Centre Hospitalier Régional Universitaire de Lille

Evaluation of the efficacy of antibiotic therapy combined with corticosteroids in severe alcoholic hepatitis. AntibioCOR- HAA

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DATES ET SIGNATURES

COORDINATING INVESTIGATOR SIGNATURE

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Synopsis

SPONSOR	CHRU de Lille Délégation à la Recherche Clinique et à l'innovation 6 rue du Professeur Laguesse 59037 LILLE Cedex Tel : 03 20 44 59 69
TITLE	Evaluation of the efficacy of antibiotic therapy combined with corticosteroids in severe alcoholic hepatitis.
STUDY COORDINATOR	Pr Philippe Mathurin
NUMBER OF CENTERS	32 centers
STUDY TYPE	Randomized controlled double-blind trial.
OBJECTIVES	Main objective of the study is to demonstrate that a combination of corticosteroids and antibiotics improve 2-month survival more than corticosteroids alone in the subgroup of patients with severe alcoholic hepatitis who have a high short-term risk of death (MELD score≥21). This time-point was chosen because most deaths occur in patients with severe alcoholic hepatitis within the first two months (e.g. mean time until death 49.7 days in the most recent multicentre study). Secondary objectives are : - to show that patients treated with antibiotics and corticosteroids develop fewer infections than patients treated with corticosteroids alone - to show that patients treated with antibiotics and corticosteroids develop hepatorenal syndrome less frequently than patients treated with corticosteroids alone - to show that patients treated with antibiotics and corticosteroids have a greater improvement in liver function than patients treated with corticosteroids alone, assessed by the MELD score <17 at two months - and to show that the probability of response assessed by the Lille model is higher in patients treated with an association of antibiotics and corticosteroids than corticosteroids alone.
STUDY DESIGN	This is a prospective, controlled, randomized, double-blind, multicentre study, phase 3.
EVALUATION CRITERIA	Evaluation criteria regarding the primary objective will be 2-month survival. Evaluation criteria regarding the secondary objectives will be: - Incidence of infection during follow-up - Incidence of hepatorenal syndrome during follow-up - Percentage of patients who have a greater improvement in liver function, assessed by the MELD score and the Lille model - Prediction of outcome by the Lille model

INCLUSION CRITERIA	- Patients aged 18-75 - Recent onset of jaundice (<3 months) - Biopsy proven alcoholic hepatitis (transjugular liver biopsy) - Maddrey's discriminant function above 32, defining severe alcoholic hepatitis - MELD score ≥21 - Alcohol consumption of more than 40g/day (women) and 50g/day (men) - Written informed consent
EXCLUSION CRITERIA	Previous severe allergy or hypersensitivity to amoxicillin or clavulanic acid (anaphylactic shock, Quincke edema, severe urticaria) - Hypersensitivity to Prednisolone or Augmentin® - Allergy to beta-lactam - History of liver injury to amoxicillin and/or clavulanic acid - Phenylketonuria, because of the presence of aspartame the experimental drugs - Type 1 hepatorenal syndrome before the initiation of treatment. - Severe extrahepatic disease - Any history of tumor during the 2 previous years - Psychosis uncontrolled by treatment - Live vaccine - Chronic renal insufficiency with baseline creatinine level ≥ 20 mg/L - Acute pancreatitis - Uncontrolled gastrointestinal bleeding - Ongoing viral or parasitic infection - Untreated bacterial infection. - Withdrawal from any antibiotic treatment less than 7 days. Patients who are currently being treated or were previously treated with antibiotics* for diagnosed infection or after a positive screening for infection may be included after wash-out period of 7 days (time from withdrawal of antibiotics to randomization). - Tuberculosis < 5 years - Positive blood PCR in patients with positive antibodies against HCV - Patient carrying HBV or HIV - Treatment with corticosteroids, immunosuppression therapy or budesonide within 6 months before the study -pregnant/nursing woman *use of beta-lactams is preferred, when considering a low accumulation in the body
NUMBER OF PARTICIPANTS	280 patients
STATISTICAL METHODS	All analyses will be assessed according to the intention-to-treat principle. The survival rate at 2 months with a 95% confidence interval will be estimated using the Kaplan Meier method. For the primary analysis, the survival rate will be compared between the two groups using the log-rank test. Hazard ratio for death (experimental vs. control group) and its 95% confidence interval will be determined using the Cox proportional hazard model. A secondary analysis will be performed to adjust the effect of treatment for the status of infection. This will be done by using the Cox model with the group, the infection status and the interaction (infection*group) as independent variables
RESEARCH BENEFITS	<u>Expected benefits to patients</u> The main expected benefit to the patient is improvement in 2-

AND RISKS EVALUATION	month survival. The study is also expected to improve liver function, prevent hepatorenal syndrome and decrease the risk of infection during follow-up. -Group Benefits Optimize the management of severe alcoholic hepatitis. - Risks and constraints The only constraint is that patients must be followed up according to a precise schedule. In France and Belgium, transjugular liver biopsy is required for the diagnosis of alcoholic hepatitis and is not for research purposes. There are no additional risks according to the study design. - Expected risks of the corticosteroids At therapeutic doses, corticosteroids can cause diabetes. Insulin treatment may then be administered and corticosteroids can be continued. In rare cases, corticosteroids may have psychiatric side-effects and treatment should be discontinued. Corticosteroids may lead to bacterial infections. The clinician may decide to discontinue corticosteroids in that case. We recommend stopping corticosteroids in case of pneumonia, endocarditis, bone infections or severe sepsis whatever the site of infection. The clinician may continue corticosteroids in case of non severe urinary infection, spontaneous peritonitis or bacteriemia. For fungal and viral infections, corticosteroids should be interrupted
EXCLUSION PERIOD	Patients will not participate in any studies assessing the efficacy of a new pharmaceutical agent for a 6-monthperiod. This corresponds to the 6 months of participation in research. Patient is allowed to participate in other studies – observational or interventional – that does not assess a new pharmaceutical agent.
CONSTITUTION OF A SUPERVISION COMMITTEE	An organizing committee was set up for this study. It will consist of statisticians from the team of Prof. A. Duhamel, members of the Federation of Clinical Research of the Lille University Hospital and pharmaceutical establishment of CHRU Lille. It will define the general organization and conduct of the trial and coordinate information.
DATA SAFETY AND MONITORING BOARD:	This trial will be supervised by an independent Data and Safety Monitoring Board (DSMB) consisting of five independent members. Their roles, missions, responsibilities and the frequency of their meetings are defined in the DSMB Convention (cf. appendix 1).
STUDY DURATION	~~3 ½ years~~ **4 ½ years**

I. BACKGROUND AND STUDY RATIONALE

Alcoholic hepatitis is one of the most severe forms of alcoholic liver disease. Major progress has been made in the management of these patients since the development of the Maddrey's discriminant function (1), which uses biological variables to distinguish patients with a severe form of alcoholic hepatitis from patients with a non-severe form who are at low risk of mortality. The Maddrey's discriminant function is calculated using the following formula: 4.6 x (patient prothrombin time in seconds – laboratory reference prothrombin time) + (serum bilirubin in mg/dl). Since the development of Maddrey's discriminant function, several tools have been proposed to predict patients with a higher risk of mortality despite appropriate medical treatment. The MELD score is one of the most frequently used of these. The formula is the following: (9.57 × log creatinine in milligrams per deciliter) + (3.78 × log bilirubin in milligrams per deciliter) + (11.20 × log international normalized ratio) + 6.43. At least two studies (2, 3) have shown that the MELD score is of interest in the prediction of outcome in severe alcoholic hepatitis.

AASLD and EASL Clinical Practice guidelines recommend the use of corticosteroids or pentoxifylline for 28 days as first line therapy for severe alcoholic hepatitis (4, 5). In France, most centres use corticosteroids instead of pentoxifylline. Although the death rate at 2 months has decreased from 40% to 20%, the death rate at 6 months is still approximately 35% (6). To more specifically predict the effect of treatment, our team developed a composite score called Lille model (7), which integrates biological improvement during therapy, evaluated by the progression of bilirubin after the first 7 days of treatment. However, the Lille model must be calculated after 7 days and therefore cannot be used to assess the severity of disease at baseline.

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 (4, 8-12). 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. It should be remembered that some studies used 6-month survival as a primary endpoint, while others used 1 or 2-month survival. 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 should be modified to select the subgroup of patients at high risk of death within 2 months. Conversely, the subgroup of patients with a low risk of death should not be included in studies evaluating the impact of a new strategy at 2 months. In a recent French multicentre study (9), we observed that the baseline MELD score effectively classified patients in high and low-risk of death at 2 months. Indeed, in this study, patients with a MELD score below 21 had a 2-month survival at 91.8%, whereas those with a MELD score greater than 21 had a 2-month survival at 73%. Those data were confirmed in a cohort of more than 600 patients treated with corticosteroids collected by our centre. 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.

The association of antibiotics and corticosteroids is one of the most promising strategies for severe alcoholic hepatitis. Indeed, several animal studies have shown that bacterial compounds from Gram positive and negative bacteria are associated with the development of alcohol-induced liver injury, in particular by triggering the inflammatory process (6, 13, 14). Use of antibiotics and knock-out mice for receptors to bacterial compounds has confirmed the implication of bacterial motives in the pathogenesis of alcoholic liver disease. In addition, experimental studies have also suggested that bacterial products and activation of their receptors impair liver regeneration, thus limiting tissue repair (6, 13, 14).

Bacterial infection is a frequent event in severe alcoholic hepatitis in patients. About 25% of patients have bacterial infections at their admission to hospital (15). After treatment with antibiotics, the outcome of treatment with corticosteroids in these patients is similar to that of those not infected at their admission. Then, another 25% (who are not the same as the latter patients) develop infection after corticosteroid treatment is initiated, with a drastic impact of survival as compared to those who remain infection-free: 46 vs. 78%, $p<0.01$. As median time for development of infection is 14 days, the early use of antibiotics may prevent such life-threatening event but also may reduce liver injury as suggested by experimental studies.

Antibiotic molecules must respect specific rules to prevent potential adverse events. Because most patients are admitted directly from home, infection is primary community acquired. This bacteriological profile allows the use of molecules like β-lactams in first line. In fact it seems difficult to propose fluoroquinolones as long as these molecules are recommended to prevent recurrence of spontaneous bacterial peritonitis. The chosen molecule will need to have a limited number of adverse events and limited toxicity, to be able to be used for long periods and to have a minimal effect on host gut microbiota. Based on the above mentioned criteria, combined treatment with amoxicillin clavulanate seems to be a rational choice. This association is adapted to community acquired infections and is recommended in community acquired pneumonia, intra-abdominal infection or skin and soft tissue infections (16-18). The pharmacokinetic/pharmacodynamics profile of this association is also interesting with a more than 40% availability by oral administration allowing use in most clinical conditions. Nevertheless, amoxicillin clavulanate treatment has been shown to disrupt the microbiota with an increase in the level of *Enterobacteriaceae* and *Bacteroides* groups and a decrease of the *C. coccoides-E. rectale* group decreased (19). One of the main consequences could be an alteration of the gut microflora leading to a potential proliferation of *Clostridium difficile* (20, 21). Nevertheless, the use of Amoxicillin clavulanate is considered a safe option, in particular when given for 1 month when considering that treatment with amoxicillin-clavulanate for 3-4 weeks is recommended in complicated pyelonephritis due to sensitive bacteria (22, 23).

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This scientific context provides a compelling evidence of data suggesting that combination of antibiotics and corticosteroids should be tested in patients with severe alcoholic hepatitis.

II. Objectives of the study

2.1 Main objective

Main objective of the study is to demonstrate that a combination of corticosteroids and antibiotics improve 2-month survival more than corticosteroids alone in the subgroup of patients with severe alcoholic hepatitis who have a high short-term risk of death (MELD score≥21). This time-point was chosen because most deaths occur in patients with severe alcoholic hepatitis within the first two months (e.g. mean time until death 49.7 days in the most recent multicentre study, (9).

2.2 Secondary objectives

Secondary objectives are :

- to show that patients treated with antibiotics and corticosteroids develop fewer infections than patients treated with corticosteroids alone
- to show that patients treated with antibiotics and corticosteroids develop hepatorenal syndrome less frequently than patients treated with corticosteroids alone
- to show that patients treated with antibiotics and corticosteroids have a greater improvement in liver function than patients treated with corticosteroids alone, assessed by the MELD score <17 at two months
- and to show that the probability of response assessed by the Lille model is higher in patients treated with an association of antibiotics and corticosteroids than corticosteroids alone.

III. Study design

3.1 Experimental Plan

This is a phase III study, because the benefit of adding antibiotics to corticosteroids has not been confirmed. This is a multicentre randomized controlled double-blind clinical trial of antibiotics vs. placebo groups.

This is a multicentre double-blind randomized controlled study with two parallel groups.

Once inclusion and exclusion criteria are confirmed and after having obtained patient written consent, participating centres will manage inclusion in the trial. Corticosteroids and antibiotics or their placebo will be begun orally. Patients will be managed in the hospital for at least 7 days after treatment has begun, which corresponds to the evaluation of response to treatment based on the Lille model. Clinical and biological follow-up after the 7-day period will be on days 14, 21, 30, 45, 60 (primary endpoint), 90 and 180. Tolerance and efficacy criteria will be assessed during each visit, as well as the presence of infection and hepatorenal syndrome (secondary endpoints).

3.2 Study population

95% of the patients suffering of severe alcoholic hepatitis have underlying cirrhosis; the remaining 5% are at pre-cirrhotic stage. Therefore, there is no particular adaptation to cirrhotic patients as they are considered as target population.

Transjugular liver biopsy is routinely performed to confirm the diagnosis of severe alcoholic hepatitis. Therefore, this technique is a part of routine practice to affirm the disease and tailor treatment. An information letter describing the procedure of transjugular liver biopsy will be given to all patients. Accordingly, this technique should not be considered as inclusion criterion but as a confirmation of diagnosis.

3.2.1Inclusion criteria

- Patients aged 18-75
- Recent onset of jaundice (<3 months)
- Biopsy proven alcoholic hepatitis (transjugular liver biopsy)
- Maddrey's discriminant function above 32, defining severe alcoholic hepatitis
- MELD score ≥21
- Alcohol consumption of more than than 40g/day (women) and 50g/day (men)
- Written informed consent

3.2.2 Exclusion criteria

- Previous severe allergy or hypersensitivity to amoxicillin or clavulanic acid (anaphylactic shock, Quincke edema, severe urticaria)
- Hypersensitivity to Prednisolone or Augmentin®

- Allergy to beta-lactam
- History of liver injury to amoxicillin and/or clavulanic acid
- Phenylketonuria, because of the presence of aspartame in the experimental drugs
- Type 1 hepatorenal syndrome before the initiation of treatment.
- Chronic renal insufficiency with a baseline creatinine level ≥ 20mg/L
- Acute pancreatitis
- Severe extrahepatic disease
- Any history of tumor within the 2 previous years or ongoing tumor
- Psychosis uncontrolled by treatment
- Live vaccine
- Uncontrolled gastrointestinal bleeding
- Ongoing viral or parasitic infection
- Untreated bacterial infection.
- Withdrawal from any antibiotic treatment less than 7 days. Patients who are currently being treated or were previously treated with antibiotics* for diagnosed infection or after a positive screening for infection may be included after wash-out period of 7 days (time from withdrawal of antibiotics to randomization).
- Tuberculosis < 5 years
- Positive blood PCR in patients with positive antibodies against HCV
- Patient carrying HBV or HIV
- Treatment with corticosteroids, immunosuppression therapy or budesonide within 6 months before the study
- Pregnant/nursing woman.

*use of beta-lactams is preferred, when considering a low accumulation in the body

Note: Patients with a past history of spontaneous peritonitis are to be treated with norfloxacin (recommended antibiotic for secondary prophylaxis). These patients can be included in the present study without any change in the protocol and this treatment must be continued. In case of spontaneous bacterial peritonitis occurring during treatment course and follow-up, norfloxacin will be started without any change in the protocol (except description of the serious adverse event).

3.3 Primary End point

Primary endpoint is the percentage of patients alive at 2 months in the experimental arm compared to the percentage of patients alive in the control arm

3.4 Secondary Endpoints

Secondary endpoints are :

- the incidence of infection during the 2-month study in the antibiotic+corticosteroid arm compared to the control arm

- the incidence of the hepatorenal syndrome during the first two months in the two treatment arms
- the percentage of patients who have a greater improvement in liver function, assessed by a MELD score <17 at two months in both treatment arms
- percentage of patients with a response to treatment according to the Lille model (<0.45) in both treatment arms

3.5 Assessments

3.5.1 Efficacy Assessments

Patients will be followed-up for 6 months. The patient's condition at 2 months, 3 months and 6 months will be scored 0 (alive) or 1 (deceased). This information will be obtained from follow-up data or in case of missing information by contacting the death registry at the patient's birthplace. Cause of death will be recorded if available.
During each visit, tolerance and efficacy criteria will be assessed, as well as the presence of infection and hepatorenal syndrome (secondary endpoints).

3.5.2 Safety Assessments

During treatment and for two months, treatment side-effects will be identified and recorded.
Tolerance criteria will be evaluated at each visit. Subjects will be given a diary during hospitalization or ambulatory period to facilitate compliance to treatment (day7, day14, day21, day30).
The duration of treatment is 30 days. The investigator will meet patients after the first week of treatment in the clinical centre during hospitalization.

The presence of infection and hepatorenal syndrome (secondary endpoints) will be assessed during each visit using clinical and/or biological examination.
A rectal swab will be obtained at the beginning of treatment and at days 30 and 60 of follow-up. These results will only be recorded to monitor changes in gut microflora, but will not influence treatment protocol.

3.6 Number of patients

The estimated survival in the control group (corticosteroids alone) at 2 months follow up is at 73% (see Scientific rationale section for justification). The expected increase in survival in the experimental group (combination antibiotics and corticosteroid therapy) is 14%. The 14% increase in survival is based on the hypothesis that combination therapy will result in an improvement and a survival rate close to that in patients with severe alcoholic hepatitis and a MELD score <21 [75% of the difference in survival observed between the two groups according to the 21 MELD cut-off: 0.75 * (91.8%-73%)=14%]. Based on a type I error of 0.05, a power of 80%, a lost to follow-up rate of 5%, a total of 54 events are required corresponding to 139 patients per group, rounded off to 140 (computation made using the PASS 2013, Log-rank test Freedman).

3.7 Method and analysis Strategy

No statistical analysis will be performed until the last included patient has reached the 2-month time point.

For baseline criteria, continuous variables will be expressed as means with the 95% confidence interval; categorical variables as frequencies, percentages with a 95% confidence interval.

Analyses will be assessed based on to the intention-to-treat principle. The intention-to-treat population is defined as all subjects who are randomized independent of whether they receive treatment or not.

Primary objective :

The survival rate at 2 months with a 95% confidence interval will be estimated using the Kaplan Meier method. For the primary analysis, the survival rate will be compared between the two groups using the log-rank test. Hazard ratio for death (experimental vs. control group) and its 95% confidence interval will be determined using the Cox proportional hazard model.

A secondary analysis will be performed to adjust the effect of treatment for the status of infection. This will be done by using the Cox model with the group, the infection status and the interaction (infection*group) as independent variables.

Secondary objective :

The incidence of infection during the first two months of treatment will be determined in each group using the Kalbfleish and Prentice method to take into account death as a competing risk. These will be compared by using the Gray's test. This analysis will also be performed for the incidence of hepatorenal syndrome.

The percentage of patients in the two treatment arms with a MELD score <17 (patients who have the greatest improvement in liver function) will be estimated with the 95% confidence intervals and compared with a Chi-square test or a Fisher Exact test. This analysis will also be performed for the response to treatment determined by the Lille model (<0.45).

The prognostic factors of 2-month survival will be identified by bivariate analysis using the Cox proportional hazards model. All variables with a <0.1 significance will be introduced in a multivariable Cox proportional hazards model to adjust the treatment effect for prognostic factors. Colinearities among prognostic factors will be investigated using the variance inflation factor (VIF). A VIF value below 3 will be considered acceptable. In case of multicolinearity, prognostic factors that are considered to be redundant will be excluded from the model based on expert opinion. The adjusted hazards ratio of treatment effect and the 95% confidence interval will be determined. The validity of the Cox model will be studied by Schoenfeld residuals and its match with the Cox model described by Therneau (Therneau T, Grambsch P. Modeling Survival Data: Extending the Cox Model. New York, NY: Springer; 2000).

IV- Logistic of the study

4.1 Places of conducting the research

The study will be performed by Prof. Philippe Mathurin (coordinating investigator), Service des maladies de l'appareil digestif et de la nutrition, CHRU de Lille, France, together with the local research direction (Délégation à la Recherche Clinique et à l'Innovation).

There will be a network of 32 participating centres in France and Belgium (Hepatology units). This network will include most of the centres that participated in the previous multicentre French trial published in September 2013 in the Journal of the American Medical Association (9).

Participating centres are listed on page 2 of the present protocol.

The study will be sponsored by The Centre Hospitalier Régional et Universitaire de Lille, France.

4.2 Study organization

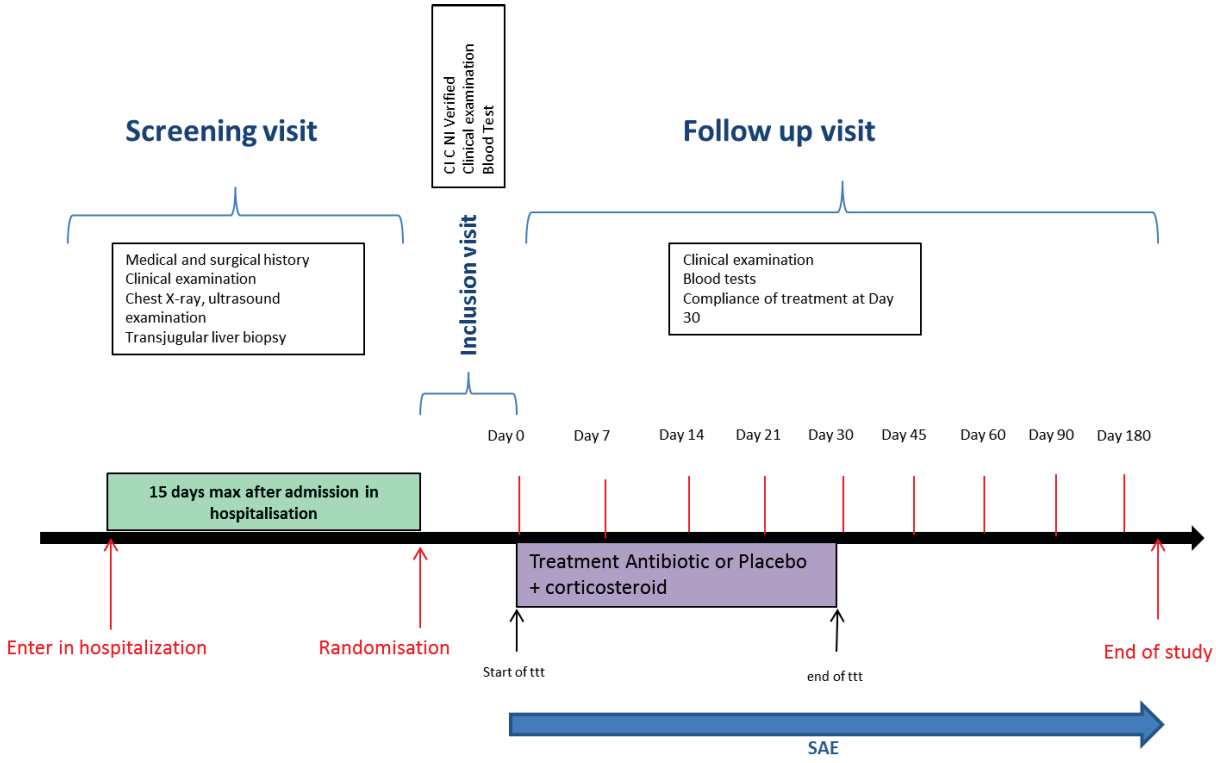

Fig 1 Design of the study

4.2.1 Screening phase Information to subjects

A letter of information letter will be given to subjects during routine hospitalization. The study will be presented and the entire process of the study will be explained in detail to potential subjects. Patients will have enough time to decide whether or not they wish to participate.
In case of severe hepatic encephalopathy, a person representing the patient may be given information about the study.

The investigator will explain the purpose and conditions of the study. Once they have read the information letter and their questions have been answered, they will inform the investigator if they agree to participate in the study. The informed consent form will be signed by the patient or if necessary a signatory may be added:

- A representative of the patient's guardian
- The family member or representative as defined by the Decree dated March 4, 2002 (for emergency consent or if it is impossible to obtain consent because of the patient's clinical condition).
- A witness (if informed consent cannot be obtained, verbal consent is obtained and witnessed)

After signing these documents, the subject is included in the study.

Eligibility for inclusion in the study will be assessed during the pre-inclusion screening phase.
A pre-inclusion screening visit should take place within 15 days before the inclusion phase. The following tests will be performed and information will be obtained:

- Medical and surgical history, including a history of alcoholic hepatitis
- Clinical examination
- Blood tests
- Pregnancy test for all women of childbearing age (WOCBA)
- Radiological examination (chest X-ray, ultrasound examination to exclude hepatocellular carcinoma)
- Screening for infection will include blood culture, urine analysis, examination of ascites fluid in patients without current antibiotic therapy. Other tests may be performed if the investigator wishes.
- Patients who are or have been treated with antibiotics for diagnosed infection or after a positive screening for infection may be included after a wash-out period of 7 days (time from withdrawal of antibiotics to randomization).
- Transjugular liver biopsy

46 mL of blood will be collected at pre-inclusion visit (4 x 7mL dry tube, 1 x 5mL EDTA tube, 1x 3mL citrate tube, 1x 5mL heparin tube, 1 x 5 mL fluorinated tube)

To confirm inclusion criteria, two scores will be calculated based on biological test results:
- The Maddrey's discriminant function will be calculated using the following formula:

4.6 x (patient prothrombin time in seconds – laboratory reference prothrombin time in seconds) + (serum bilirubin in mg/dl)

- The MELD score will be calculated using the following formula:
(9.57 × log creatinine in milligrams per deciliter) + (3.78 × log bilirubin in milligrams per deciliter) + (11.20 × log international normalized ratio) + 6.43.

4.2.2 Inclusion Visit / Randomization

All patients who consume excess quantities of alcohol (defined as more than 40 g/day of pure alcohol for women and 50g/day for men) and have had jaundice for less than 3 months with a Maddrey's discriminant function above 32 are eligible for the study.
Patients who meet all inclusion criteria are eligible for the study. The inclusion visit will be scheduled after all pre-inclusion test results are obtained.
The diagnosis of alcoholic hepatitis will be based on the histological findings and the clinical and biological criteria described in the inclusion criteria section.

Subjects, who meet all the inclusion criteria, are eligible for the study. Randomization will be performed immediately before treatment administration is begun.
The inclusion visit will take place within 15 days after admission in the participating center (except if the patient is being treated for infection because of the wash-out period). As the screening visit may be performed 15 days before the beginning of treatment, another pregnancy test will be required for WOCBA at the inclusion visit.

Once the patient has been included in the study, s/he will be randomized into one of the two treatment arms (Antibiotics or Placebo). Assignment to study arm will be based on the randomization list drafted by the sponsor. The investigator will send a fax to the sponsor for each included patient. The study randomization number returned to the investigator as well as the number of the box to be administered to the patient. The period from screening to the inclusion visit should be less than 15 days. Randomization will be centralized and balanced (blocks of 4). Because randomization is performed on a centre by centre basis, to obtain the correct proportion of treatments A and B each randomized group must be completed by the centre once it is begun.

The date of the beginning of treatment and follow-up visits will be programmed for each patient. Antibiotics or Placebo will be provided for a 30-day period.
A clinical examination will be performed during this visit associated with laboratory tests including a complete blood count with platelet count, liver function tests (albumin, prothrombin time in seconds, INR, AST, ALT, GGT, ALP, bilirubin), ionogram, urea, creatinine, C-reactive protein. A rectal swab in search of multiresistant bacteria will be performed.

25 mL of blood will be collected at inclusion visit (1 x 5 mL heparin tube, 1 x 3mL citrate tube, 1 x 5mL EDTA tube, 1 x 5mL fluorinated tube, 1x 7 mL dry tube).

Moreover, after the patient has agreed to a biological collection, and regardless of its participation to AntibiocorHAA, the following samples will be collected on the first day of treatment:

-1 EDTA tube (5mL). On the receipt of the tube, 1 mL of the whole blood will be aliquoted and placed at -80°C.

-1 heparin tube (7mL). This tube will be centrifuged within 2 hours of collection (2500 rpm for 15min).). Once the centrifugation, aliquot 1 ml of supernatant in 2 cryotubes. Eliminate the remaining supernatant and gently raise the white ring composed of PBMCs (buffy coat) present above the blood pellet and transfer to a third cryotube and stored at -80°C.

-1 dry tube (7mL). This tube will be centrifuged between 30 min and 2 hours of collection (2500 rpm for 15min). Collected from the supernatant, 2 samples of 1mL will be aliquoted in 2 cryovial tubes and stored at -80°C.

These samples will be stored for a maximum of 20 years. The details concerning the management and logistics of the biological collection are specified in section VI.

4.2.3 Follow-up Visits

After inclusion, patients will have the following follow-up visits: the first month after inclusion, patients will have a weekly follow-up. After day 30, visits will be scheduled for days 45, day 60, day 90 and day 180 (M6).

It is suggested that the patient remain hospitalized for the first week of treatment (from Day 0 at Day 7).

At Day 7, the Lille score will be calculated in both groups (see screening visit).

The MELD score will be calculated at each visit using to the same formula as above (see screening visit)

During each of the visits during the treatment phase (days 7, 14, 21, 30), compliance will be assessed by questioning the patient. A diary will be given to the subjects to facilitate treatment compliance during the ambulatory period and hospitalization (days 7, 14, 21, 30).

A clinical examination will be performed during each follow-up visit as well as laboratory tests including a complete blood count with platelet count, liver function tests (albumin, prothrombin time in seconds, INR, AST, ALT, GGT, ALP, bilirubin), ionogram, urea, creatinine, C-reactive protein). A rectal swab in search of multiresistant bacteria will be performed at day 30, another one will be performed at Day 60.

18 mL of blood will be collected at follow up visits (1x 5mL heparin tube, 1x 5 mL EDTA tube, 1 x 3mL citrate tube, 1 x 5mL fluorinated tube)

Moreover, after the patient has agreed to a biological collection, and regardless of its participation to AntibiocorHAA, the following samples will be collected at D7, D30 and D60:

-1 heparin tube (7mL). This tube will be centrifuged within 2 hours of collection (2500 rpm for 15min).). Once the centrifugation, aliquot 1 ml of supernatant in 2 cryotubes. Eliminate the remaining supernatant and gently raise the white ring composed of PBMCs (buffy coat) present above the blood pellet and transfer to a third cryotube and stored at -80°C.

-1 dry tube (7mL). This tube will be centrifuged between 30 min and 2 hours of collection (2500 rpm for 15min).). Collected from the supernatant, 2 samples of 1mL will be aliquoted in 2 cryovial tubes and stored at -80°C.

These samples will be stored for a maximum of 20 years. The details concerning the management and logistics of the biological collection are specified in section VI.

An end of participation visit (6 months) will be planned after 180 days.

Patient status patient will be scored 0 (alive) or 1 (deceased) in the protocol folder. Alcohol (in gram/day) consumption will be estimated at each visit based on a discussion between the physician and the patient.

	Screening visit	Inclusion Day 0	Day 7	Day 14	Day 21	Day 30	Day 45	Day 60	Day 90	Day 180
Inclusion criteria / Exclusion criteria	X									
Consent	X									
Clinical examination	X	X	X	X	X	X	X	X	X	X
Chest X-ray	X									
Abdominal Ultrasound examination	X									
Infection screening blood culture, urinary analysis, examination of ascitic fluid	X									
Blood Test	X	X	X	X	X	X	X	X	X	X
Hematology, ionogram	X	X	X	X	X	X	X	X	X	X
Chemistry Group	X	X	X	X	X	X	X	X	X	X
Liver tests	X	X	X	X	X	X	X	X	X	X
Ethanolemia	X	X	X	X	X	X	X	X	X	X
α feto-protéine	X									
HBV, HCV, HIV 1+2	X									
Biological collection*		X	X			X		X		
Pregnancy test	X	X								
Rectal swab		X				X		X		
Randomization		X								
Treatment is delivered		X								
Compliance assessment			X	X	X	X				
Return pharmacist and destruction of treatment						X				

*with the consent of patient

Fig 2. flow-chart

If infection occurs during treatment with corticosteroids and experimental treatment:

The expected rate of infection during the treatment course is 25%, at least in the corticosteroid+placebo arm (15).

The investigator will receive the following recommendations:

- Corticosteroids can be continued in case of bacteraemia, spontaneous peritonitis, urinary tract infection in the absence of evidence for severity (in particular septic shock and renal failure)
- Corticosteroids should be discontinued in case of pneumonia, endocarditis or bone infection
- When considering that patients with severe alcoholic hepatitis will be hospitalized, investigators should consider as a first-line treatment antibiotics such as imipenem or ticarcillin+tazobac tam.
- Once the antibiogram has been received, antibiotics can be decremented
- Treatment with amoxicillin and/or clavulanic acid should be avoided (Clamoxyl®, Augmentin®, Ciblor®, Claventin®…)
- The decision to stop experimental treatment (antibiotics or placebo) or not is left up to the investigator.
- After having stopped corticosteroids and/or experimental treatment, once infection has been controlled with antibiotics, both treatment options can be begun again according to the investigator's decision.
- Using of immunosuppressant of pentoxifylline or Budézonide type will not be allowed during the study.

In case of infection upon treatment, and if the investigator considers this relevant, a phone call to the Pr Guery infection division is possible at +33320445743 or at +33320444962 (line 30015 B. Guéry, line 30237 T. Galperine). In case of difficulties to reach them, the phone line of the intensive care physician of Lille digestive diseases unit is available 24h / 24 (+33320445962 line 32143)

4.2.4 List of treatments that are not allowed during this trial

- Any antibiotic treatment in the last 7 days
- Treatment with corticosteroids or immunosuppression therapy within 6 months before study
- Pentoxifylline or Budézonide

4.3 Study duration

Each patient will be included for 6 months. Patient recruitment is expected to last ~~36~~ **48** months, which means that the duration of the study is expected to be ~~42~~ **54** months.
The study will end after 280 patients have been included.

4.4 Rules for study termination

Treatment will be stopped if side effects can be attributed to treatment. In case of bacterial infection, the choice of stopping corticosteroid treatment is left to the investigator. In that case, corticosteroid treatment can be stopped and begun again once the infection has been controlled.

If corticosteroid-induced diabetes develops, insulin treatment can be begun with no need to discontinue corticosteroids. However the patient will continue to be included in his/her group for intention to treat analysis.

Each patient may leave the study by decision of the competent administrative authority, the sponsor and the coordinating investigator but also by decision of a co-investigator or by decision of the firm itself in accordance with regulations and as mentioned in the form of obtaining consent.

4.5 Interdiction for simultaneous participation – Exclusion period

Patients will not participate in any studies assessing the efficacy of a new pharmaceutical agent for a 6-month period. This corresponds to the 6 months of participation in research. Patient is allowed to participate in other studies – observational or interventional – that does not assess a new pharmaceutical agent.

4.6 Benefits, risks and study constraints

- Expected benefits to patients

The main expected benefit to the patient is improvement in 2-month survival. The study is also expected to improve liver function, prevent hepatorenal syndrome and decrease the risk of infection during follow-up.

-Group Benefits

Optimize the management of severe alcoholic hepatitis.

- Risks and constraints

The only constraint is that patients must be followed up according to a precise schedule. In France and Belgium, transjugular liver biopsy is required for the diagnosis of alcoholic hepatitis and is not for research purposes.

There are no additional risks according to the study design.

- Expected risks of the corticosteroids

At therapeutic doses, corticosteroids can cause diabetes. Insulin treatment may then be administered and corticosteroids can be continued. In rare cases, corticosteroids may have psychiatric side-effects and treatment should be discontinued.

Corticosteroids may lead to bacterial infections. The clinician may decide to discontinue corticosteroids in that case. We recommend stopping corticosteroids in case of pneumonia, endocarditis, bone infections or severe sepsis whatever the site of infection. The clinician may continue corticosteroids in case of non severe urinary infection, spontaneous peritonitis or bacteriemia. For fungal and viral infections, corticosteroids should be interrupted.

Note: there is no formal recommendation to stop or continue the cortocosteroid treatment in case of infection. In this protocol, the continuation of corticosteroids is considered as an option when the infection is related to liver disfunction (e.g. spontaneous peritonitis, bacteriemia), whitch means that the resolution of infection can be improved by an improvement in liver function upon steroids.

The proposal of discontinue corticosteroids for pneumonia, endocarditis ans bone infection is based on the fact that these 3 localizations justify a prolonged antibiotic therapy and that it is difficult to obtain bacteriological documentation in this setting. Moreover, we lack arguments suggesting that an improvement in liver function could contribute to the resolution of the infectious episode.

4.7 Data and Safety Monitoring Board

An organizing committee was set up for this study. It will consist of statisticians from the team of Prof. A. Duhamel, members of the Federation of Clinical Research of the Lille University Hospital and pharmaceutical establishment of CHRU Lille. It will define the general organization and conduct of the trial and coordinate information.
This trial will be supervised by an independent Data and Safety Monitoring Board (DSMB) consisting of five independent members. Their roles, missions, responsibilities and the frequency of their meetings are defined in the DSMB Convention (cf. appendix 1).

V – Pharmacological agents

5.1 Investigational Medicinal Product (IMP)

5.1.1 Description of the IMP being tested

AUGMENTIN 1 g/125 mg pwp susp buv Ad
*Powder for oral suspension adult 1 g/125 mg *: Bags-dose boxes of 8 and 12.*

Indication: **They describe the antibacterial activity and pharmacokinetic properties of this drug. The indications take into account both clinical studies, and the place of this product in the spectrum of currently available antibacterial products .** Augmentin is indicated in infections caused by bacteria known to be sensitive to this drug especially when multiple bacterial species may be responsible and/or resistant to currently available antibiotics. Recommended management will follows the general guidelines for the use of the drug outside the present trial. No dose adjustment is required in patients with creatinine clearance (CrCl) greater than 30 ml / min. In case of renal insufficiency with a clearance of creatinine below 30 ml/min, based on the adaptation of dose from the website www.sitegpr.com (Guide de Prescription et Rein, French reference for the use of drugs in patients with renal failure), the following dose will be used: 1g/125mg/day of amoxicillin/clavulanate, which means 1 dose per day of the experimental drug, instead of 3 doses per day.

Patient's at risk of acute pneumonia patient including chronic alcohol consumption, chronic smoking, patients older than 65 years or with swallowing disorders.

Contraindication:
Absolute:
• Allergy to beta-lactam antibiotics (penicillins, cephalosporins) because of the risk of cross sensitivity to cephalosporins.
• Hypersensitivity to any ingredient in the medication.

• History of liver damage related to the combination of amoxicillin-clavulanic acid.

• Powder for oral suspension (all dosages): phenylketonuria, because of the presence of aspartame (E 951).

In relation to:

• Methotrexate (see Interactions).

Use: Powder for oral suspension in sachets: The contents of sachet is dispersed in half a glass of water before being taken

Adverse reactions: diarrhoea, nausea and vomiting.

5.1.2 Description of the IMP used as comparator

A placebo of the IMP will be manufactured to fulfil recommendations for double blind randomized controlled trials.

5.2 Medicinal non experimental (MNE)

Solupred (Prednisolone) 20 mg orodispersible tablets

Orodispersible tablet 20 mg (white): Box of 20, blisters.

Indications: Histologically proven severe acute alcoholic hepatitis, .

Contraindications:

Any infections excluding specific indications.

Certain progressive viruses (including hepatitis, herpes, chickenpox, shingles).

Psychoses that are not controlled by treatment.

Live vaccines.

Hypersensitivity to any of the ingredients.

Use: Orodispersible tablets: anorodispersible tablet disolves rapidly in the mouth with saliva. Place the orodispersible tablet in the mouth, let it melt, swallow it and drink a glass of water

Adverse reactions:

There is a greater risk of adverse reactions with high doses or with prolonged treatment over several months.

Electrolyte Disorders: hypokalemia, metabolic alkalosis, fluid retention, arterial hypertension, congestive heart failure

Endocrine and Metabolic Disorders: decreased glucose tolerance revealing latent diabetes

Neuropsychiatric disorders: frequent: euphoria, insomnia, excitement; rare: manic episodes, confusion, convulsions (general or intrathecal)

It should be ensured at the time of prescription of antibiotic and corticosteroid compliance including against-indications, warnings and precautions, having a special regard on drug interactions. Refer to the information available on the public database of drugs, accessible via the Internet at the following address: http://base-donnees-publique.medicaments.gouv.fr/

5.3 Description and justification of the route of administration, dose, dosage schedule and treatment duration

Medication will be delivered to patients for 30 days.

5.3.1 Antibiotherapy

⇒ **Pharmaceutical Form**: 1g/125 mg per sachet or placebo supplied by pharmaceutical provider

⇒ **Dose of drug per administration**: amoxicillin+clavulanic acid at a daily dose of 3 grams (amoxicillin) and 375 mg (clavulanic acid) in three daily doses of 1g/125mg. (0 mg for Placebo)

⇒ **Route and method of administration**: oral route. Sachet mixed in approximately 120 mL of plain water and swallowed when sitting or standing position.

⇒ **Timing**: During the 7 days of hospitalization in the clinical center and the 23 day ambulatory period, Augmentin or placebo will be taken in the morning, midday and evening. At home, patients will be instructed to take the treatment in the morning.

⇒ **Duration of treatment**: a total of 30 days

⇒ **Packaging**: sachets

⇒ **Specificity**: This pharmacological agent is administrated to prevent development of infection. It is used outside its AMM (Autorisation de Mise sur le Marché) indication.

5.3.2 Corticosteroid treatment

⇒ **Pharmaceutical Form**: 40 mg per orodispersible tablet supplied by the pharmaceutical provider

⇒ **Dose of drug per administration**: 40 mg/d of prednisolone in a single daily dose in the morning.

⇒ **Route and method of administration**: oral route. Place the orodispersible tablet in your mouth, let it melt, swallow and drink a glass of water

⇒ **Timing**: During the 7 days of hospitalization in the clinical center and the 23 day ambulatory period Prednisolone should be taken at in the morning, At home, patients will be instructed to take the treatment in the morning.

⇒ **Duration of the treatment**: a total of 30 days

⇒ **Packaging**: Blisters

5.4 Description of the dosage and administration of the study drug(s) Description of the unit dose, packaging and labelling of the study drug(s)

Therapeutic units IMP / placebo will be in the form of a box labelled treatment with 4 cases labelled 23 sachets. For the MNE is in a form of case of 30 sachets labelled.

Amoxicillin/clavulanic acid will be labelled in compliance with good manufacturing practice (GMP).

Study drug packaging bear a label with the identification, the protocol number, batch number, storage conditions, drug identification and dosage and the statement “For clinical use Only” and “Keep away from children”

The box treatment is delivered at once with the patient.
The patient will come with box treatment at each follow visit to verify adherence to treatment.

Due to the powder form as an oral solution of experimental drugs (amoxicillin / clavulanic acid - placebo), the pharmacy for internal use of the Lille University Hospital does not have the technical means to achieve a placebo powder for oral solution.

The developer therefore uses an external provider for the design of therapeutic units and their expeditions in all investigational sites of France.

The provider will produce experimental drugs and placebo by subcontracting. It will take delivery of ambient boxes and pack oral suspension into sachet.

Thereafter, pack of experimental drugs (or placebo) will be send by the provider to each pharmacy for internal use of investigational sites. Since then, investigator can prescribe treatment to patient. As pharmacy receives the instruction, it can distribute the treatment.

In the same time, number of batch and treatment will be gathered by pharmacy for internal use and investigator (according the CRF instruction) in order to ensure protection of patients and to observe compliance. Meanwhile pack must be stored between 15 and 25°C.

As the patient takes his treatment, he will keep all sachets (taken and non-taken). During each of the visits during the treatment phase, investigator will get the pack back and keep stickers from sachet.

Then, sent back pack will be gathered by pharmacy for internal use of the investigator site. Finally, the provider will recover those one at pharmacy and destroy them.

<u>For an extension of the expiry date :</u>
The investigational site will labelled each boxes with an extension expiry date label. The old expiry date will be barred. The sachets will not be labelled because they are not mention of expiry date.
This operation will be related on a relabeling procedure.
The provider sent to the pharmacy the labels.

5.5 Drugs and treatments that are permitted and non-permitted in the study, including those required in an emergency

Others medicinal product are allowed (provided that their use they are not a contraindicated medication) and will be recorded in case report forms.

5.6 Methods of monitoring treatment compliance

Treatment will be dispensed to patients by name and boxes will be returned during follow-up visits. Treatment boxes contain enough sachets for 30 days of treatment.

To facilitate compliance to treatment throughout the 30 days of the study the following approaches will be used:

- Subjects will receive a diary.
- Compliance to the drug schedule will be checked during the follow-up visit after the first week of treatment. All participants will be instructed to return any unused drugs to the investigator on the last follow-up visit after treatment.

5.7 Methods of storing the investigational drug(s)

Once treatment units are received, the pharmacist of the participating center must immediately return the form acknowledging receipt after completing and signing the letter (the date the compounds were received should be clearly stated).
The pharmacist makes sure that treatment units are stored as defined on the label, in a locked area. Only the pharmacist and/or a member of his/her staff has access to this room.
Throughout the study, the investigator must keep a record of the number of treatment units supplied to subjects (drug inventory).
The powder for oral suspension in sachet has a shelf life 2 years and has no need special storage.
But to be in conformity with ICH, all Investigational Products will be stored at room temperature between 15 and 25°C and protected from humidity in an appropriate locked area which is managed by the center pharmacist.

VI – Biology

Laboratory tests will be carried out in centers in the usual operation of the management of blood samples from each hospital.
Laboratory tests including complete blood count with platelet count, liver function tests (ALT, AST, GGT, PAL, bilirubin), renal function, and ethanol will be made at follow-up visits, that is, the first month: D7, D14, D21, D30, second month: D 45, D 60, third month : D90 and sixth month : D180.

Laboratory Test	Viral tests
Blood count including platelets (5mL EDTA tube)	
Prothrombin time in seconds, INR (3mL citrate tube)	HBsAg, HBsAb and HBcAb, HCV-Ab (7mL dry tube)
C-reactive protein (5 mL heparin tube pool)	HIV 1 and 2 (7mL dry tube)

Albumin (5 mL heparin tube pool)	
Ionogram (5 mL heparin tube pool)	
Urea, creatinine (5 mL heparin tube pool)	
AST, ALT, GGT, ALP, bilirubin (5 mL heparin tube pool)	
AFP (7 mL dry tube)	

In order to better understand the pathogenesis of alcoholic hepatitis, a biological collection will be done, in order to get new insights on genetics, epigenetics, serum and plasma markers.
All patients must have given their free and informed consent to the AntibiocorHAA protocol. A dedicated paragraph of the written consent will explain the issues and interests of the ancillary collection. This collection will not result in any additional venipuncture. The tubes of biological collection will be taken during venipuncture for the monitoring biological balance detailed in the protocol.
The following tubes will be taken for ancillary collection:

- start of the protocole (D0) : 1 dry tube (7mL), 1 heparin tube (7mL) and 1 ETDA tube (5mL)
- after seven days of treatment (day 7): 1 dry tube (7mL), 1 heparin tube (7mL)
- at the end of treatment (D30): 1 dry tube (7mL), 1 heparin tube (7mL)
- at the end of follow-up (D60): 1 dry tube (7mL), 1 heparin tube (7mL)

A specific square in the consent will allow the patient to give consent to collect cells for the purpose of collecting DNA, regardless of its participation in AntibiocorHAA protocol.

Sample	Purpose of sampling	Delay between sampling and centrifugation	Time and speed of centrifugation	Number of cryotubes
1 dry tube	Serum	Between 30 min and 2 hour at ambient temperature	2500 rpm for 15 min at ambient temperature	2 of 1 ml
1 heparin tube	Plasma	Upon receipt until 2 hour at ambient temperature	2500 rpm for 15 min at ambient temperature	2 of 1 ml
1 EDTA tube	Whole blood	Aliquoting receipt		1 of 1 ml

- Cryovial will be provided by sponsor.
- No centrifugation for EDTA tube. Upon receipt of the tube, aliquot 1 mL of whole blood in a cryovial.
- For serum (dry tube), once the centrifugation, aliquot 1 ml of supernatant in 2 cryotubes.
- For serum (heparin tube), once the centrifugation, aliquot 1 ml in 2 cryotubes. Eliminate the remaining supernatant and gently raise the white ring composed of PBMCs (buffy coat) present above the blood pellet and transfer to a third cryotube.
- Label the cryotubes with a permanent waterproof marker with the sampling date, the day of visit, initials, patient number and type of the collection tube used (dry, EDTA or heparin) as following

AntibiocorHAA
« JJ/MM/AAAA »
« J0 »
Initials « First Name-Last name »
Patient number « _ _ /_ _ _ »
« EDTA »

- Freeze in the box provided by coordinating center. Cryovials should be frozen at -80°C.If the center does not have a feezer at -80°C, cryovials may exceptionally be stored at -20°C
- Complete table of data collection for the serum bank including the sampling dates, freezing and shipment. When sending cryotubes, join the table to the samples

- An annual group shipment will be done for storage before use under the supervision of Laurent Dubuquoy, INSERM Unit 995, Lille, 03.20.97.42.08, mail: Laurent.dubuquoy@inserm.fr

VII – Management of adverse events

7.1 Definition

Adverse event: any untoward event occurring in a subject who is a participant in biomedical research regardless of whether the untoward event is related to the research or the research product.

Adverse reaction: any untoward effect related to research

Severe adverse events or reactions (SAE or SAR): any adverse event or effect that leads to:
- death
- is life threatening to subject participating in the research
- leads to hospitalization or requires longer than expected hospitalization
- leads to a long-lasting or severe handicap
- leads to a birth defect regardless of the administered dose
- any effect that is considered medically severe by the investigator

Unexpected adverse reaction: any adverse effect that differs from the information provided on the nature, the progression and the severity of effects expected by the products, applications and methodologies used during the study.

7.2 Adverse effects and risks related to the protocol

The most commonly reported adverse drug reactions are diarrhea, nausea and vomiting.

Gastrointestinal disorders	
Diarrhea	Very common (1/10)
Nausea	Common (1/100 to <1/10)
Abdominal pain	Common (1/100 to <1/10)
Vomiting	Uncommon (1/1,000 to <1/100)
Indigestion	Uncommon (1/1,000 to <1/100)
Antibiotic-associated colitis	Not known (cannot be estimated from available data)
Black hairy tongue	Not known (cannot be estimated from the available data)
Hepatobiliary disorders	
Increase in AST and/or ALT	Uncommon (1/1,000 to <1/100)
Hepatitis	Not known (cannot be estimated from available data)
Cholestatic jaundice	Not known (cannot be estimated from available data)

Nausea is more often associated with higher oral doses. If gastrointestinal reactions occur, they may be reduced by taking Augmentin at the beginning of a meal.
A moderate increase in AST and/or ALT has been noted in patients treated with beta-lactam class antibiotics, but the significance of these findings is unknown.

7.3 Procedures to collect and report adverse effects

The investigator will inform the sponsor of all SAE occurring during the study within 24 hours by faxing the SAE form to the "Cellule Vigilance de la Fédération de Recherche Clinique" at 03 20 44 57 11.
The Investigator will provide the information on severity, beginning and end dates of each SAE, the relationship to the study drug, actions taken in relation to the study drug, and outcome.

The sponsor will provide the appropriate Regulatory Authorities and the Ethics Committee:

- with all relevant information about serious unexpected adverse events that are suspected to be related to the study drug, that are fatal or life threatening as soon as possible but no later than seven days receiving the information. Relevant follow-up information these cases will be submitted within an additional eight days.
- with information about any other serious unexpected events that are suspected to be related to the study drug as soon as possible, but no more than fifteen days after the investigator learns about these events.

7.4 Final report and publications

The study data belong to the sponsor of the study. Partial and final reports will be written by the study coordinator.

VIII – Access to data

The investigators will provide access to data sources to members of the competent administrative authority for auditing, monitoring or inspection purposes.
Investigators agree to be audited and provide access to study data sources(medical records, computer files, study documents ...). During these visits subject to agreement by the investigator, the following may be audited:
- Compliance with the protocol and associated procedures
- Quality assurance of collected data in case reports: accuracy, missing data, data consistency, control of source documents.

IX – Control and quality assurance

- Trial schedule

A kick-off meeting to begin the study will be organized in each center with the Principal Investigator (PI) and a sponsor representative. The aim of kick-off meeting is to explain the protocol and trial schedule as well as the roles and responsibilities of the investigator and sponsors (CGP, monitoring, safety). The PI will keep the sponsor informed of the enrollment schedule

- Study monitoring

To ensure accurate data collection, laboratory notebooks will be checked regularly by the sponsor's Clinical Research Associate (CRA). During trial site monitoring visits CRAs will have free access to:
- laboratory notebooks with data from patients included in the study;
- medical files and nursing files for these patients;
- the investigator's notes.
This protocol has been assigned to the low risk research category. the goal of monitoring will therefore be to :

- confirm that patients are enrolled and have signed the informed consent form;
- confirm that inclusion criteria are respected;
- confirm the main primary end-point
- follow and report SAE
- identify any unexpected events that may require amendment.
- monitor the management of the pharmacy

- Data collection

To facilitate data analyses, all data collected in laboratory notebooks will be recorded in a computerized data base. To reduce the number of errors, this procedure will be performed twice.

- End of the study

At the end of the trial there will be a closing procedure. Documents and data will be classified. Once the final data analysis has been performed and validated, all of the data will be sealed and archived in a locked room.

X – Ethical and legal considerations

The research will be performed in accordance with the protocol, good clinical practices, and the legislative and regulations in effect.

The investigator is responsible for the progression of the trial.

The investigator agrees:

- To keep data sources as well administrative documents in relation to the protocol,
- Not to include patients before receiving official approval from the Ethic's Committee and the expert authorities,
- To follow the protocol,
- To perform the trial in accordance with moral, regulatory, ethical and scientific principles governing clinical research,
- To obtained the informed, written consent from each participant,
- To report any serious adverse events.

The subjects will receive complete oral and written information explaining the steps of the trial. A letter of information will be given to the subject by the investigator or the doctor representing the investigator before the patient is included in the study. In case of severe hepatic encephalopathy, a person representing the patient can be given information about the study.

An informed consent form (enclosed to the protocol) will be obtained from each patient before they are included in the study.
The informed consent form will be signed by the patient or if necessary another signatory may be added:

- A representative of the patient's guardian
- The family member or representative as defined by the Decree dated March 4, 2002 (for emergency consent or if it is impossible to obtain consent because of the patient's clinical condition).
- The witness (if it is impossible obtain written informed consent, verbal consent is obtained and witnessed)

No specific act of the protocol may begin without first obtaining the signed consent of the patient or his/her representative.
Three copies of the letter of information and the consent form will be drafted and 1 copy will be given to the patient or his/her representative.

- The consent form will be signed by the investigator or the doctor representing the investigator and by the patient or his/her representative.

Registration in the national register for individuals participing in -biomedical research

Any person that accepts to participate in the study will be registered by the investigator, in the national register of individuals participating in biomedical research.

Registration will be performed in accordance with decree dated November 14, 2006 in relation to gathering data in the national register of individuals participating in biomedical research.

Approval from the expert authorities and the opinion of Ethics Committee

The sponsor will submit a request for approval to the competent national authorities a and obtain approval form the Ethics Committee (CCP) before beginning the study, in accordance with article L1121-4 of the Code of Public Healthcare Code.

Modifications to the protocol:

The sponsor is the only person that is authorized to modify the protocol after consulting with the coordinating investigator.

"Substantial modifications" mean modifications that significantly influence any aspect of the trial, in particular the protection of patients participating in the trial, including their safety, the validity of the study, the quality and safety of the tested products, the interpretation of the scientific documents explaining the phases of the study or its .

A request for a substantial modification shall be submitted by the sponsor either to the competent national authority or to the Ethics committee, or both, depending on the case, for approval and/or and opinion. As soon as the approval and/or positive opinion is/are received, an amended version of the protocol will be transmitted to the investigators by the sponsor.

A "non-substantial modification" means minor modifications or clarifications that do not affect the performance of the trial. These modifications are not submitted to the competent expert authorities but do require the agreement of the sponsor and the investigator and will be clearly documented (in the study file).

XI – Data analyses and storage of trail data

Personal data collected during the study will be reported on a laboratory notebook. After, it will be entered into a computerized data analysis system. Data will be confidential, in accordance with the law dated January 6, 1978.

Data will be analyzed in accordance with methodology described in MR 06001 of the CNIL in the Department of Neurology and Vascular Pathology of the CHU Lille.

Access to data will be restricted to individuals who are directly involved in the study. Data may be modified by any physician participating in the study or a fellow working with a physician participating in the study.

Trial data will be archived for at least 15 years after the trial has ended.

XII – Trial funding and insurance

12.1 Funding

Grant pending (Programme Hospitalier de Recherche Clinique)

12.2 Insurance

The sponsor has signed an insurance contract covering his liability and that of all the other participants in the study, in accordance with Article L1121-10 of the Code of Public Health.

XIII – Publication and valorisation

The investigator study coordinator will prepare the final report of the study as required by law, and send to the developer.

In accordance with Article R 5121-13 of the Code of Public Health, the tests may not be subject to any written comments or oral without the joint agreement of the investigator and sponsor. Any publication must mention that the University Hospital of Lille sponsor. In any event, the University Hospital of Lille,

sponsor of the study, the control of the first publication. The investigator shall send a copy of its publications to the proponent.

The sponsor is the exclusive owner of the results of the study. These results, as well as all data relating to the research, should never be given to third parties without previously negotiated by the Delegation for Research counterpart. Any such solicitation must be submitted as soon as possible for Legal Affairs of the Delegation for Research.

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