Statistical Analysis Plan for AntibioCor-HAA study : Evaluation of the efficacy of antibiotic therapy combined with corticosteroids in severe alcoholic hepatitis.

Version : 2.0 Date: 06/01/2021

This document is written according to Guidelines for the Content of Statistical Analysis Plans in Clinical Trials. (1)

Trial registration: 2014-002536-13 / NCT02281929

Protocol version: The SAP has been written based on information contained in the study protocol version 6, dated 26 June 2017.

SAP revision history

Protocol Version	SAP version no.	Section number changed	Description of and reason for change	Date
6.0	1.0	NA	First SAP version signed	19/11/2020
6.0	2.0	5.2. Analysis methods	During the blind review, the occurrence of liver transplantation was discussed. For primary analysis, patients with liver transplantation will be treated as censored events (according a cause-specific model) and as competing event in a sensitivity analysis.	06/01/2021

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Protocol title: Evaluation of the efficacy of antibiotic therapy combined with corticosteroids in severe alcoholic hepatitis. AntioBioCOR-HAA

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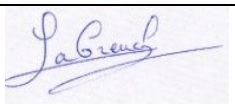
Julien Labreuche (Biostatistician)		06/01/2021
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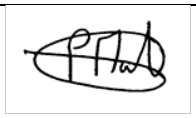
Philippe Mathurin, MD, PhD (Chief investigator)		06/01/2021
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List of Abbreviations

Abbreviations	Label
AH	Alcoholic Hepatitis
CI	Confidence limit
HR	Hazard ratio
ITT	Intent to treat
MELD	Model for end-stage liver disease
PP	Per-protocol

1 INTRODUCTION

1.1 Background and rational

Alcoholic hepatitis (AH) is one of the most severe forms of alcoholic liver disease. AASLD and EASL Clinical Practice guidelines recommend the use of corticosteroids or pentoxifylline for 28 days as first line therapy for severe alcoholic hepatitis (1, 2). Because of the high risk of mortality with this entity, EASL Clinical Therapeutic Guidelines recommend that new strategies be evaluated (4). Numerous trials have assessed the impact of treatment strategies in combination with corticosteroids including anti-TNF antibodies, antioxidants and pentoxifylline (1, 3-7). None of these combined therapies improved survival compared to corticosteroids alone, although there was a trend towards improvement with N-acetylcysteine with corticosteroids at 6 months that was not significant. The validity of a 6-month endpoint is debatable because it is far from the end of the therapeutic period (treatment given for only 1 month). However, use of survival at 1 or 2-month as the primary outcome time period requires modifying the study design because of the low mortality at 2 months (approximately 15-20%). As a consequence, to show an impact of any new therapeutic strategy, the inclusion criteria. Thus, we propose a study design targeting patients most likely to die within 2 months consisting in a 2-step screening: first to select patients with a Maddrey function ≥32 and second to include only those with a baseline MELD score greater than 21, who represent about 75% of patients admitted for severe alcoholic hepatitis.

1.2 Research hypothesis

The null hypothesis is that, in severe AH patients, there is no difference in the 2-month overall survival between the intervention strategy (a combination of corticosteroids and antibiotics) and control strategy (corticosteroids alone). The alternative hypothesis is that there is a difference between the two treatment strategies of severe AH patients.

1.3 Study Objectives

The primary objective of the AntioBioCOR-HAA trial is to determine the efficacy (superiority) of corticosteroids and antibiotics combination versus corticosteroids alone for improving the 2-month overall survival) in patients with severe AH who have a high-short-term risk of death defined as a MELD score ≥21.

Secondary objective are:

To determine the efficacy of corticosteroids and antibiotics combination versus corticosteroids alone to:

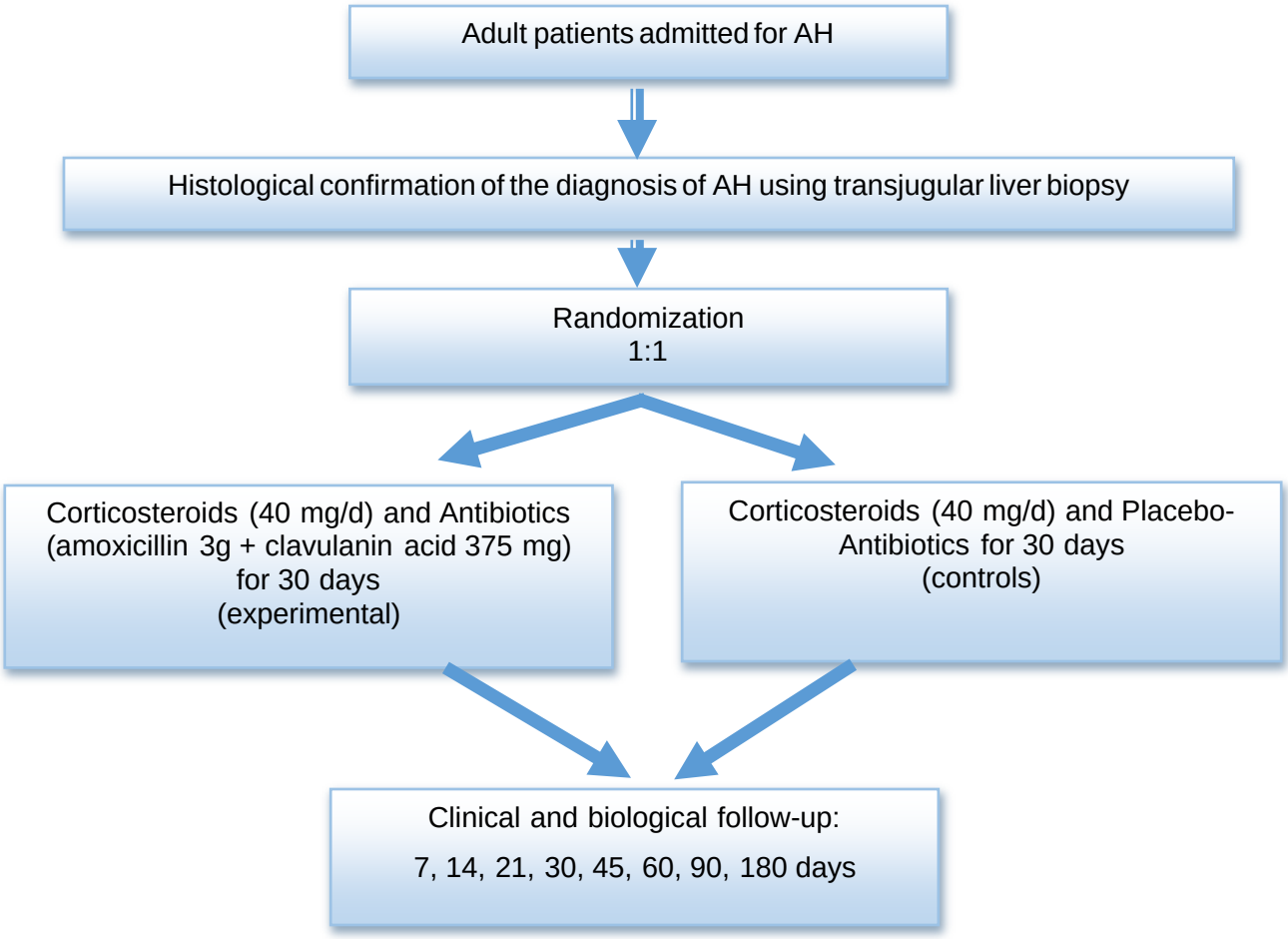
- improve the 3-month overall survival
- improve the 6-month overall survival
- decrease the incidence of infection at 2-month
- decrease the incidence of hepatorenal syndrome at 2-month
- increase the rate of patients with liver function improvement at 2-month
- increase the rate of patients with treatment response

2 TRIALS METHODS

2.1 Trial design

AntibioCOR-HAA is multicentre, randomized, balanced-parallel group, placebo-controlled, double-blind trial conducted in France. It as an academic trial designed to answer the question: is a combination of corticosteroids and antibiotics combination is superior to corticosteroids alone to reduce the short term mortality in patients with severe AH and high risk of early mortality. Adults patients admitted in 32 of participating centres with a recent onset of jaundice (<3 months), a biopsy proven AH, a Maddrey's discriminant function above 32 (defining severe AH), a MELD score ≥21 (defining high risk of early mortality), and alcohol consumption of more than 40g/day (women) and 50g/day (men) are included and randomized to be received antibiotics or placebo for 30 days with a treatment allocation ratio of 1:1. All patients received corticosteroids for 30-days.

Overview of Study Flow



2.2 Randomisation

The randomization process is described in detail within the clinical trial protocol. To be brief, a centralized real time randomization procedure is performed by using randomization list implemented. The randomization list was provided by an independent statistician (who did not take part in assessing the patients at any point in the study) using computer-generated random numbers (PLAN procedure of SAS software) with block sizes of four and stratified by centre.

2.3 Sample size

Full details of the sample size are described within the clinical trial protocol. 140 patients (54 events) are needed to have a power >80% to detect, with a two-sided log-rank test at 0.05 significance level, an absolute increase of 14% in 2-month overall survival in intervention arm compared to control arm, by assuming a two-month overall survival of 73% in control arm, considering a follow-up time of 2 months for all patients and a loss of follow-up rate of 5%.

2.4 Framework

Primary and secondary objectives of AntibioCOR-HAA trial are tested for superiority.

2.5 Statistical interim analyses and stopping guidance

No statistical interim analysis was planned.

2.6 Timing of analysis

Final analysis is planned to take place in December 2020 after the database is cleaned and locked according to data management plan. The first main report/publication of the AntibioCOR-HAA trial will be prepared at the same time.

2.7 Timing of outcome assessments

All included patients are followed 7, 14, 21, 30, 45, 60, 90 and 180 days after randomization. The time points at which outcomes are measured is provided in table 1. Full detail of the schedule of the study visits are described within the clinical trial protocol.

Table 1. The schedule of follow-up study visits

Outcomes	7 days	14 days	21 days	30 days	45 days	60 days	90 days	180 days
Vital status & physical examination	X	X	X	X	X	X	X	X
Labs	X	X	X	X	X	X	X	X
Presence of infection	X	X	X	X	X	X	X	X
Hepatorenal syndrome	X	X	X	X	X	X	X	X
MELD score	X	X	X	X	X	X	X	X

Lille model	X							
Adverse events	X	X	X	X	X	X	X	X

3 STATISTICAL PRINCIPLES

3.1 Confidence intervals and p-values

All applicable statistical tests will be 2-sided and will be performed using a 5% significance level.

No correction for multiple comparisons will be applied; all secondary objectives will be considered as exploratory and only effect sizes estimates with their confidence intervals (CI) will be reported.

All CIs presented will be 95%CI and 2-sided.

3.2 Adherence and Protocol Deviations

Patient's adherence was evaluated during the first month at each time point (7, 14, 21, 30 days) by clinicians and if available by sachet count and/or diary entries. Adherence levels will be reported by the rate of adequate adherence assessed by physician and if available the median (IQR) number of days without missed dose of study products divided by the number of days alive from randomization to 30 days will be reported.

Protocol deviations will be identified and classified as major or minor in blind reviews before the database freezing. The number and % of patients with major and minor protocol deviations will be provided by treatment group, with details of the type of deviation. No formal statistical comparison will be done.

3.3 Analysis population

Intent-to-treat (ITT): The ITT population will include all randomized patients, regardless of their eligibility and any protocol deviations, according to the intervention group to which they were assigned at randomization. The ITT population will be the primary analysis population for primary and secondary efficacy outcomes.

Per-protocol (PP): The PP population will include all randomized patients excluding patients with major protocol deviations.

PP population will be considered only for primary efficacy outcome as a secondary analysis.

Safety: The safety population will include all randomised patients who have received at least one dose of study treatment (antibiotics or placebo). Patients will be analysed according to the treatment they actually received. Safety population will be considered for assessment of adverse events.

4 TRIAL POPULATION

4.1 Screening data

The overall recruitment period will be provided in months. The number of screened patients, number of randomized patients and the reason for non-randomization will be reported for overall population according to consort flow diagram (figure 1) compliant with the CONSORT 2010 standard.

4.2 Eligibility

The trial inclusion and exclusion criteria are full detailed in clinical trial protocol. The number of ineligible patients screened and not randomized will be provided. The number of ineligible patients randomized will be reported by treatment group according to consort flow diagram (figure 1) compliant with the CONSORT 2010 standard.

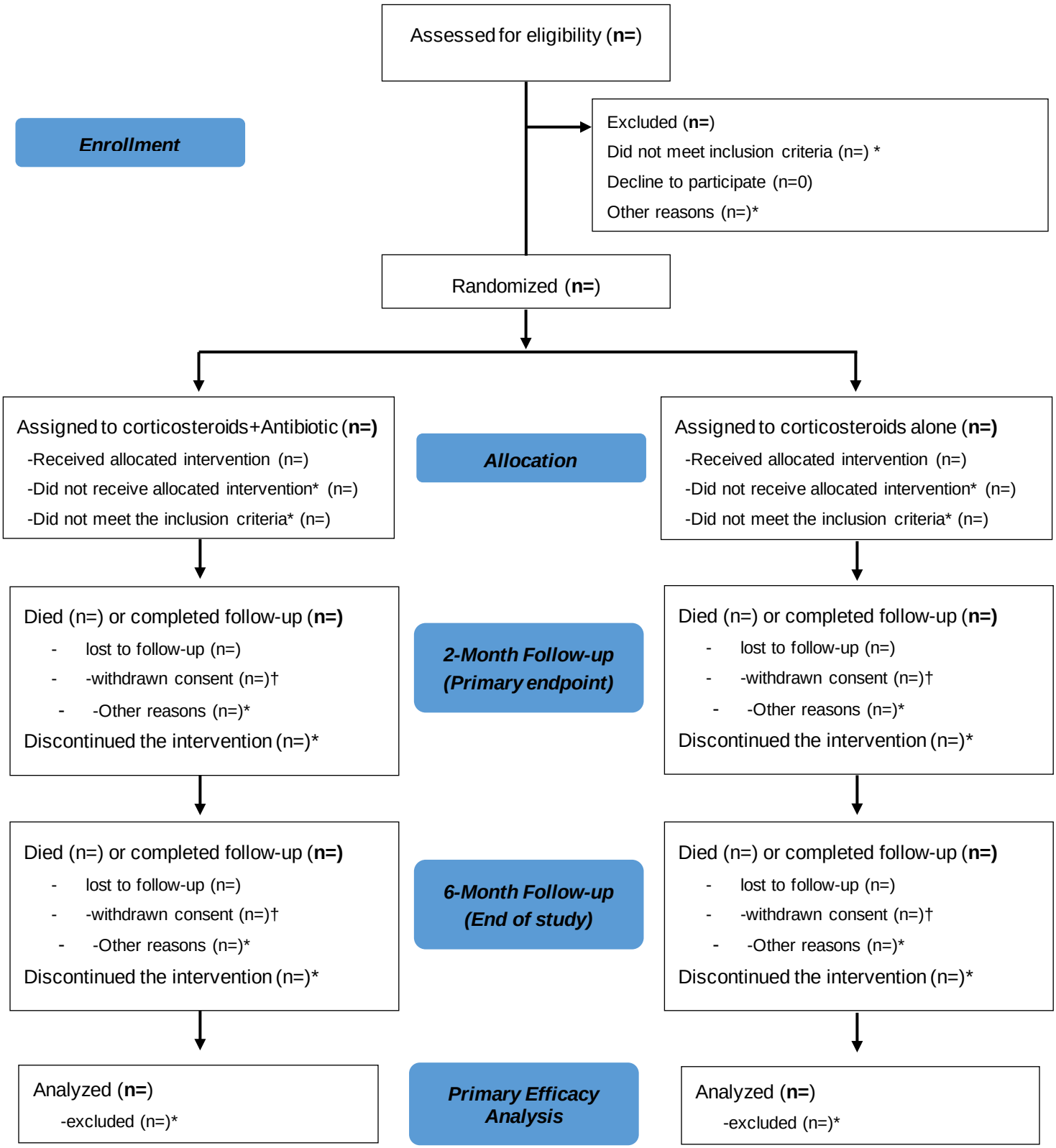
4.3 Withdrawal/Follow-up-level of withdrawal

The level of withdrawal will be tabulate and classified as:

1) Withdrawn consent from follow-up but allow data to be used until the date of consent withdraw
2) Withdrawn consent from follow-up and did not allow data to be used until the date of consent withdrawal
3) Withdrawal due to lost to follow-up
4) Withdrawal due to investigator decisions

The timing of withdrawal and reasons for withdrawal will be provided by treatment group according to consort flow diagram (figure 1) compliant with the CONSORT 2010 standard.

Figure 1. Flow of participation in the AntibioCOR-HAA trial.



* Reasons will be provided. † Level of consent withdrawal will be provided

4.4 Baseline patient characteristics

Detail of baseline characteristics are reported in table 2. Baseline characteristics will be described, in overall and according treatment groups. Quantitative variables will be expressed as mean (standard deviation) or median (interquartile range) for non-Gaussian distribution. Categorical variables will be expressed as frequencies and percentages. Normality of distribution will be assessed graphically and using the Shapiro-Wilk test. The number of missing data will be also reported. No formal statistical comparisons will be done; clinical importance of any imbalance will be noted.

Table 2. Baseline patient's characteristics

Characteristics	**Corticosteroids and Antibiotics (N=)**	**Corticosteroids Alone (N=)**
Age, years		
Male sex		
Ascites		
Encephalopathy		
Leukocyte count, /mm^3		
Neutrophil count, /mm^3		
Prothrombin time, seconds		
INR		
Bilirubin, mg/dl		
Creatinine, mg/dl		
Albumin, g/l		
AST, IU/l		
Maddrey's DF		
MELD score		

5 Analysis

Data analysis will be performed by an academic statistician (Julien Labreuche) from Biostatistics Department of University of Lille (France) under the responsibility of Professor Alain Duhamel.

5.1 Ouctome definitions

5.1.1. Primary efficacy outcome: The two-month overall survival defined as time from date of randomization to the death for any cause or the two-month time point. Death that occurred after the two-month follow-up period will not be included in this analysis.

5.1.2. Secondary clinical efficacy outcomes are defined as

- The six-month overall survival defined as time from date of randomization to the death for any cause or the six-month time point. Death that occurred after the six-month follow-up period will not include in this analysis

- The three-month overall survival defined as time from date of randomization to the death for any cause or the three-month time point. Death that occurred after the three-month follow-up period will not include in this analysis. This secondary endpoint, not specified in the protocol, was added regarding the recent recommendation from the NIAAA Alcoholic Hepatitis Consortia (8).

-The two-month incidence rate of infection defined as time of first occurrence of any infection from date of randomization to the two-month time point (or last available follow-up for death and loss of follow-up that occurred before the two-month time point). Infection that occurred after the two-month follow-up period will not be included in this analysis.

- The two-month incidence rate of hepatorenal syndrome defined as time of first occurrence of hepatorenal syndrome from date of randomization to the two-month time point (or last available follow-up for death and loss of follow-up that occurred before the two-month time point). Hepatorenal syndrome that occurred after the two-month follow-up period will not be included in this analysis.

- The alive patients who have a greater improvement in liver function at two-month follow-up visit defined by a MELD score <17 and using the overall distribution of MELD score

-The alive patients with a response to treatment at 7 days defined by a Lille model <0.45

5.1.3. Safety outcomes

-The rate of severe adverse events (defined by death or any event threatening life, requiring hospitalization or prolongation, resulting in injury or irreversible damage or considered important from a medical point of view) that occur from date of randomization to the six-month of follow-up visit.

- The rate of digestive disorders (diarrhoea, nausea, vomiting, abdominal pain) from date of randomization to the 30-day follow-up visit (treatment duration).

5.2 Analysis methods

- Primary efficacy outcome

The overall survival rate at two-month with will be estimated in each arm using the Kaplan Meier method. Any patients withdrawn from the trial or lost to follow-up before the two-month time point will be censored at the last available follow-up. Patients with liver transplantation within two-months of follow-up will be considered as censored events at the time of liver transplantation. Patients alive at the two-month time point will be censored at the two months datepoint. The absolute between-arm difference (intervention versus control arm) of the two-month overall survival will be calculated with its 95%CI. The primary efficacy analysis will be conducted using the log-rank test. Hazard ratio (HR) for death (intervention vs. control arm) and its 95%CI will be estimated using the Cox proportional hazard model as relative treatment effect size.

The proportional hazard assumption will be checked by examining the plot of scaled Schoenfeld residuals against time and by adding a time-dependent covariate into the Cox proportional hazard model. In case of evidence of non-proportional hazard, the interpretation of HR is debated, and we will use the methods proposed by Royston and Parmar (to provide the estimate of treatment effect based on restricted mean survival time) (9).

- Secondary efficacy outcomes

The same method described to analyse the primary efficacy outcome will be employed to compare the 3- and 6-month survival between the two arms.

The cumulative incidences of infection and hepatorenal syndrome during the first two months of treatment will be estimated in each arm using the Kalbfleish and Prentice method by considering any death as a competing risk (10). Subhazard ratio of event of interest (intervention vs. control arm) and its 95%CI will be estimated using Fine and Gray model (11). Proportional subhazard assumption will be checked by examining scaled Schoenfeld residuals and by adding a time-dependent covariate. In case of evidence of non-proportional hazard, the effect of intervention vs. control will be modelled by using time-dependent coefficients, with a specification of time-varying effects guided by visual inspection of Scaled Schoenfeld residuals plots.

Among patients alive at two-month follow-up visit, the relative risk in percentage of patients with a MELD score <17 and the mean difference in MELD score at two month follow-up visit (intervention vs. control arm) will be calculated with theirs 95%CI as treatment effect sizes.

Among patients alive at 7-day follow-up visit, the percentage of patients with a Lille model <0.45 at 7-day follow-up visit (patients who have a response to treatment) will be calculated and relative risk (intervention vs. control arm) will be calculated with its 95%CI as treatment effect size.

For treatment sided effects, the rate of specific adverse events will be evaluated only descriptively (based on subject counts and not on event counts) for each treatment arm.

5.3 Subgroup analyses

A subgroup analysis for primary outcome only, is planned according to presence or not of infection treated with antibiotics just before randomization.

Heterogeneity in treatment effect size on primary outcome according to infection status at randomization will be evaluated by including the corresponding multiplicative interaction terms in the multivariable Cox proportional hazard model. From this model, treatment effect sizes (HR) will be estimated in each subgroup by using linear contrasts.

5.4 Missing data

The primary efficacy analysis will be done using survival analysis by treating missing information (withdraw or loss of follow-up) as censored information at the last available follow-up. No missing data procedure will be done for secondary outcomes

5.5 Sensitivity analyses

A sensitivity analysis will be conducted in PP population for primary efficacy outcome only. We also perform sensitivity analyses for mortality outcomes, by using Fine and Gray models (11) to account liver transplantation as competing events; effect sizes will be estimated by subhazard ratio.

5.6 Additional analyses

No additional analyses will be done.

5.7 Statistical software

Data will be analysed using the SAS software (Version 9.4. SAS Institute Inc, Cary, NC, USA). Other package such as R software may be used if necessary.

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