2; miRNAs; factor V; thrombocytopenia, and sCD163. The ALFSG also has reported on 4 susceptibility biomarkers: keratins 8 and 18 (K8/K18) gene variants; polymorphisms of genes encoding putative APAP-metabolizing enzymes (*UGT1A1*, *UGT 1A0*, *UGT 2B15*, *SULT1A1*, *CYP2E1*, and *CYP3A5*) as well as *CD44* and *BHMT1*; single nucleotide polymorphisms (SNPs) of genes associated with human behavior, rs2282018 in the arginine vasopressin (*AVP*) gene and rs11174811 in the AVP receptor 1A gene. Finally, rs2277680 of the *CSCL16* gene in HBV-ALF patients. In conclusion, we have reviewed the prognostic and susceptibility biomarkers studied by the ALFSG. We suggest that a better approach to predicting the clinical outcome of an ALF patient will require a combination of biomarkers of pathogenic processes such as cell death, hepatic regeneration, and degree of inflammation that could be incorporated into prognostic models such as KCC, MELD or ALFSG PI.

KEYWORDS: acute liver failure; prognostic biomarkers; susceptibility biomarkers; prognostic models

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Acute liver failure (ALF) is an infrequent condition characterized by a rapid onset of severe liver injury in the absence of prior liver disease with hepatic encephalopathy and synthetic dysfunction. ALF is associated with substantial morbidity and mortality (1,2). Management aims to control or prevent cerebral edema/intracranial hypertension (CE/ICH), correct metabolic abnormalities, and conserve hemodynamic stability (3,4). For those not responding to medical therapy, liver transplantation (LT) is frequently indicated. However, the severity of multiorgan failure and coincident psychosocial factors may hinder eligibility for LT (1,5,6). Although up to 75% of acetaminophen (APAP)-induced ALF patients will spontaneously recover without LT, other etiologies of ALF carry a ≤40% of transplant-free survival (7). Clinicians caring for ALF patients face the challenge of making the decision whether a patient will need LT vs supportive care within a matter of hours or days and often without complete information. Outcomes in ALF patients are determined by the interplay of the extent of hepatocyte necrosis, hepatic regeneration, and presence and severity of multiorgan failure, including CE/ICH, vasoplegia, and sepsis (7).

CURRENT EXISTING PROGNOSTIC SCORES

Currently, the limitations of several prognostic models are tolerated because they are used in clinical practice. The King's College criteria (KCC) were developed to identify patients with APAP-ALF or non-APAP-ALF who were more likely to recover spontaneously (8), whereas the model for end-stage liver disease (MELD) score is used in the listing of patients with decompensated cirrhosis for LT and is also used in ALF patients (9). These prognostic models are predominantly used for clinical decision-making regarding LT indication in an ALF patient. McPhail et al (9) performed a meta-analysis to determine the accuracy of the KCC vs MELD scores in predicting hospital mortality among patients with APAP-ALF vs non-APAP-ALF. They found that among patients with APAP-ALF, the KCC more accurately predicted hospital mortality, whereas for patients with non-APAP-ALF, the MELD scores more accurately predicted mortality.

Recently, the Acute Liver Failure Study Group (ALFSG) reported a new prognostic index (ALFSG PI) that was developed to assess the 21-day transplant-free survival (TFS) (10). They reported that the ALFSG PI accurately discriminated clinical outcomes (*c*-statistic 0.84) and, importantly, rarely predicted

[1]Division of Gastroenterology and Hepatology, Mayo Clinic in Arizona, Phoenix, Arizona, USA; [2]Division of Gastroenterology (Liver Unit), Division of Critical Care Medicine, University of Alberta, Edmonton, Alberta, Canada; [3]Division of Gastroenterology and Hepatology, Medical University of South Carolina, Charleston, South Carolina, USA; [4]Department of Internal Medicine, Mayo Clinic in Arizona, Phoenix, Arizona, USA; [5]Division of Digestive and Liver Diseases, University of Texas Southwestern, Dallas, Texas, USA. **Correspondence:** Jorge L. Rakela, MD. E-mail: rakela.jorge@mayo.edu.
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spontaneous survival (SS) when death or LT (D/LT) occurred. In this analysis of the ALFSG registry, the ALFSG PI also outperformed KCC and MELD. The authors cautiously stated that future studies are required to clarify applicability outside tertiary referral transplant centers. Notably, none of these prognostic models are required by United Network for Organ Sharing for listing ALF patients for LT.

One of the common limitations of previously published ALF prognostic scores is that many have been developed based on the findings at 1 single time point. There have been several efforts to improve these scores by making them more "dynamic" (i.e., reflect more than 1 time point) with the premise that prognostic discrimination would be improved in ALF. Bernal et al (11) developed a dynamic outcome prediction model for patients with APAP-ALF. Early and accurate discrimination of survival is crucial in the management of APAP-ALF, as identifying those patients who require emergency LT can be a challenge. Bernal et al included metadata from 2 consecutive days: 1 set from the first day including age, Glasgow coma scale, arterial pH lactate, creatinine, international normalized ratio (INR), and circulatory failure and a second set of data from day 2 including additional changes in lactate and INR. A derivation model using data from day 1 (n = 350) found that the area under the receiver operating characteristics curve (AUROC) for 30-day survival was 0.92 (95% confidence interval [CI] 0.88–0.96), whereas the AUROC for 30-day survival using the day 2 model was 0.93 (0.88–0.97). In the validation data set (n = 150), the day 1 model found that the AUROC for 30-day survival was 0.89 (0.84–0.95), and using the day 2 model, it was 0.90 (0.85–0.95). Interestingly, when they applied their model to patients who received LT (n = 116), the median predicted 30-day survival was 51% (95% CI 33–85), suggesting that a significant number of patients who underwent LT may have survived with medical management alone. This reflects an ongoing challenge with APAP-ALF patients; improved intensive care unit management may result in patients recovering without LT (12,13). Patients who fail to recover often present with severe multiorgan failure and have an increased risk of death while on the waiting list and potentially missing the "window to transplant" (14,15).

Furthermore, patients with APAP liver injury are more likely to develop CE/ICH that may preclude LT. Earlier recognition and new improved dynamic prognostic models are needed to improve listing decisions in APAP-ALF patients (10,16,17).

NOVEL PROGNOSTIC BIOMARKERS

There has been a growing interest in identifying prognostic biomarkers that alone or when added to available prognostic models would improve the identification of ALF patients in need of an emergency LT. Our review mainly describes the prognostic and susceptibility biomarkers that have been evaluated and published by the ALFSG.

The prognosis of a patient with ALF is determined by the extent of cell necrosis, the accompanying complications such as in the interplay of the systemic inflammatory syndrome response (SIRS) and the compensatory anti-inflammatory response, the timing and magnitude of the hepatic regeneration in response to hepatocyte necrosis, and clinical complications such as CE/ICH, sepsis, and multiorgan failure. Many biomarkers evaluated by the ALFSG are associated with cell death by necrosis or apoptosis, and hepatic regeneration as well as measures of inflammatory response.

The ALFSG has evaluated 15 potential prognostic biomarkers: serum alpha-fetoprotein (AFP) (18); apoptosis-associated proteins (19); serum actin-free Gc-globulin (20); serum glycodeoxycholic acid (21); soluble receptor for advanced glycation end products (sRAGE)/receptor for advanced glycation end product (RAGE) ligands (22); plasma osteopontin (OPN) (23); circulating mannan-binding lectin (MBL), M-, L-, H-ficolin and collectin-liver-1 (CL-1) (24); plasma galectin-9 (Gal-9) (25); serum fatty acid-binding protein 1 (FABP1) (26,27); serum hepcidin (28); serum leukocyte cell-derived chemotaxin-2 (Lect2) (29); microRNAs (miRNAs) (30); factor V (FV) (31); thrombocytopenia (32) and soluble CD163 (sCD163) (33). Most of these studies have evaluated the clinical value of these biomarkers in 1 time point in the clinical course of the disease; this limitation probably reflects a very accelerated clinical course that would not allow obtaining multiple samples in time; if the clinical evolution of a patient would allow obtaining multiple determinations in time, this limitation would be overcome, and the results may allow a more precise assessment of the patient's prognosis.

Biomarkers reflecting regeneration and hepatocellular death

Schiødt et al (18) observed that the rise of serum AFP between day 1 and day 3 indicated a better prognosis. By day 3, the AFP ratio was 2.2 among the SSs vs 0.87 among nonsurvivors ($P < 0.001$). An increasing AFP level, indicated by an AFP ratio ≥1, was observed in 70 of 98 (71%) survivors, whereas a ratio <1 was observed in 51 of 64 (80%) nonsurvivors. Interestingly, this large prospective study found that higher absolute values of AFP did not predict survival, instead a rising level of AFP over the first 3 hospital days frequently indicated survival.

The liver plays a role in iron homeostasis through the synthesis of the serum transporter transferrin and serum hepcidin. Spivak et al (28) studied parameters of iron metabolism in a cohort of 121 adult patients with ALF, including 66 APAP-associated ALF from the ALFSG. The serum sample from these patients was assayed to determine baseline serum levels of ferritin, transferrin, iron, and hepcidin. At 3 weeks after enrollment, outcomes were categorized as SS vs D/LT. Patients with ALF had increased ferritin and lower serum hepcidin, resulting in a smaller hepcidin/ferritin ratio. Patients in the SS group had lower iron (29.1 vs 34.5 μmol/L; $P < 0.05$) and less transferrin saturation (60.9% vs 79.1%; $P < 0.01$) but had higher hepcidin levels (8.2 vs 2.7 ng/mL; $P < 0.001$) and larger hepcidin/ferritin ratio (0.0047 vs 0.0009; $P < 0.001$) compared with patients in the D/LT group. The authors used multivariate analysis to show that a log-transformed hepcidin-containing model displayed similar prognostic power to the ALFSG PI (*c*-statistic 0.87 vs 0.85) and was better than the MELD score (*c*-statistic 0.76). They showed that several serum iron parameters were significantly associated with 3-week outcomes in adults with ALF. Specifically, a decrease in hepcidin serum levels was an independent predictor for reduced survival in adult patients with ALF at 3 weeks, suggesting that hepcidin levels should be included in future prognostic scores.

The authors proposed an iron model that incorporated log transformation of INR, platelet count, log transformation of hepcidin, APAP etiology, and advanced coma grade (3 or 4). This iron model showed a similar prognostic value to ALFSG PI and was better than MELD score.

Patidar et al (31) evaluated the efficacy of FV in predicting the outcome of patients with ALF. They studied serum samples from 90 patients (56% with APAP) collected by the ALFSG and reported that the median FV was significantly higher in SS patients D/LT patients (31% vs 15%, respectively; $P = 0.001$). They

developed a FV model that incorporated FV, vasopressor use, total bilirubin, APAP etiology, and advanced coma grade and used logistic regression analysis to show that the AUROC was 0.77 for APAP-ALF patients (10.5% cutoff, 79% sensitivity, and 69% specificity) and 0.77 for non-APAP-ALF patients (22% cutoff, 85% sensitivity, and 67%, specificity). They evaluated their findings in a validation cohort of 51 patients whose samples were also collected by the ALFSG and included 59% with APAP. The AUROC for the FV model was 0.75 for APAP (81% sensitivity and 44% specificity) and 0.95 for non-APAP (90% sensitivity and 73% specificity). In the derivation cohort, the AUROC for the FV model was 0.86. Furthermore, because of FV's hepatic synthesis and its short half-life in plasma, it could be useful in identifying ALF patients who may be more likely to recover spontaneously.

Karvellas et al (26) evaluated FABP1 as a potential prognostic biomarker in APAP-ALF patients. Their rationale was that FABP1 is abundant in hepatocytes and may be released with hepatocyte necrosis. In the study, they evaluated serum FABP1 levels in day 1 (early) and day 3–5 (late) in 198 APAP-ALF patients (nested case-control study with 99 survivors, 99 nonsurvivors) collected by the ALFSG. They found that at early stages, APAP-ALF survivors had significantly lower serum FABP1 levels compared with nonsurvivors (238.6 vs 690.8 ng/mL, $P < 0.0001$), which was also true at the late stage (148.4 vs 612.3 ng/mL, $P < 0.0001$). They reported that a serum FABP1 level greater than 350 ng/mL was associated with significantly higher risk of death at early ($P = 0.0004$) and late ($P < 0.0001$) time points. Increased serum FABP1 at both early (log FABP1 odds ratio [OR] = 1.31, $P = 0.027$) and late (log FABP1 OR = 1.50, $P = 0.005$) was associated with increased 21-day mortality.

The AUROC for early and late multivariate models was 0.778 and 0.907, respectively. With the addition of FABP1, the AUROC for the KCC at early and late time points significantly improved ($P < 0.002$ for both). They concluded that patients with APAP-ALF, FABP1 may be a useful prognostic biomarker to discriminate survivors from nonsurvivors and may improve models—such as MELD, ALFSG PI, and KCC—currently used in clinical practice.

More recently, they have reported FABP1 as a prognostic biomarker in other etiologies of ALF (27). They studied 384 ALF patients, included those with autoimmune hepatitis (n = 125), drug-induced liver injury (DILI, n = 141), and hepatitis B (HBV, n = 118). Of this cohort, 177 patients received LT (46%). They reported that FABP1 levels at early stages were significantly higher in ALF patients requiring vasopressor support (203.4 vs 76.3 ng/mL) and renal replacement therapy (203.4 vs 78.8 ng/mL; $P < 0.001$ for both). Whereas at late stages, FABP1 levels were significantly higher in patients requiring mechanical ventilation (77.5 vs 53.3 ng/mL) and vasopressor support (116.4 vs 53.3 ng/mL) and in patients with grade 3/4 hepatic encephalopathy (71.4 vs 51.4 ng/mL; $P = 0.03$ for all). Late FABP1 levels were significantly lower in TFS patients (TFS 54 ng/mL vs NTFS 66 ng/mL; $P = 0.049$) but not on admission (TFS 96 vs NTFS 87 ng/mL; $P = 0.67$). However, after adjusting for significant covariates, serum FABP1 did not discriminate significantly between TFS and patients who died or received LT at day 21 on either admission ($P = 0.29$) or late (days 3–5, $P = 0.087$) time points. In conclusion, FABP1 serum levels were not as useful as a prognostic biomarker in the setting of non-APAP-ALF because they were previously reported in APAP-ALF.

Salehi et al (34) described a unique hepatic miRNA signature that was associated with hepatic regeneration in an experimental model of auxiliary LT, which was characterized by downregulation of miRNA-23a, -150, -200b, -503, and -663 and upregulation of miRNA-20a. Later, they evaluated whether this specific regeneration-linked miRNA signature was associated with clinical outcomes in acute and chronic liver disease (35). They found that this regeneration-linked miRNA signature was associated with clinical recovery after ALF.

More recently, they studied 194 patients with APAP-ALF enrolled in the ALFSG at early (day 1–2) and late (day 3–5) time points (30). Their early time-point model (AUROC = 0.78, 95% CI 0.71–0.84) contained the previously described regeneration-linked miRNA signature, and their late time-point model (AUROC = 0.83, 95% CI 0.76–0.89) contained a microRNA signature associated with cell death. The respective models improved with the addition of the vasopressor use and MELD score and both outperformed the KCC alone. The early time-point model (combined with the MELD score and vasopressor use) outperformed the ALFSG PI and the MELD score alone. They concluded that this regeneration-linked microRNA signature combined with MELD score and vasopressor use can outperform existing prognostic models for ALF—MELD, ALFSG PI, and KCC alone—in identifying patients who may benefit from LT.

Other biomarkers reflecting regeneration and hepatocellular death include M30 antigen (19), a neo-antigen after cleavage of cytokeratin-18. A M30 antigen was found to be elevated among patients who died or underwent LT (21,830 vs 1,004 U/L, $P = 0.026$). The serum M30 antigen level was found to correlate with coma grade and admission MELD.

Other circulating apoptosis-associated proteins of interest are serum levels of soluble Fas (sFas), tumor necrosis factor-alpha, and hepatocyte growth factor (HGF) which were higher among patients who died.

Another biomarker of interest is Gc-globulin, which is part of the actin scavenger system. After cell injury, actin is released and may lead to intravascular obstruction and tissue hypoxia. Schiødt et al (20) studied the predictive value of serum actin-free Gc-globulin in 252 ALF patients. Receiver operating characteristics curve analysis demonstrated that 40 mg/L as a cutoff value provided the best prognostic information with a positive predicted value and a negative predictive value of 68% and 67%, respectively. However, the results were not better than the information provided by KCC alone in the same set of patients.

Biomarkers of inflammation

OPN is a phosphoglycoprotein expressed in Kupffer cells that plays a role in activating natural killer cells, neutrophils, and macrophages. Srungaram et al (23) compared OPN levels in patients with ALF to elucidate the function of OPN in the context of massive hepatocyte necrosis. Plasma OPN levels were measured in 105 consecutive ALF patients enrolled by the ALFSG, as well as control samples from patients with rheumatoid arthritis (n = 40) and healthy subjects both before, and 1 and 3 days after undergoing spine fusion surgery (n = 35) which served as a model for acute inflammation. When compared with healthy controls, the median plasma OPN levels across all etiologies of ALF patients were elevated 1,055 ng/mL, range 33–19,124, the overall median plasma OPN median level for healthy prespine fusion surgery individuals was 41 ng/mL, range 2.6–86.4, within the ALF patient group, and the median OPN levels were highest in APAP (3,603 ng/mL) and ischemia-related ALF (4,102 ng/mL) as opposed to viral hepatitis (706 ng/mL), DILI (353 ng/mL), or autoimmune hepatitis (436 ng/mL). These values correlated with the degree of hepatocellular damage, as reflected by aminotransferase values (R value: 0.47 for aspartate aminotransferase,

$P < 0.001$). The authors concluded that elevated levels were associated with hyperacute injury and clinical outcomes.

Laursen et al (24) studied lectin levels in ALF patients and its association with clinical outcome. The lectin pathway of complement is initiated by soluble pattern recognition molecules which are synthesized in the liver: MBL, M-, L-, and H-ficolin, and CL-L1. The authors assayed serum samples from 75 patients enrolled by the ALFSG, which were collected on days 1 and 3. For control samples, the authors included healthy blood donors (n = 75) and cirrhotic patients (n = 20). On day 1, the MBL level in ALF patients was 40% lower compared with healthy controls (0.72 vs 1.15 mug/mL, respectively; $P = 0.02$). The M-ficolin level on day 1 was 60% lower (0.54 vs 1.48 mug/mL; $P < 0.0001$). The CL-L1 level at day 1 was increased, but not significantly higher compared with healthy controls (3.20 vs 2.64 mug/mL; $P = 0.11$). Spontaneous ALF survivors had higher levels of MBL at day 1 (0.96 vs 0.60 mug/mL; $P = 0.02$) and lower levels of L-ficolin by day 3 compared with patients who died or were transplanted (1.61 vs 2.17 mug/mL; $P = 0.02$). In conclusion, the authors reported elevated lectin levels in ALF patients, which suggests that the lectin pathway of complement may play a role in ALF pathogenesis.

Experimental work has implicated the RAGE and RAGE-dependent mechanisms in APAP-induced liver injury. Basta et al (22) investigated whether circulating levels of sRAGE or RAGE ligands, including extracellular newly identified receptor for advanced glycation end product-binding protein (EN-RAGE), high-mobility group box 1 (HMGB1), and N-epsilon-(carboxymethyl) lysine adducts, may be prognostic biomarkers in APAP-ALF. The authors retrospectively studied APAP-ALF patients (n = 60; 30 SS and 30 D/LT patients) who were enrolled in the ALFSG. When ALF patients were compared with normal controls, the ALF patients had higher levels of sRAGE, EN-RAGE, and HMGB1, but not N-epsilon-(carboxymethyl) lysine adducts ($P < 0.001$). The levels of sRAGE ($P = 0.03$), HMGB1 ($P < 0.01$), and EN-RAGE ($P = 0.03$) were significantly higher in patients with a SIRS score >2 vs patients with a SIRS score ≤2. However, when comparing D/LT subjects with SS subjects, only sRAGE levels were higher in D/LT patients ($P < 0.001$). The authors postulated that RAGE-ligand axis activation may interfere with liver regeneration and further evaluation of the RAGE-ligand axis as a prognostic biomarker is warranted.

sCD163 is a scavenger receptor released into the serum during activation of macrophages by inflammation. Møller et al (36) tested whether sCD163 was increased in patients with ALF and whether sCD163 levels could predict ALF patient outcome. They assayed the serum and clinical data collected on days 1 and 3 from 100 consecutive patients enrolled in the ALFSG. They reported that the median level of sCD163 was significantly increased in ALF patients compared with healthy controls or when compared with patients with compensated cirrhosis (ALF group = 21.1 mg/L, range 3.6–74.9; control group = 2.3 mg/L, range = 0.65–5.6, $P < 0.0001$; and compensated cirrhosis group = 9.8 mg/L, range 3.6–16.9, $P = 0.0002$). sCD163 on day 1 correlated significantly with alanine aminotransferase, aspartate aminotransferase, bilirubin, and creatinine. Importantly, sCD163 concentrations on day 3 were elevated in patients with fatal outcome of disease compared with SSs, 29.0 mg/L (7.2–54.0) vs 14.6 mg/L (3.5–67.2), respectively ($P = 0.0025$). The authors reported that an elevated level of sCD163 (>26 mg/L) was significantly correlated with a fatal outcome. Therefore, the authors suggested that sCD163 might be used to determine prognosis in ALF patients.

sCD163 has also been evaluated in Wilson disease (WD). Glavind et al (33) investigated sCD163 levels in patients with acute and chronic WD; 28 patients with WD-ALF were from the ALFSG registry and 147 patients with chronic disease from a German WD registry and 19 healthy individuals were included as a control group. In the ALF cohort, median sCD163 was 10-fold higher than in healthy controls (14.6 mg/L, range 2.5–30.9, vs 1.5 mg/L, range 1.0–2.7, $P < 0.001$). The ALF group also had higher levels of sCD163 when compared with the chronic group, median sCD163 was 2.6 mg/L, range = 0.9–24.9; $P < 0.001$. There was no difference in sCD163 among cirrhotic patients according to their initial clinical presentation (asymptomatic, neurologic, hepatic, or mixed). Patients with cirrhosis at the time of diagnosis had higher sCD163 compared with those without cirrhosis (3.0 [1.2–24.9] vs 2.3 [0.9–8.0] mg/L, $P < 0.001$), although both cohorts had significantly lower levels than the ALF patients. Their findings suggest that sCD163 may reflect disease severity in WD patients.

Other biomarkers

APAP-induced ALF remains a major clinical problem. Although most patients (~75%) recover after severe liver injury, a subset of patients may evolve into ALF. Woolbright et al (21) studied whether individual bile acid levels could predict the clinical outcome in patients with APAP-ALF. They reported that on day 1 after the overdose, bile acid levels were elevated 5-fold–80-fold above control values in APAP patients on day 1 after the overdose and decreased subsequently. Glycodeoxycholic acid (GDCA) was significantly increased in nonsurvivors compared with survivors. GDCA values compared at day 1 of admission and at peak serum alanine aminotransferase levels indicated that GDCA could predict survival in these patients (AUROC = 0.70 for day 1, AUROC = 0.68 for peak alanine aminotransferase). These data suggest that assaying levels of GDCA in APAP-ALF early on may predict outcome and may serve as a prognostic biomarker.

Rosen et al (25) evaluated Gal-9 in patients with DILI-ALF. Gal-9 is produced by Kupffer cells; these cells are probably engaged in the pathogenesis of ALF and may be involved in regulating immunity. The authors investigated whether plasma levels of Gal-9 were associated with outcomes of patients with ALF. They studied plasma samples collected at the time of hospital admission from 149 patients collected by the ALFSG (110 had APAP-ALF and 39 had non-APAP-ALF). The authors found that patients with ALF had statistically higher plasma levels of Gal-9 than control subjects, and these levels did not differ significantly between patients with APAP-ALF vs patients with non-APAP-ALF. The authors reported that for patients with ALF, Gal-9 levels above 690 pg/mL with MELD score were informative in predicting the outcome of patients; the survival rate at 21 days of patients with a MELD ≥30 and Gal-9 ≥690 ng/mL was less than 30% (Table 1).

SUSCEPTIBILITY BIOMARKERS

The ALFSG also reported on 4 susceptibility biomarkers in patients with ALF. Strnad et al (37) studied keratins 8 and 18 (K8/K18) gene variants in patients with ALF. Their rationale was that K8/K18 provide a cytoprotective function, specifically anti-apoptotic effect, in liver injury. They analyzed the entire coding regions of *KRT8* and *KRT18* genes (15 total exons and their exon-intron boundaries) to determine the frequency of K8/K18 variants in ALF patients (n = 344, 49% APAP-related) and 2 control

Table 1. Prognostic biomarkers evaluated by the ALFSG (2006–2021)

Biomarker		Diagnosis	Clinical outcome	*P* value	Reference
AFP day 3/AFP day 1 ratio		ALF	SS > D/LT	<0.001	Schiødt et al (18)
M-30 Ag		ALF	SS < D/LT (1,004 vs 21,830 U/L)	0.026	Rutherford et al (19)
Actin-free Gc-globulin		ALF	SS > D/LT (53 vs 37 mg/L)	0.002	Schiødt et al (20)
GDCA		APAP-ALF	SS < D/LT	<0.004	Woolbright et al (21)
sRAGE		APAP-ALF	SS < D/LT SIRS ≤2 < SIRS > 2	0.0005 0.029	Basta et al (22)
Osteopontin		APAP-ALF (APAP, ischemia)	SS > D/LT (1,470 vs 825 ng/mL)	0.067	Srungaram et al (23)
Gal-9		DILI-ALF	SS < D/LT	<0.002	Rosen et al (25)
FABP-1		APAP-ALF	SS < D/LT Early 238.6 vs 690.8 ng/mL Late 148.4 vs 612.3 ng/mL	<0.0001	Karvellas et al (26)
Serum hepcidin		ALF	SS > D/LT 8.2 vs 2.7 mM/L	<0.001	Spivak et al (28)
Lect2		ALF	SS < D/LT 14.8 ng/mL vs 21.6 ng/mL	0.024	Slowik et al (29)
sCD163 macrophage activator marker		Wilson-ALF	Wilson-ALF > chronic WD 14.6 vs 2.6 mg/L	<0.001	Glavind et al (33)
Factor V		ALF	SS > D/LT 31% vs 15%	0.001	Patidar et al (31)
miRNA (miR-150, -27a, -149, -191)	(Days 1–2, regeneration-linked) + MELD score and vasopressor use	APAP-ALF	Outperformed the MELD score, ALFSG PI with and without a threshold value and the KCC in predicting 21-d mortality	<0.001	Tavabie et al (30)

ALF, acute liver failure; ALFSG, Acute Liver Failure Study Group; APAP, acetaminophen; D/LT, death or liver transplantation; DILI, drug-induced liver injury; FABP1, fatty acid-binding protein 1; Gal-9, Galectin-9; GDCA, glycodeoxycholic acid; K8/K18, keratins 8 and 18; KCC, King's College criteria; Lect2, leukocyte cell-derived chemotaxin-2; MELD, model for end-stage liver disease; miRNA, micro-RNA; PI, prognostic index; sCD163, soluble CD163; SIRS, systemic inflammatory syndrome response; sRAGE, soluble receptor for advanced glycation end products; SS, spontaneous survivor; WD, Wilson disease.

groups collected by the ALFSG (African American, n = 245 and previously analyzed of White subjects, n = 727). A proportion of ALF patients had significant amino-acid-altering K8/K18 variants (n = 45), including those with K8 R341H (n = 23) and those with K8 G434S (n = 11).

K8 variants were significantly more common than K18 variants. The authors also reported that White patients with K8/K18 variants were less likely to survive ALF without transplantation ($P = 0.02$). They concluded that the presence of K8/K18 variants may predispose to adverse ALF outcome in White patients.

Court et al (38,39) investigated genotype frequency differences in genes related to APAP metabolism. The cohort included patients who had developed ALF intentionally from a single time-point overdose of APAP (n = 78), unintentionally after chronic high doses of APAP (n = 79), or from causes other than APAP (n = 103). The authors looked specifically into genetic polymorphisms in genes encoding APAP-metabolizing enzymes (*UGT1A1, UGT1A6, UGT1A9, UGT2B15, SULT1A1, CYP2E1*, and *CYP3A5*) as well as *CD44* and *BHMT1*. They found that the *CYP3A5* rs776746 A allele were overrepresented among ALF patients who had intentionally overdosed with APAP when compared with all other ALF patients (OR 2.3, 95% CI 1.1–4.9; $P = 0.034$). The *CD44* rs1467558 A allele was also overrepresented among patients who had unintentionally developed ALF from chronic APAP use when compared with all other ALF subjects (OR 4.0, range 1.0–17.2; $P = 0.045$).

However, these 2 genetic associations were considered weak, and they were not statistically significant after adjustment for multiple comparison testing.

Another approach has been to investigate distribution of gene single-nucleotide polymorphisms (SNPs) of genes that may be associated with human behavior. Randesi et al (40) examined a series of 21 SNPs in 9 genes associated with impulsivity and/or stress responsivity. They studied 229 patients with APAP-ALF collected by the ALFSG. The genotype frequencies of 2 SNPs were significantly different between the APAP-ALF patients and the controls: SNP rs2282018 in the arginine vasopressin gene (*AVP*, OR 1.64) and SNP rs11174811 in the AVP receptor 1A gene (*AVPR1A*, OR 1.89). Interestingly, these have been reported to be linked to a drug-use disorder.

Ajmera et al (41) reported that the G allele of the SNP rs2277680 of the *CXCL16* gene has an approximately 50% frequency in the general population but is overrepresented in the HBV-ALF population (~91%). On the other hand, the A allele of this variant is underrepresented in the general population (~9%), when compared with chronic hepatitis HBV or acute HBV populations (~50%). This polymorphism may lead to increased adhesion of natural killer T cells, which have been described as a

Table 2. Susceptibility biomarkers evaluated by the ALFSG (2006–2021)

Biomarker	Mechanism	Variants	Patients	Outcome	*P* value	Reference
K8/K18 variants	Provide antiapoptotic function	R341H in White patients G434S in Blacks	ALF	D/LT > SS ALF > controls	0.02 0.02	Strnad et al (37)
UGT1A1	APAP glucuronidation	rs8830	Unintentional APAP-ALF	Lower prevalence in unintentional APAP-ALF overdose	0.003	Court et al (38)
Arginine vasopressin gene (*AVP*); AVP receptor 1A gene (*AVPR1A*)	Stress responsivity	rs2282018 rs11174811	APAP-ALF	Patients > controls	0.028 0.015	Randesi et al (40)
CXCL16	Adhesion of NKT cells, inflammation mediators	rs2277680	HBV-ALF	HBV-ALF > controls 91% vs ~50%	8.8×10^{-5}	Ajmera et al (41)

ALF, acute liver failure; ALFSG, Acute Liver Failure Study Group; APAP, acetaminophen; D/LT, death or liver transplantation; HBV, hepatitis B virus; K8/K18, keratins 8 and 18; NKT, natural killer T; SS, spontaneous survivor; UDP-UGT, uridine 5′-diphospho-glucuronosyltransferase.

critical component of inflammation in models of hepatitis. This report suggested a potential mechanism leading to severe acute HBV (Table 2).

BIOMARKERS IN ALF PROGNOSTIC MODELS

Since biomarkers may represent the underlying pathophysiologic processes occurring in ALF, several biomarker candidates have been added to prognostic models in hopes of improving their performance (Table 3).

Although the MELD score was not primarily derived to predict outcomes in ALF, it is the model most often used in combination with biomarkers in existing studies. Specifically, MELD has been combined with (i) 7S domain of type IV collagen (4COL7S) and ammonia (non-APAP-ALF) that were evaluated in a publication from Japan (42), (ii) measures of iron metabolism (ferritin and transferrin in mixed etiologies) (28), (iii) regeneration-linked and cell death-linked miRNA (mixed etiologies) (30), and (iv) Gal-9 (GAL9 in APAP and DILI-ALF) (25). The miRNA models and iron metabolism models were superior to both the MELD score and the KCC in predicting ALF mortality, but formal statistical comparisons were not performed for the ferritin and transferrin models. 4COL7S was only compared with the MELD score, whereas GAL-9 that was not compared with any existing prognostic scores. The regeneration-linked (early) miRNA model with MELD and vasopressor use also outperformed the ALFSG PI, while the cell death-linked (late) model outperformed KCC, MELD, and ALFSG PI with a defined threshold. Only 1 study used the ALFSG PI (43) in a combined biomarker model with serum liver-type FABP1 (26). In this study, FABP1 was added to the

Table 3. Biomarkers in ALF prognostic models

Biomarker used in prognostic modeling	Additional variables	ALF population studied	Model performance	Comparison results
4COL75	MELD + ammonia	Non-APAP	AUROC 0.9	Outperformed MELD
Transferrin	MELD + age	ALF	AUROC 0.947	AUROC higher than MELD or KCC*
Ferritin	MELD + age	ALF	AUROC 0.893	AUROC higher than MELD or KCC*
Early miRNA	MELD + vasopressor use	ALF	AUROC 0.85	Outperformed MELD, KCC, and ALFSG prognostic score
Late miRNA	MELD + vasopressor use	ALF	AUROC 0.89	Outperformed MELD and KCC
GAL9	MELD	APAP and DILI-ALF		
M30	Phosphorus, INR, bilirubin, coma	ALF	AUROC 0.82	Outperformed MELD and KCC
Hepcidin	Log INR, platelet count, APAP etiology, coma	ALF	AUROC 0.87	Outperformed MELD
Factor V	Vasopressor use, bilirubin, APAP etiology, coma	ALF	AUROC 0.86	Outperformed KCC-APAP, KCC-non-APAP, and MELD
FABP1	KCC or MELD	ALF	AUROC 0.778 (early) AUROC 0.907 (late)	Outperformed KCC and ALFSG prognostic score for APAP-ALF

ALF, acute liver failure; ALFSG, Acute Liver Failure Study Group; APAP, acetaminophen; AUROC, area under the receiver operating characteristics curve; GAL9, galectin-9; INR, international normalized ratio; KCC, King's College criteria; MELD, model for end-stage liver disease; miRNA, micro-RNA.

KCC and the ALFSG PI. These hybrid, multivariable models calculated either early (day 0–2) or late (day 3–56) were superior to KCC and ALFSG Prognostic scores alone for APAP-ALF patients, but this was not the case in non-APAP-ALF (27).

Although these studies used existing prognostic models, additional attempts were made to combine biomarkers with clinical variables to create unique, dynamic prognostic scores. These include hepcidin (log INR, platelet count, log hepcidin, APAP etiology, and coma grade) (28), FV (bilirubin, vasopressor use, FV level, and coma grade) (31), and an ALFSG index that combined log-transformed M30 with serum phosphorus, INR, bilirubin, and coma grade (43). The hepcidin and FV models both outperformed KCC and MELD, but not the ALFSG PI. However, the ALFSG index with M30 was only compared with MELD and KCC because it predated the ALFSG PI (43).

Combining biomarkers with existing prognostic models is an attractive option to hopefully identify ALF patients more quickly and accurately at risk for death and who need to be considered for transplant. These newer biomarkers in combination with prognostic models, such as KCC, MELD, and ALFSG PI, would need to be validated in large and diverse patient populations. An important aspect if these novel biomarkers would also become widely available in medical centers where patients with ALF are taken care.

LESSONS LEARNED

The ALFSG evaluated 15 circulating prognostic biomarkers in patients with ALF (Table 1). One of our observations was that a circulating prognostic biomarker may be useful in 1 etiological group and not in another: Circulating FABP-1 had a clear prognostic value in APAP-ALF patients and when evaluated in non-APAP-ALF patients was not as useful as a prognostic biomarker.

Four of these prognostic biomarkers were only evaluated in the setting of APAP-ALF: Serum glycodeoxycholic acid, sRAGE, OPN, and a specific miRNA signature were found to have prognostic value in this subset of patients. Gal-9 was found to have a prognostic value in APAP-ALF and non-APAP DILI-ALF patients.

Eight prognostic biomarkers were evaluated in ALF patients that included APAP-ALF in addition to other ALF etiologies. The median serum AFP levels were higher among non-APAP-ALF patients as compared with APAP-ALF patients; the AFP levels were the highest in AIH-ALF, followed by WD, HCV, and indeterminate etiologies.

Serum apoptosis markers—sFas, HGF, interleukin 6, and M-30 antigen—were significantly higher in ALF patients. sFas and HGF levels were significantly higher in non-APAP DILI-ALF and APAP-ALF patients.

Lect2 is considered a biomarker of hepatic regeneration. This study did not report any serum level differences in the various etiological groups.

These observations point toward the *etiology of ALF* as a factor to be taken into consideration when interpreting the clinical significance of these prognostic biomarkers.

Two other biomarkers, thrombocytopenia, and components of the lectin pathway were evaluated on their *potential pathogenic role* they may have in determining a poorer outcome, and the possibility of developing specific therapies in the management of ALF.

The circulating prognostic biomarkers evaluated by the ALFSG reflected various cellular processes associated with the ALF syndrome. For example, a specific miRNA signature including M-30 Ag, hepcidin, FABP1, GDCA, Gal-9, actin-free Gc-globulin, and FV were associated with *markers of cell death*. Whereas a specific miRNA signature associated with *markers of hepatic regeneration* included serum APF levels and Lect2. In addition, the development of thrombocytopenia was associated with the *development of multiorgan failure* that poses a poor prognosis.

The studies referenced were limited in that 1–2 time points were used in the study design. This limitation occurs when clinical samples are unavailable at other time points or if the accelerated clinical course phenotype of the patient precluded obtaining more samples. Bypassing this limitation would require a specificity for and sensitivity to circulating biomarkers that is not feasible at this time.

Nevertheless, several of the studies showed that combining a circulating biomarker level improved the accuracy of traditional prognostic indices, such as KCC, MELD, and ALFSG PI. This is an approach that may need to be explored further.

In conclusion, the etiology of ALF, the accelerated clinical course, the utilization of more than 1 biomarker reflecting different pathogenic mechanisms, and the combination of biomarkers with prognostic indices may improve the accuracy of our current clinical decision-making regarding the need and timing of LT in patients with ALF.

CONFLICTS OF INTEREST

Guarantor of the article: Jorge L. Rakela, MD.
Specific author contributions: J.L.R. conceived the idea of summarizing all biomarkers evaluated by the ALFSG in the setting of acute liver failure and invited C.J.K., D.G.K., and W.M.L., who have contributed in this area. J.L.R., C.J.K., D.G.K., S.V., and W.M.L. contributed to data acquisition, data interpretation, manuscript writing, critical review, and analysis of the manuscript. All authors have approved the final version of the manuscript.
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