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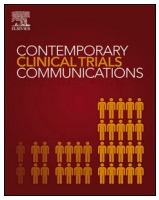

Design of a multicenter randomized clinical trial for treatment of Alcohol-Associated Hepatitis☆,☆☆,☆☆☆

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ABSTRACT

Background: Mortality is high for severe alcohol-associated hepatitis (AH). Corticosteroids are the standard of care for patients without contraindications. Recent data showed that interleukin-1β receptor antagonist anakinra attenuated inflammation and liver damage. We designed a multicenter, double-blind, randomized controlled trial to assess the safety and efficacy of anakinra compared to prednisone.

Methods: Patients meeting the clinical and biochemical criteria for severe AH with MELD scores between 20 and 35 were recruited at eight clinical sites. Eligible patients enrolled in the study were randomized to anakinra, 100 mg subcutaneous injection for 14 days, plus zinc sulfate 220 mg for 90 days, vs. prednisone 40 mg PO daily for 30 days. Matching placebos for anakinra, zinc, and prednisone were provided to mask the treatment. Participants were followed for 180 days. The primary outcome was overall survival at 90 days. An unadjusted log-rank test was used to compare the survival of the two treatments in the first 90 days. Between July 10, 2020, and March 4, 2022, we screened 1082 patients with severe AH, and 147 eligible patients were enrolled and randomized. The average baseline MELD score was 25 [range 20–35], Maddrey discriminant function (MDF) was 59.4 [range 20.2–197.5]. The mean aspartate transaminase (AST)-to-alanine transaminase (ALT) ratio was 3.5. The baseline characteristics were not statistically different between the two treatment groups.

Conclusions: The study provided a direct comparison of the survival benefits and safety profiles of anakinra plus zinc vs. prednisone in patients with severe AH.

1. Introduction

Alcohol-associated hepatitis (AH), an acute clinical syndrome resulting from prolonged heavy alcohol exposure, carries significant mortality and morbidity [1,2]. While patients with mild-to-moderate AH can recover with abstinence and supportive medical management,

☆ **Trial:** Trial of Anakinra (plus Zinc) or Prednisone in Patients with Severe Alcoholic Hepatitis (AlcHepNet).☆☆ **Trial registration**: Date: First post on August 28, 2019; last update on September 16, 2022. Number: NCT04072822.☆☆☆ **Role of** sponsor**:** Dr. Samer Gawrieh contributed to the design and overall conduct of the trial and is the IND holder. He provided critical review of the current manuscript.

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severe AH often requires inpatient care, thus incurring significant healthcare costs [3]. By some estimates, the total care costs for a commercially insured patient with AH averaged over $145,000 in five years [4]. Practice guidelines from the American Association for the Studies of Liver Diseases (AASLD), the American College of Gastroenterology (ACG), and the European Association for the Study of the Liver (EASL) recommend that AH patients with a Maddrey discriminant function (MDF) $\geq$ 32 or the Model for End-stage Liver Disease (MELD) $\geq$ 20 be treated with corticosteroids [5–7]. Still, the short-term mortality rate remains high, and the long-term prognosis is poor [8]. The Steroids or Pentoxifylline for Alcoholic Hepatitis (STOPAH) trial reported a 28-day mortality of 17% in the placebo group, 14% in the prednisolone group, 19% in the pentoxifylline group, and 13% in the prednisone and pentoxifylline group [9]. In the STOPAH trial, prednisolone was associated with a reduction in 28-day mortality that did not reach statistical significance and no improvement in outcomes at 90 days or one year. Subsequent analysis of the trial showed a statistically significant increase in serious infections in participants treated with corticosteroids compared to those treated with pentoxifylline or placebo [10]. A meta-analysis of 11 controlled clinical trials confirmed that corticosteroids reduced the risk of death within 28 days of treatment but not in the following six months [11], thus highlighting the need for new therapeutic strategies to improve medium-term (90 and 180 days) outcomes.

Preclinical studies showed that multiple cytokines were elevated in patients with severe AH [12]. Among the cytokines, IL-1β induced liver inflammation and hepatic cell injury but did not interfere with liver regeneration [13]. As a result, blocking the IL-1β receptor may reduce liver injury without attenuating liver repair [14]. Animal models provide further supporting evidence for the benefits of IL-1β blockage [15,16]. This strategy was put to the test in a recent clinical trial. The Defeat Alcoholic Steatohepatitis (DASH) trial was a multicenter randomized controlled trial evaluating the effects of a combination of anakinra, an IL-1β receptor antagonist, pentoxifylline, and zinc vs. methyl-prednisolone on 30 and 90-day mortality in patients with severe AH [17]. When DASH investigators designed their trial, STOPAH results had not been published. Pentoxifylline, therefore, was included in the combination therapy based on earlier evidence showing pentoxifylline improved survival and reduced the risk of hepatorenal syndrome (HRS) [18]. Zinc was included to improve gut barrier function [19]. The trial found that the combination treatment provided survival benefits similar to corticosteroids with a lower risk of serious fungal infections but not overall infections. The analytical power of the DASH trial was limited by its modest sample size. After the initiation of the DASH trial in 2014, the STOPAH trial reported in 2015 that pentoxifylline did not improve survival in patients with severe AH, nor did it reduce the incidence of HRS and acute kidney injury (AKI). Whether the IL-1β receptor antagonist anakinra, in the absence of pentoxifylline, provides better protection against medium-term (90-day) mortality than corticosteroids remained unanswered.

We conducted a clinical trial to address the above question. This manuscript describes the design of this new clinical trial, which was proposed and conducted by investigators of the Alcoholic Hepatitis Network (AlcHepNet) consortium and funded by the National Institute on Alcohol Abuse and Alcoholism (NIAAA). This multicenter, double-blind clinical trial compared the survival benefit of anakinra plus zinc versus prednisone and examined the drug metabolism and safety in patients with severe AH.

2. Methods

2.1. Overview of trial design and study setting

We conducted a Phase 2b, two-arm, double-blind, multicenter randomized clinical trial (RCT) with two parallel treatment arms in patients with severe AH. Key design features of AlcHepNet are presented in Table 1 in contrast to STOPAH and DASH. The trial was conducted concurrently at eight clinical sites within the AlcHepNet consortium.

Eligible patients who consented to participate in the trial were enrolled. Patients who did not meet inclusion/exclusion criteria or were unwilling to participate in the RCT were encouraged to participate in a companion observational study. Clinical data were managed by the Data Coordinating Center (DCC) at Indiana University. Blood, urine, and stool samples were stored and processed by a central repository at the University of Massachusetts, coordinated by the DCC at that institution. The study was registered in clinicaltrials.gov (NCT04072822).

2.2. Informed consent and data and safety monitoring plan

The study protocol and informed consent forms, as well as subsequent amendments to these documents, were approved by a central institutional review board (IRB). Changes were discussed at the monthly AlcHepNet Clinical Subcommittee meetings and then communicated in writing to investigators at all clinical sites in a timely fashion.

The conduct of the trial was monitored by a Data Safety Monitoring Board (DSMB). The study DSMB included two hepatologists, one alcohol use disorder specialist, and one clinical trialist/biostatistician that were not associated with any of the participating institutions in AlcHepNet. The composition of the DSMB was reported to and approved by the funding agency. An Investigational New Drug (IND) application was registered with the United States Food & Drug Administration.

Table 1
Comparison of AlcHepNet trial design features with those of recently completed trials.

Features	STOPAH	DASH	AlcHepNet
Trial period	January 2011–February 2014	February 2014–March 2018	July 2020–August 2022
Patients	Severe AH, 18 years or older, Maddrey's DF > 32	Severe AH, 21–70 years of age, MELD ≥20, Maddrey DF > 32.	Severe AH, 21 years of age or older, MELD 20-35
Design & intervention	2X2 factorial design of four treatment groups: Placebo vs. prednisolone 40 mg q.d for 28 days vs. Pentoxifylline 400 mg t.i.d. for 28 days vs. prednisone 40 mg q.d. + pentoxifylline 400 mg t.i.d for 28 days Evaluation at 7, 14, 21, 28 days. Follow up at 90 days and 1 year Sample size: 300 per treatment group; total 1200	Direct comparison of two treatment arms: methylprednisolone 32 mg daily for 28 days vs. a combination of pentoxifylline 400 mg t.i.d. for 28 days plus zinc sulfate 220 mg oral q.d for 90 days plus anakinra 100 mg daily subcutaneous injection for 14 days Sample size: 65 per treatment group; total 130 No treatment-stopping rules except for due to AE	Direct comparison of two treatment arms: Prednisone 40 mg oral q.d 30 days vs. Anakinra 100 mg daily subcutaneous injection for 14 days, plus zinc sulfate 220 mg oral q.d for 90 days. Evaluation at 3, 7, 14, 28, 60, 90 days. Follow up at 180 days Sample size: 129 per treatment group; total 258 Treatment stopping: Lille>0.45 on day 7, MELD increased by 5 and > 20, Maddrey's DF increased by 5 and > 32 or due to AE
Outcomes	Primary: 28-day mortality Secondary: Mortality or liver transplant at 90 days or 1 year	Primary: 180-day mortality Secondary: 30 and 90-day mortality; changes in MELD at 30, 90, and 180 days	Primary: 90-day overall survival Secondary: 90-day transplant-free survival, 30-day, and 180-day overall survival

Note: STOPAH = Steroids or Pentoxifylline for Alcoholic Hepatitis; DASH = Defeat Alcoholic Steatohepatitis; AlcHepNet = Alcoholic Hepatitis Network.

2.3. A concurrent observational study

Concurrent with the clinical trial, AlcHepNet also recruited and followed an observational cohort for studies of the pathogenesis and natural history of AH. The cohort included cases of AH, heavy drinking controls without AH, and healthy controls. The observational study collected clinical data and biological samples. Because the cohort was conducted concurrently with the clinical trial, the observational data provided valuable information on the incidence of mortality, infection, and serious adverse events of AH patients at the participating sites during the trial period.

2.4. Participants

Participants were patients diagnosed with severe AH [20]. Full details of the inclusion and exclusion criteria are presented in Table 2. Briefly, participants were 21 years or older, with severe AH, defined as having a Model for End-Stage Liver Disease (MELD) [21,22] score 20–35, with onset of jaundice (total bilirubin>3 mg/dL), regular consumption of alcohol with an intake of >40 gm daily or >280 gm weekly on average for women and >60 gm daily or > 420 gm weekly on average for men for six months or more, with less than eight weeks of abstinence before the onset of jaundice, AST >50 IU/L, AST/ALT >1.5 and both<400 IU/L.

2.5. Interventions

The trial had two parallel treatment arms: prednisone vs. anakinra + zinc. Prednisone is considered a standard of care for patients with severe AH [7]. Anakinra is an IL-1β receptor antagonist approved by the FDA for treating rheumatoid arthritis, neonatal-onset multisystem inflammatory disease, and autoinflammatory disease caused by IL-1 receptor antagonist deficiency. In AH, substantial evidence points to the activation of innate immune response and the elevation of inflammatory cytokines, including IL-1β. Anakinra is thought to have the potential to ameliorate liver injuries by blocking the IL-1β receptors [15,23]. The recent DASH trial confirmed that together with pentoxifylline and zinc, anakinra produced survival benefits similar to corticosteroids [17]. We included zinc supplements in the treatment as it is often deficient in patients with ALD [24]. Zinc deficiency impairs gut mucosal integrity in heavy drinkers [25] and is associated with oxidative stress that may exacerbate liver injuries [26].

The two treatment groups were:

Group 1 (Prednisone): Standard of care plus prednisone 40 mg orally once daily for 30 days and matching anakinra placebo syringes containing sterile saline for Days 1–14 and zinc placebo pills for Days 1–90.

Group 2 (Anakinra + Zinc): Standard of care plus Anakinra syringes (100 mg s.c.) once daily for Days 1–14, and zinc sulfate 220 mg (ZnSO4) once daily for Days 1–90, and matching placebo for prednisone for Days 1–30.

2.6. Randomization and treatment allocation concealment

The trial was conducted in a parallel, double-blind, placebo-controlled manner. Enrolled subjects were randomized to the two treatment groups in equal proportions, stratified by site and MELD score. Treatment assignments were generated by the REDCap system as programmed by the Indiana University DCC. Both participants and investigators were blinded to the treatment assignments. The study pharmacy dispensed medication according to assigned treatments.

We masked the treatment assignments by providing matching placebos for anakinra, zinc, and prednisone. The treatment plans for the two groups are depicted in Fig. 1. Patients and study personnel were blinded on treatment assignment. Study drugs were packaged by a central pharmacy at Indiana University and distributed to the study participants through participating sites. Active drugs and placebos provided to the participants had identical appearances and schedules for administration.

A new feature of this RCT was that prednisone or the prednisone placebo was discontinued if the Lille score calculated on Day 7 was >0.45. The Lille model was developed as a prognostic tool; values greater than 0.45 indicate patients that would not benefit from corticosteroids [27]. In other recent trials (DASH, STOPAH, etc.), corticosteroids were continued for 28 days regardless of Lille score.

Table 2
Inclusion and exclusion criteria for the clinical trial.

Inclusion Criteria
1. Alcoholic hepatitis, as defined by the NIAAA pan-consortia
   A. Onset of jaundice *(defined as serum total bilirubin* > 3 mg/dL*)* within the prior 8 weeks to screening visit
   B. Regular consumption of alcohol with an intake of >40 gm daily or >280 gm weekly on average for women and >60 gm daily or >420 gm weekly on average for men for 6 months or more, with less than 8 weeks of abstinence before onset of jaundice
   C. AST >50 IU/l
   D. AST: ALT >1.5 and both values < 400 IU/l
   E. and/or histological evidence of AH*
2. MELD 20–35 on day of randomization.
3. Ages ≥21

**In patients with possible AH or AH with confounding factors such as possible ischemic hepatitis, possible DILI, uncertain history of alcohol use (e.g., patient denies excessive alcohol use), and atypical/abnormal laboratory tests (e.g., AST < 50 IU/L or > 400 IU/L, AST/ALT ratio < 1.5), antinuclear antibody > 1:160 or SMA > 1:80, a standard of care liver biopsy may be performed during current hospital admission to confirm AH and exclude competing etiologies*

Exclusion Criteria
1. MELD SCORE <20 or >35
2. Active sepsis (positive blood or ascitic cultures) with Systemic Inflammatory Response Syndrome (SIRS) or hemodynamic compromise requiring intravenous pressors to maintain tissue perfusion
3. Pneumonia as evidenced by radiological exam
4. Multi-organ failure
5. Renal failure defined by GFR <35 mL/min by CKD- EPI
6. Clinically active C. diff infection
7. History of imaging of the liver (ultrasound, computerized tomography, or magnetic resonance) showing other causes of jaundice
8. History of other liver diseases including hepatitis B (positive HBsAg or HBV DNA), hepatitis C (positive HCV RNA), autoimmune hepatitis, Wilson disease, genetic hemochromatosis, alpha1-antitrypsin deficiency, or strong suspicion of drug Induced Liver Injury (DILI). Previously treated hepatitis C that was cured (sustained virological response with negative RNA ≥24 weeks following treatment) is not an exclusion.
9. History of HIV infection (positive HIV RNA or on treatment for HIV infection)
10. History or presence of cancer (including hepatocellular carcinoma) other than non- melanoma skin cancer
11. History of other significant medical problems such as autoimmune diseases, severe asthma, psoriasis, Inflammatory Bowel Disease (IBD), etc. that might require immunosuppressive treatments
12. Pregnancy or breastfeeding
13. Prior exposure to experimental therapies in last 3 months
14. Prior exposure to systemic corticosteroid (glucocorticoid) or immunosuppressive therapy for more than 4 days within previous 30 days
15. Need for inotropic pressor support to maintain perfusion to critical organs within prior 48 h before randomization and initiation of experimental treatment
16. Clinically significant pancreatitis- abdominal pain, elevated lipase (>3 X ULN) and at least edema of pancreas with fat-stranding on CT scan
17. Total WBC count >30000/mm3
18. Known allergy or intolerance to therapeutic agents to be tested
19. Inability to voluntarily obtain informed consent from participant or guardian
20. Perceived inability to follow study procedures and comply with protocol
21. Platelet count <40,000 k/cumm
22. Positive PCR test for COVID -19 within 7 days prior to the baseline day 0 visit*
23. Active gastrointestinal bleeding defined as hematemesis or melena with a decrease in hemoglobin more than 2 g/dl in 24 h. Due to gastrointestinal bleeding, or with a decrease in mean arterial BP to <65 mmHg

*Positive PCR test for COVID-19 is exclusionary only during screening period. If a patient tests positive any time after baseline randomization, a positive PCR test for COVID-19 will be considered as a SAE.

Fig. 1. Treatment schedules in the trial arms. Arm 1 is the prednisone group. Arm 2 is the anakinra + zinc group. Matching placebos were provided for the designed lengths of the treatments.

2.7. Primary and secondary outcomes

Patients with severe AH represent a heterogeneous population in disease severity, end-organ involvement, care received, and alcohol consumption. Death often involves failures of multiple organs, making it difficult to ascertain the primary cause of death. We used the overall survival at 90 days as the primary outcome in comparing the efficacies of the two treatments. Secondary survival outcomes included transplant-free survival at 90 days, as well as overall survival at 30 and 180 days.

Other secondary endpoints included: (a) Changes in Lille and MELD scores, development of acute kidney injury (AKI), multi-organ failure, systematic inflammatory response syndrome (SIRS), transfer to ICU, and changes in liver function as evaluated at 7, 30, and 90 days. (b) Organ dysfunction: Changes in Sequential Organ Failure Assessment (SOFA)

Table 3
Study visits and data collection schedule.

	Screening	Treatment Phase							Follow-up phase
		D0	**D3**	**D7**	**D14**	**D28**	**D60**	**D90**	**D180**
Window (days)		±2	±2	±2	±2	±7	±7	±7	±7
Informed consent	x								
History and physical	x	x	x	x	x	x	x	x	x
Vital signs, weight[f]	x	x	x	x	x	x	x	x	x
Alcohol consumption history	x	x			x	x	x	x	x
Randomization		x							
Concomitant medicines	x	x	x	x	x	x	x	x	x
Dispense Study Drug		x		x[d]					
Adverse events			x	x	x	x	x	x	x
Electrocardiogram[h]	x								
Evaluation of compliance			x	x	x	x	x	x	
CBC, PT/INR, Hepatic Function Panel, BMP	x	x	x	x	x	x	x	x	x
Test for COVID-19[g]	x								
Sepsis studies, as indicated	x	x	x	x	x	x	x	x	x
Pregnancy test, serum[e]	x								
Pregnancy test, urine[e]		x			x	x	x	x	x
Blood collection for trough Anakinra levels		x	x	x	x				
Specimen Banking[a]		x		x	x	x	x	x	x
Saliva Banking[b]		x						x	
Questionnaires [c]	x					x	x	x	x

Abbreviations: CBC: Complete blood count, BMP: Basic metabolic, INR: International normalized ratio and PT: Prothrombin Time.
*****In response to COVID-19, we are lessening restrictions if applicable or necessary such as: allowing wider windows D14, D28, D60 to ± 10 days. Allowing replacement of protocol mandated in-person study visits with one or more of the following, phone calls, telemedicine virtual visits, implement digital technology to record responses to questionnaires. Lastly, allowing blood draws at remote or commercial laboratories.

[a] Specimen banking includes blood and stool (when possible) D0, D7, D14, D28, D60, D90, D180, and, when available, urine and liver biopsy/tissue. Blood will be used to extract germline DNA at baseline and at Day 180 as well as to extract serum/plasma/PBMC at all visits. *Type of specimen collection is site specific.*

[b] Saliva collection at D0 and D90. Collected until an adequate number of samples are collected.

[c] Questionnaires include Alcohol Use Disorders Identification Test (AUDIT), Alcohol Timeline Follow Back (TLFB), and Chronic Liver Disease Questionnaire (CLDQ). The timeline follow back questionnaire will be the only questionnaire administered at days 28, 60, 90, and 180. All are given at screening.

[d] Day 7 study drug dispensation will be unscheduled and only on an as-needed basis, based on safety lab evaluations **(outpatient only.)**.

[e] if applicable.

[f] height at screening only.

[g] Test for COVID-19 PCR only if not done as SOC with 7 days prior to the baseline Day 0 visit.

[h] ECG can be done as standard of care within 7 days of screening.

scores and proportions requiring hemodynamic support for MAP <65 mm Hg and lactate >2 mmol/L, renal replacement therapy, or mechanical ventilation. We modified and re-evaluated the SOFA score without platelet counts, given that these are usually low in AH. (c) Infections and Sepsis: We assessed the types of infection and identified the proportions of those with sepsis, septic shock, or quick-SOFA (qSOFA) criteria based on SEPSIS-3 guidance [28,29], thus capturing the key parameters needed for various sepsis-related endpoint construction aligned by the recent guidance from the European Drug Development Hub (http://eddh-cro.wixsite.com/fdtsfv). (d) Renal dysfunction: AKI development and progression to chronic kidney disease. (e) Care escalation: Proportion of participants requiring transfer to ICU for care. (f) Indicators of the gut permeability (endotoxin and bacterial 18S DNA) and pro-inflammatory cytokine/chemokines (TNFa, MCP1, IL-6, IL-1β) in serum/plasma samples.

2.8. Follow-up protocol

After the initial screening, enrolled participants were followed for up to 180 days. Study visits were scheduled for 3, 7, 14, 28, 60, 90, and 180 days. Study visits and data/biosample collection schedules are presented in Table 3. Biosamples were collected to assess the metabolism of the study drugs and were also used for translational studies of AH. In addition to the data elements collected at planned study visits, the study also recorded concomitant care provided to treat complications related to AH and/or portal hypertension, including treatment not specified by the protocol, including but not limited to antibiotics, intravenous (IV) fluids, albumin, vasopressors, kidney and liver transplants.

2.9. Treatment discontinuation

In addition to stopping prednisone (or prednisone placebo) based on Day 7 Lille score, study drugs were held for 3 days in patients with documented infection (pneumonia defined as new infiltrate by chest X-ray or CT, positive blood/ascites culture for bacteria or fungi, positive fungal culture >50,000 colonies/ml in urine, CNS infection defined as positive culture or WBC >5 in CSF, severe soft tissue or bone infection including clinical diagnosis of cellulitis) or SIRS (defined as two or more abnormalities in temperature, heart rate, respiration, with WBC count) with an increase in SOFA ≥2 points. Patients were reassessed on the third day with resumption of study drugs if infection was controlled within 3 days; otherwise, study drugs were discontinued.

All adverse events (AEs) and serious adverse events (SAEs) were recorded and coded using MedDRA terms. Study drugs were withheld for cases of persistent infection, pregnancy, clinical deterioration (defined as an increase in MDF by 5 and >32, an increase in MELD score by 5 and >20), development of severe leukocytosis (WBC≥100,000/mm3), or suspected drug-induced liver injury (DILI). Other reasons for treatment discontinuation included patient withdrawing consent or refusing to comply with the trial requirement for participation, adverse events, and DILI. The safety officer was notified when a patient withdrew or treatment was discontinued.

2.10. Adherence to the trial protocol

Variations in protocol implementation and clinical care can have a major impact on trial outcomes. To minimize protocol deviations, we standardized the trial operation by implementing the following: (1) GCP training and certification of all study personnel, (2) investigator/coordinator training and certification prior to site activation, (3) monthly performance reports, (4) development of manual of operations that provide best practices, and (5) annual investigator meeting to review developments and clinical and trial practice patterns. (6) DSMB convened every 6 months to ensure relevant study procedures were followed.

2.11. Safety monitoring, auditing, and confidentiality

Safety was assessed in terms of adverse events (AEs). All AEs, including serious adverse events (SAEs), whether observed by investigators, reported by patients, noted from laboratory findings, or identified by other means, were recorded from Day 0 until the participant completed the trial (Day 180). AEs were graded for severity (mild, moderate, severe), reported on the AE case report form (CRF), and adjudicated for their relationship with the study medication as "definite," "probable," "possible," "unlikely," "not related."

SAEs that are common in severe AH include AKI (defined as 50% or more increase in creatinine, of 0.3 mg/dL within a period of 48 h, or renal failure requiring dialysis), sepsis, infection, decompensation of liver disease, hepatic, cerebral, renal, respiratory, circulatory, or multiple organ failures.

All AEs and SAEs were reported to the Indiana University DCC within two working days of occurrence. SAEs were recorded in the REDCap system within two working days of the investigator becoming aware of the event. The initial telephone report was followed by the completion of SAE CRF. We have put in place a risk-based monitoring (RBM) plan for efficient and timely monitoring of the accumulated data on adverse events of interest, namely AKI and infection. AE/SAEs were reported to WIRB on a continuing basis, to DSMB in an unblinded format for semi-annual review, and to NIAAA and FDA for annual review. The Indiana DCC had an auditor who reviewed all trial records, including those pertaining to AEs and SAEs, reported by the clinical sites. Protected health information from the study participants was removed before analysis to ensure confidentiality. Analyses were based on validated and de-identified data.

2.12. Sample size and power of the trial

The sample size of the trial was determined to ensure adequate power for the analysis of the primary endpoint – 90-day survival. We planned for one interim analysis, scheduled to be performed when 50% of the enrolled participants completed the 90-day follow-up so that the primary endpoints could be assessed. Because repeated testing inflates the type 1 error rate, we used the group sequential method to control the type 1 error rate of the trial. Assuming that the prednisone group had a 90-day overall survival of 80%, we calculated the power of the log-rank test in a group-sequential setting with two sequential looks. Analysis showed that with 258 patients (129 in each treatment group), we would have 85% power to detect a hazard ratio of 0.325 (log(0.93)/log(0.8)) by using a two-sided log-rank test at 0.05 significance level. We used O'Brien and Fleming α-spending function to determine the stopping boundaries for the sequential tests [30]. Based on this calculation, we would reject the null hypothesis and claim a significant difference between the two treatment groups if the test statistic of the log-rank test ≥2.963 or ≤ −2.963. The trial would be stopped, and a significant difference declared. Otherwise, we would continue enrollment until the full sample size was reached for the final analysis. For the final analysis, a significant difference would be declared if the test statistic ≥1.969 or ≤ −1.969. Otherwise, no statistically significant difference would be declared.

2.13. Analytical methods

All analyses on treatment efficacy were based on the intention-to-treat (ITT) principle. In this framework, randomized participants were assumed to receive the assigned intervention, regardless of their adherence to the assigned regimen. Per-protocol analyses were to be performed controlling for the actual levels of medication adherence.

The analysis for the primary endpoint, i.e., the overall survival at 90 days, was based on a two-sided log-rank test, which compared the survival functions of the two treatment groups at a 0.05 significance level with survival censored at 90 days. Kaplan-Meier estimates were used to

depict the survival functions of the two treatment groups. Cox proportional hazard regression was used in a secondary analysis to control for the effects of the demographic and clinical characteristics of the study participants. Variables of interest included but were not limited to age, sex, race, BMI, baseline MELD, serum creatinine, total and direct bilirubin, AST, ALT, the international normalized ratio (INR), etc. Estimated effects were expressed as adjusted hazard ratios (aHR). Analyses for other survival endpoints were analyzed similarly. P values less than 0.05 were considered statistically significant.

Other secondary endpoints included the occurrence of clinical events such as AKI and infection. Times from baseline to the occurrence of the event of interest were also analyzed with proportional hazard models. Event counts were analyzed with count data regression models. Laboratory values were analyzed with mixed effects models with random subject effects.

2.14. Baseline characteristics of trial participants

As of March 4, 2022, we have screened 1082 patients with severe AH. We enrolled and randomized 147 subjects that met the eligibility criteria. The baseline characteristics of the participants are reported in Table 4. Briefly, 60% of the participants were male, and 82% were white. The average age was 45 years, and the average body mass index (BMI) was approximately 30. The baseline clinical profiles of the participants, as shown by the prognostic scores and laboratory test results, were typical for a population of severe AH. The mean MELD score was 25. The mean discriminant function score was 59.4. The mean albumin was below the normal range (2.7 g/dL), bilirubin was elevated (18.9 mg/dL), creatinine was normal (0.8 mg/dL), AST/ALT was greater than 3.5, alkaline phosphatase was mildly elevated (182.5 IU/L), the mean corpuscular volume (MCV) was just above the upper threshold of the normal range (100.7 fL), INR was high (2.0), and prothrombin time as high (22 s). The two treatment groups were well-balanced on these variables, indicating that the randomization scheme performed as designed.

The baseline demographic characteristics and laboratory values above were representative of a severe AH population. They were similar to the recently published DASH trial [17], and broadly comparable to that of the STOPAH subjects [9], except that our participants were slightly younger with higher MELD and MDF scores.

Table 4
Baseline demographic and clinical characteristics of the study participants.

Variable	Mean (SD) (n = 147)
Male sex, n(%)	88 (60%)
Age (years)	44.7 (9.9)
Race white, n(%)	121 (82%)
Body mass index	29.6 (7.0)
MELD Score	25.0 (3.6)
Maddrey discriminant function	59.4 (26.6)
Alcohol consumption (g/day)*	84.7 (85.2)
Glucose- fasting (mg/dL)	92.9 (17.8)
Albumin (g/dL)	2.7 (0.5)
Total Bilirubin (mg/dL)	18.9 (8.6)
Creatinine (mg/dL)	0.8 (0.3)
ALT (IU/L)	45.1 (25.8)
AST (IU/L)	138.5 (71.9)
Alkaline Phosphatase (IU/L)	182.5 (81.7)
Total Protein (g/dL)	5.9 (0.9)
Hemoglobin (g/dL)	9.7 (1.9)
Total WBC (10^9/L)	11.6 (6.2)
Platelet Count (10^9/L)	168.8 (104.5)
MCV (fL)	100.7 (9.0)
INR	2.0 (0.5)
Prothrombin time (PT) (sec)	22.0 (5.7)

Note: * Calculated from the timeline follow-back (TLFB) assessment. Alcohol consumption (g/day) by using (TLFB total number of drinks for 30 days)*14/30 under the assumption that a standard drink contains 14 g of pure alcohol.

2.15. Data dissemination plan

Study data were managed centrally by the Indiana University DCC during the course of the trial. Analytical proposals by the AlcHepNet investigators were reviewed by the network's Publication Committee and implemented by the DCC analysts. Results were disseminated to the wider scientific community in the form of published abstracts and manuscripts and then made accessible to the general public via PubMed Central within 12 months of publication.

3. Discussion

This is the first clinical trial that directly compared the survival benefit of anakinra against prednisone in severe AH. Corticosteroids are currently considered the standard of care, although the survival benefits associated with the treatment are often short-lived ($\leq$ 30 days) and gained at the expense of increased infection risk [10,31]. An alternative treatment, pentoxifylline, a phosphodiesterase inhibitor, showed a potential to reduce the incidence of AKI and hepatorenal syndrome [32,33]. The STOPAH trial formally compared the efficacy of pentoxifylline, prednisolone, and the combination of the two, against placebo [9]. Trial data suggested that pentoxifylline was neither superior to prednisolone nor to placebo and that prednisolone was associated with improved 28-day survival, but the improvement did not reach statistical significance. More recently, hope has been raised about prospects of using IL-1β antagonist anakinra to treat severe AH. The newly published DASH trial compared a combination therapy of pentoxifylline, anakinra, and zinc against prednisolone [17]. Results of the DASH trial showed that the combination conferred a survival benefit similar to prednisolone, although the incidence of AKI was lower in the combination treatment group for those with MELD scores between 20 and 25. Because pentoxifylline was included in the combination, a direct comparison of anakinra and prednisone remained unavailable. The current study will offer insights into the relative efficacy and safety of these two therapies in AH.

A novel feature of the corticosteroid treatment protocol in the current trial is the use of the Lille score at 7 days >0.45 as a stopping rule for prednisone or prednisone placebo. This essentially added a decision point for the futility of continuing the corticosteroid treatment. The Lille score was developed to identify "non-responders" to corticosteroids [34,35]. An earlier report using historical controls suggested that switching from prednisolone to pentoxifylline did not improve survival in patients who were considered non-responders to prednisolone based on the early change in bilirubin (a precursor to the Lille score) [36]. Louvet and colleagues reported that the difference in rates of infection after initiation of corticosteroids in patients with AH was higher in those who were non-responders (Lille >0.45) regardless of whether steroids were continued for a full 28 days or stopped after non-response was identified [37]. This finding was based on a non-randomized, sequential cohort with steroids continued for 28 days in an earlier group, and in a later treatment group, steroids were discontinued after non-response was identified. In clinical practice, corticosteroids are often stopped if the Lille score is unfavorable (>0.45). To our knowledge, the Lille score has not been used prospectively as an indication of futility or for stopping corticosteroids in clinical trials due to the difficulty of maintaining blinding. In this study, because of the use of a prednisone placebo, we were able to use an unfavorable Lille score as a formal stopping rule, thereby reducing the potential risk of infection in steroid non-responders.

The trial also presented an opportunity for assessing the safety profile of anakinra in patients with severe AH. Although the drug was approved by the FDA for other indications, its safety in patients with severe AH was investigated only in combination with pentoxifylline. Pharmacokinetic studies of anakinra have not been done in patients with liver disease [38,39]. Because anakinra is cleared primarily through the kidney, in patients with impaired renal function, including those with

AKI/HRS, whether the drug can be eliminated efficiently remains to be evaluated [40]. In summary, the current trial provided an opportunity to better understand anakinra's effects and metabolism in patients with severe AH.

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Declaration of competing interest

Dr. Samer Gawrieh provides consulting services to TransMedics and Pfizer and he receives research grant support from Cirius, Viking, and Zydus. Dr. Vijay Shah serves on Advisory boards of Akaza Bioscience Ltd, AgomAb Therapeutics, Generon Shanghai, Intercept Pharmaceuticals, Inc, Mallinckrodt Pharmaceuticals, and Surrozen. He provides consulting services Ambys Medicines, Durect Corporation, HepaRegeniX, and Novartis Pharma AG. Dr. Mack Mitchell owns stocks in Amygdala Neuroscience and Advisory at Prodigy Biotech. Dr. Douglass Simonetto provides consulting services to BioVie and Mallinckrodt. Dr. Wanzhu Tu provides consulting services to Bayer. Dr. Gyongyi Szabo provides consulting services to Alnylam, Duret, Generon, Glympse Bio, Novartis, Quest Diagnostics, Surrozen, Terra Firma, and Zomagen. Dr. Arun Sanyal owns stocks in Sanyal Bio, Exhalenz, Conatus, Genfit, Duret, Indalo, and Tiziana. He provides consulting services to Conatus, Genfit, Gilead, Mallinckrodt Pharmaceuticals, Pfizer, BI, Novartis, Merck, Lilly, Novo Nordisk, Terns, Albireo, Sanofi, Jannsen, Takeda, Northsea, Poxel, 89Bio, NGM Bio, Amgen, Genentech, Roche, Madrigal, Inventiva, Covance, Prosciento, Histoindex, PathAI, and Biocellvia. Other authors declared no conflicts of interest.

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