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Liver transplantation in patients with **C**irr**H**osis and severe **A**cute-on-Chronic Liver Failure (ACLF): i**N**dications and out**C**om**E**s (**CHANCE**) Study on behalf of the **EASL-CLIF Consortium**

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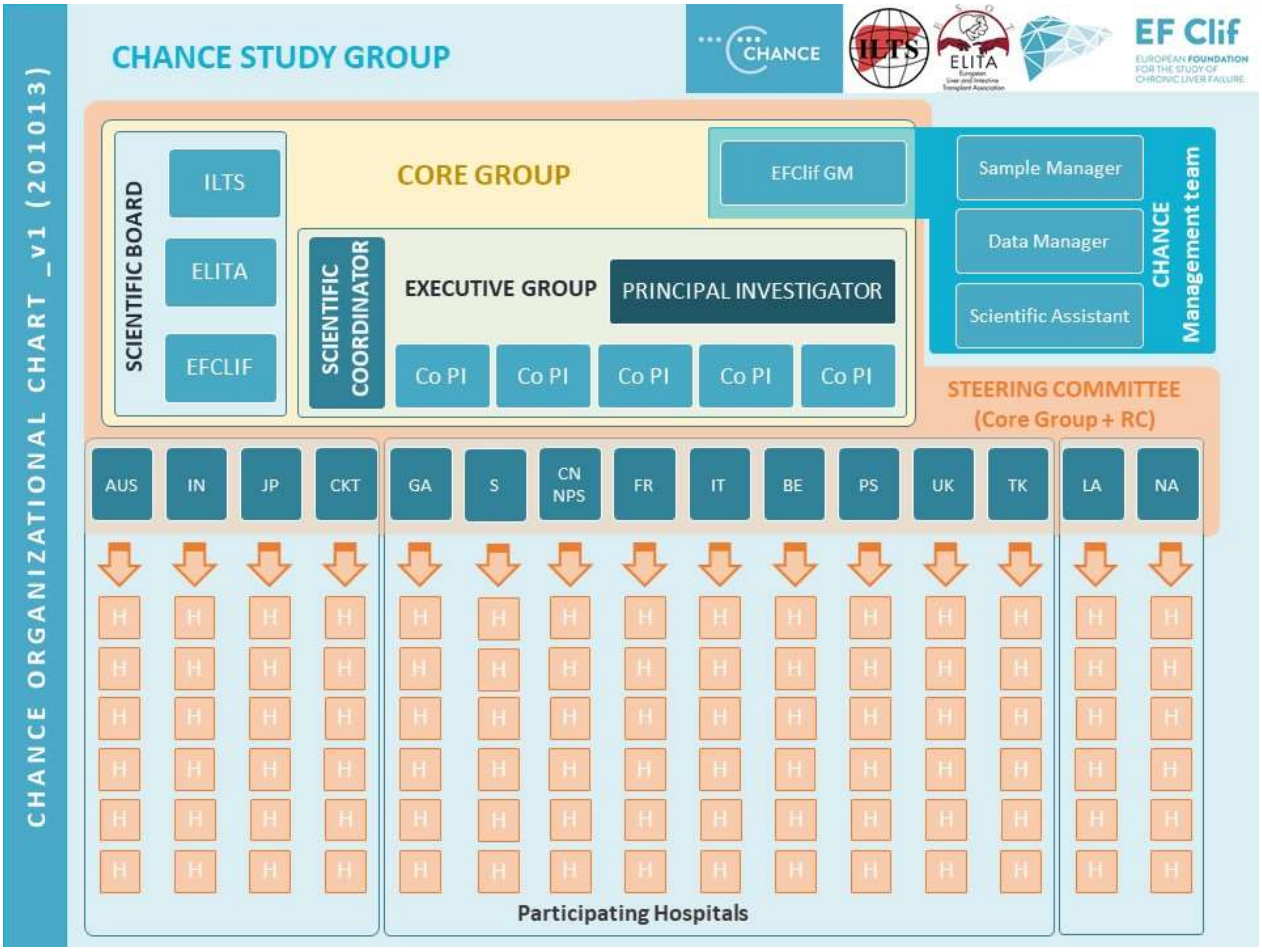

PART I: synopsis

Sponsor	European Foundation for the study of Chronic Liver Failure (EF-Clif)
Title	Liver transplantation in patients with CirrHosis and severe Acute-on-Chronic Liver Failure (ACLF): iNdications and outComEs (CHANCE)
Study centers	Multicenter and international
Primary objective	To compare 1-year graft and patient survival rates after liver transplantation (LT) in patients with ACLF-2 or 3 at the time of LT with patients with decompensated cirrhosis without ACLF-2 or 3 at the time of LT and transplant-free survival of patients with ACLF-2 or 3 not listed for LT.
Secondary objectives	Secondary objectives are as follows: - To assess the proportion of patients with ACLF-2 or 3 referred to transplant team who are listed or not and reasons of this decision. - To evaluate the outcomes of patients listed with ACLF-2 or 3 on the waiting list compared with those of patients listed with decompensated cirrhosis without ACLF-2 or 3. - To define independent predictive factors of death/delisting on the waiting list for patients listed with ACLF-2 or 3 and develop a new prognostic model based on ACLF criteria to predict mortality on the waiting list and to improve the allocation of organs. - To compare the characteristics of accepted grafts for patients listed with ACLF-2 or 3 with those of patients listed with decompensated cirrhosis without ACLF-2 or 3 and their impact on post-LT outcomes. - To explore independent predictive factors of death after LT for patients transplanted with ACLF-2 or 3 to design futility criteria for LT. - To compare post-LT survival rates of patients with ACLF-2 or 3 at listing and patients without ACLF at listing who develop ACLF-2 or 3

	on the waiting list. - To compare post-LT quality of life (QoL) for patients listed with ACLF-2 or 3 with those of patients listed with decompensated cirrhosis without ACLF-2 or 3. - To assess the resources utilization for patients listed with ACLF-2 or 3 (in intention-to-treat and per protocol) compared with patients listed with decompensated cirrhosis without ACLF-2 or 3.
Exploratory objectives	The exploratory objectives are as follows: - To assess the predictive ability of new biomarkers to predict the prognosis on the waiting list and after LT for patients with decompensated cirrhosis with or without ACLF-2 or 3. - To investigate the impact of LT on systemic disturbances (inflammation, leukocyte dysfunction, metabolic alterations) observed in ACLF. - To explore the mechanisms of liver and extrahepatic organ recovery after LT in patients with ACLF-2 or 3 and determinants of this recovery
Study duration	Duration of enrolment period: 2 years Post-LT follow-up: 1 year Total duration: 3 years
Study design	Prospective non-interventional observational study

Study population and number of patients	*Pre-screening group:* all patients with ACLF-2 or 3 referred to the transplant team (unlimited) *Group 1:* patients listed for liver transplantation with ACLF-2 or 3 at the time of listing or developing ACLF 2-3 while on the waiting list (n=2,000) *Group 2:* patients listed for liver transplantation with decompensated cirrhosis without ACLF-2 or 3 and poor liver function (MELD > 20) at the time of listing (n=500) *Group 3:* patients having ACLF-2 or 3, are assessed for inclusion in the waiting list, but are finally not listed for liver transplantation (n=500)
Main eligibility criteria	<u>Inclusion criteria</u> 1. Male or female subject ≥18 years of age. 2. Subjects with diagnosis of liver cirrhosis (based on clinical, laboratory, endoscopic, and ultrasonographic features or on histology). 3. Subjects who have been hospitalized for acute decompensation of liver cirrhosis and referred to the transplant team: - *Group 1:* patients listed for liver transplantation with ACLF-2 or 3 at the time of listing or developing ACLF 2-3 while on the waiting list. - *Group 2:* patients listed for liver transplantation with decompensated cirrhosis without ACLF-2 or 3 and poor liver function (MELD>20) at the time of listing. - *Group 3:* patients having ACLF-2 or 3, are assessed for inclusion in the waiting list, but are finally not listed for liver transplantation. 4. Patients (or trusted person, family member or close relation if the patient is unable to express consent) who have been informed and signed their informed consent

	<u>Exclusion criteria</u> A subject meeting any of the following exclusion criteria is NOT eligible for participation in the study. 1. Acute or subacute liver failure without underlying cirrhosis. 2. Patients with hepatocellular carcinoma outside Milan criteria or other active neoplasia. 3. Subjects listed for transplantation other than liver or liver-kidney transplant. 4. Subjects with previous liver transplantation. 5. Vulnerable population (person under temporary or permanent guardianship or deprived of liberty by a judicial decision). 6. Pregnant and/or breastfeeding woman 7. Patients with relevant comorbidities that could impact the prognosis: o Subjects with very severe hepatopulmonary syndrome (with PaO_2 < 50 mmHg on FiO_2 21%) or moderate to severe portopulmonary hypertension (non-reversible mPAP ≥ 35 mmHg or PVR ≥ 500 dyn.s.cm^{-5}). o Subjects with severe (grade IV) pulmonary disease (Global Obstructive Lung Disease [GOLD]). o Subjects with chronic kidney disease requiring hemodialysis o Subjects with severe heart disease (NYHA class III and IV) o Subjects with a known infection with human immunodeficiency virus (HIV) o Subjects with severe neurological or psychiatric disorders 8. Patients who cannot provide prior informed consent and when there is documented evidence that the patient has no legal surrogate decision maker and it appears unlikely that the patient will regain consciousness or sufficient ability to provide delayed informed consent. 9. Physician and team not committed to provide intensive care if needed.

Statistical methods	Group comparisons will be made using Student's t-test, the Mann-Whitney test, the Chi-squared test, or Fisher's exact test, as appropriate. In order to identify factors associated with the occurrence of death, univariate and multivariate regression analyses will be performed using the Fine and Gray competing risks proportional hazards regression model. All tests will be two-tailed and a p value of less than 0.05 will be considered to be statistically significant.

PART II: study description

I. Background

1. The concept of Acute-on-Chronic Liver Failure (ACLF) and definition

Patients with acute decompensation of cirrhosis are a heterogeneous clinical group associated with different prognoses which need to be stratified to define appropriate management. The term "Acute-on-Chronic Liver Failure (ACLF)" was first introduced to characterize a subgroup of patients with chronic liver diseases which develop organ failure(s) leading to very poor short-term outcome [1]. A large, multicenter prospective, observational study (CANONIC study performed by the EASL-CLIF Consortium) in 1343 patients with cirrhosis who were hospitalized for an acute decompensation (AD) of cirrhosis (large ascites, hepatic encephalopathy, gastrointestinal hemorrhage and/or bacterial infection), provided the first evidence-based diagnostic criteria that permitted physicians to distinguish between Acute-on-Chronic Liver Failure (ACLF) and 'mere' AD (i.e., traditional AD) [2]. In this study, 28-day and 90-day mortality rates were higher among patients who had ACLF at enrolment than among those who had traditional AD (34% and 51% vs. 5% and 14%, respectively). We developed several tools for estimating outcomes of patients with ACLF. The baseline grade of ACLF, the clinical course of the syndrome, and a specific score (CLIF-Consortium ACLF score [CLIF-C ACLFs]) can accurately predict the outcomes. The initial grade of ACLF is defined according the number of organ failures (OFs) and the presence of kidney and/or neurological dysfunction (see Appendix A). The 28-day and 90-day mortality rates are 22% and 41% for ACLF grade 1 (ACLF-1), 32% and 52% for ACLF-2, and 77% and 79% for ACLF-3, respectively.
The current accepted strategy is to treat the underlying precipitating factor of ACLF: (antibiotics for bacterial infections, corticosteroids for severe alcoholic hepatitis and nucleot(s)ide analogs for HBV flare). The management of ACLF *per se* is mainly supportive with intensive monitoring and supports of failing organs. Clinical deterioration despite maximal supportive management is associated with very poor outcomes and leads physicians to consider potential salvage liver transplantation (LT) [3,4].

2. The results of liver transplantation for patients with ACLF

This option remains highly controversial. LT in sicker recipients is unquestionably associated with an improved survival benefit but could result in less acceptable longer term survival rates

after LT. Due to the scarcity of deceased liver donors, we need a strategy of rationing where the success of deceased-donor LT (DDLT) will be maximized [5]. Alternative strategies to increase the donor pool (living-donor liver transplantation [LDLT], marginal livers, ABO incompatible donation) should be explored for patients with ACLF. LDLT is now an option with impressive results in expert Eastern centers including India. However, in Western countries, this option is restrained due to distressing experiences with severe and life-threatening complications. Moreover, the need of urgent LDLT in the case of ACLF reduces the time of the clinical donor's assessment and increases the pressure on the donor resulting in a potential coercion.

Experiences with DDLT in ACLF patients from European centers are increasingly being published. In CANONIC, DDLT of ACLF patients (38% had ACLF-3) was associated with an acceptable 1-year post-LT survival of 75% [3]. A recent retrospective study from three French liver centers reported that 73 patients with ACLF-3 received DDLT with an outstanding 1-year post-LT survival of 84%, suggesting that ACLF-3 *per se* should not be viewed as a contraindication for LT [6]. These results were confirmed in United States. Indeed, a study based on United Network Organ Sharing (UNOS) registry reported a 1-year post-LT survival of 81.8% for 6381 transplanted patients with ACLF-3 [7]. Several reports from Eastern countries have shown similar outcomes of ACLF patients receiving LDLT compared to those with DDLT [8,9]. On the other hand, several studies reported less acceptable results. In a small single center experience, 13 ACLF-3 patients (all supported by mechanical ventilation and vasopressors, and 77% by renal replacement therapy), were transplanted with a median MELDs of 38 and authors reported a 1-year survival post-LT rate of 46% [10]. Another study reported a 1-year survival post-LT rate of 43% for 30 patients with pre-LT ACLF-3 [11]. These post-LT survival rates seem to be inacceptable in the light of scarcity of grafts.

3. Areas of uncertainties

Waiting list outcomes and organ allocation for patients with severe ACLF

Patients listed with ACLF-3 had a high probability to die on the waiting list (WL) or be removed from the WL. Based on the UNOS registry, 32.7% of patients listed with ACLF-3 died or were removed from the WL at 21 days after the listing [12]. A significant proportion of patients with ACLF-3 or 2 were misclassified by the MELD-Na. This subgroup of patients had the highest rate of death or removal from the WL [7]. Due to the absence of prioritization, patients with ACLF-3 died or were removed from the WL in a higher frequency than patients with acute liver failure (status 1a in US). The objective nature of MELDs and the MELD-Na in the allocation system has largely improved outcomes of patients on the waiting list [13,14]. On the other hand, MELD

score does not take into account cerebral, circulatory, and pulmonary failures, giving low specific priority for ACLF patients. The specific scores for ACLF (CLIF-C ACLF score and AARC scores) are more accurate for prediction of short-term outcomes than MELDs. Then, we must define predictive factors of death or removal from the WL for patients with severe ACLF based on prospective data to try to design better rule for organ allocation for this group of patients.

Objective limits for LT in patients with severe ACLF

In the context of scarcity of liver donors, the potential benefit of LT in ACLF patients must also be balanced against the need for rationing of limited resources [5]. Classically, experts agree that patients should be offered LT if there is a more than 50% expectancy of 5-year survival post-LT and an acceptable QoL [15,16]. Currently, limited data about the results of LT in the sickest patients with cirrhosis are available and experiences with adverse outcomes are frequently underreported in the literature. All reports with inadequate results are retrospective and monocentric [10,11]. Some authors suggested some limit criteria (active gastrointestinal bleeding, control of sepsis for less than 24 h, hemodynamic instability requiring dose of norepinephrine > 50 µg/min, lung failure defined as a PaO_2/FiO_2 ratio < 150) but these criteria are not prospectively validated [6]. In another retrospective study, authors reported that the presence of more than two criteria (age ≥ 53 years, pre-LT arterial lactate ≥ 4 mmol/L, mechanical ventilation with PaO_2/FiO_2 ratio ≤ 200 and pre-LT leucocyte ≤ 10 G/L) are associated with only 8.3% of 1-year post-LT survival rate [17]. Active infections are recognized as contraindications for LT. Several studies have reported that patients with a fully-recovered episode of bacterial infection had similar post-LT survival rates than uninfected patients despite longer post-LT hospital stays [18,19]. This observation was also made for controlled infections but a standardized definition of controlled infections is lacking [6,20]. Prospective data from large multicenter international studies are urgently needed, aiming to resolve objectively this controversial ethical issue that results either in wasting scarce organ resources or in precluding severely sick patient's access to life-saving treatment.

Timing of LT for severe ACLF patients to improve the LT results

Another challenge for LT in the setting of ACLF is definition of the ideal timing for this option. Indeed, optimization of clinical condition by resolution or improvement of organ/system failures before LT seems to be associated with a significantly better post-LT outcome. In the CANONIC experience, liver transplanted patients with resolution of ACLF had a 1-year post-LT survival of 90% compared to 75% for those with a remaining ACLF at the time of LT [3]. Moreover, the

impressive post-LT results for ACLF-3 patients in the French study could also be due to the clinical improvement observed between ICU admission and LT [6]. On the other hand, in the case of clinical deterioration, a prompt decision for LT must be made but limits, as discussed before, defining when the patient should be considered to be too sick to be transplanted and LT as futile are currently largely unknown. The concept of a timing window for patients with severe ACLF should be is suggested addressed in the future through large studies.

The characteristics of donor organs impacting the LT outcomes for patients with severe ACLF

Alternative strategies to increase the donor pool (living-donor liver transplantation [LDLT], marginal livers, extracorporeal organ perfusion, ABO incompatible donation) should be explored for patients with ACLF. LDLT is now an option with impressive results in expert Eastern centers including India. Indeed, several reports from Eastern countries have shown similar outcomes of ACLF patients receiving LDLT compared to those with DDLT [8,9]. However, in Western countries, this option is restrained due to distressing experiences with severe and life-threatening complications. Moreover, the need of urgent LDLT in the case of ACLF reduces the time of the clinical donor's assessment and increases the pressure on the donor resulting in a potential coercion. Extracorporeal liver perfusion (normothermic or others) is now used in some centers to expand the donor pool and reduce early allograft dysfunction. Then, we must assess prospectively the impact of the characteristics of donor organ on LT outcomes in the case of patients with severe ACLF.

The post-LT outcomes and potential complications in patients with severe ACLF

In a French study, post-LT complications were more frequently reported in patients transplanted for ACLF-3 [6]. Transplant patients with ACLF-3 had a particularly high rate of neurological, pulmonary, renal and infectious complications. In this study, the rejection rate of patients with ACLF-3 did not seem to be increased in the first year after LT. It is essential to describe in detail post-LT complications (infections, rejection, primary poor function or non-function, neurotoxicity, nephrotoxicity) in patients transplanted with severe ACLF in a large prospective study.

The post-LT quality of life (QoL) for patients with severe ACLF

The goal of LT is not only to ensure the patient's survival, but also to offer an adequate QoL, ideally in the same state of health as before the onset of liver disease. Unfortunately, the

measurement of QoL in the overall LT recipient population, particularly in patients with pre-LT ACLF, has not been rigorously studied. For example, about 70% of transplanted patients develop stage 3-4 chronic kidney disease, with a cumulative risk of End-Stage Renal Disease (ESRD) requiring chronic hemodialysis and/or renal transplantation within the first 10 years after LT ranging between 3% to 9% [21]. Pre-LT renal impairment, frequently observed in patients with ACLF, is the main predictor of post-LT chronic kidney disease. Therefore, the incidence of post-LT ESRD in recipients with pre-LT ACLF is currently unknown and could greatly affect post-LT QoL. Thus, the development of a large international database collecting information on long-term survival and QoL after LT should be built, not only to confirm that a severe course of ACLF is a reasonable indicator for LT, but also to define strict selection criteria that support a good post-LT QoL.

The resources utilization for LT in case of patients with severe ACLF

Liver transplantation is a life-saving procedure but is associated with a significant utilization of resources. The care of patients with ACLF (ICU management, post-LT rehabilitation) is also expensive. In a French study, patients transplanted with an ACLF had longer length of stay in the ICU and the hospital compared to those transplanted without ACLF [6]. Moreover, the occurrence of post-LT complications was also significantly increased. Cost-effectiveness analyses that consider the health benefits are scarce in the setting of LT. Therefore, it's essential to determine the resources utilization for LT for severe ACLF patients to potentially justify this strategy.

II. Aims and goals of the study

1. Primary objective: Survival results of LT in patients with severe ACLF

To compare 1-year graft and patient survival rates after LT in patients with ACLF-2 or 3 at the time of LT with patients with decompensated cirrhosis without ACLF 2-3 and transplant-free survival of patients with ACLF-2 or 3 not listed for LT.

2. Secondary objectives

1) To define the factors in decision-making process for listing patients with severe ACLF; in particular to assess the proportion of patients with ACLF-2 or 3 referred to transplant team who are listed or not and reasons of not listing.
2) To analyze the clinical course of patients listed with ACLF-2 or 3 on the waiting list compared with those of patients listed with decompensated cirrhosis without ACLF-2 or 3.
3) To define independent predictive factors of death/delisting on the waiting list for patients listed with ACLF-2 or 3 and develop a new prognostic model based on ACLF criteria to predict mortality on the waiting list and to improve the allocation of organs.
4) To compare the characteristics of accepted grafts for patients listed with ACLF-2 or 3 with those of patients listed with decompensated cirrhosis without ACLF-2 or 3 and their impact on post-LT outcomes.
5) To explore independent predictive factors of death after LT for patients transplanted with ACLF-2 or 3 to design futility criteria for LT.
6) To compare post-LT survival rates of patients with ACLF-2 or 3 at listing and patients without ACLF at listing who develop ACLF-2 or 3 on the waiting list.
7) To compare post-LT quality of life (QoL) for patients listed with ACLF-2 or 3 with those of patients listed with decompensated cirrhosis without ACLF-2 or 3.
8) To assess the resources utilization of care and LT procedure in patients listed with ACLF-2 or 3 (in intention-to-treat and per protocol) compared with patients listed with decompensated cirrhosis without ACLF-2 or 3.

3. Exploratory objectives

1) To develop new biomarkers to predict the prognosis on the waiting list and after LT for patients with decompensated cirrhosis with or without ACLF-2 or 3.
2) To investigate the impact of LT on systemic disturbances (inflammation, leukocyte dysfunction, metabolic alterations) observed in ACLF.
3) To explore the mechanisms of liver and extrahepatic organ recovery after LT in patients with ACLF-2 or 3 and determinants of this recovery.

III. Logistical aspects of the study

1. Financial Support

This study is supported by EF-Clif. EF-Clif will cover all logistics issues related to the project: electronic CRF; Data Management Center; shipment of biological samples to a central repository biobank, biobanking expenses and investigator's meeting. There is a modest financial aid to be distributed among centers for collecting samples. Therefore, only centers with sufficient research facilities and dedicated staff will be admitted. Centers without research facilities are discouraged to apply for inclusion into the study. A careful monitoring of screening failures and patient inclusion during the first months of the study will guarantee satisfactory protocol execution.

2. Pre-screening of patients

Every consecutive patient with ACLF-2 or 3 referred to the Liver Transplant team (i.e. at the admission in the Liver Transplant Center) during the study period must be pre-screened. This pre-screen log will collect only basic clinical anonymized data (age, sex, etiology of cirrhosis, organ failures and grade of ACLF, the site of admission [ICU vs. ward]). This point is crucial to capture the burden of ACLF-2 or 3 and the LT strategy of each center for patients with ACLF-2 or 3. The pre-screening period extends from the moment of initiation of the pre-LT assessment to the time when a decision of listing or not is made (date of screening for inclusion). Death occurring before inclusion will be registered. The reason of non-inclusion will be also registered.

3. Screening and inclusion of patients

The screening and inclusion of consecutive eligible patients are crucial to meet the goals of the study. Therefore, critical points are the screening of patients referred to transplant center for ACLF-2 or 3 (G*roup 1* and *3*) or decompensated cirrhosis with MELD > 20 at the time of listing (*Group 2*), as well as inclusion of all patients without exclusion criteria. This dataset will include data on the current clinical status and on the health trajectory prior to the referral. Then, this screening visit collects clinical and laboratory data and a predefined questionnaire (Q) will interrogate potential precipitating and predisposing factors from the medical trajectory and

patient history. The inclusion visit will be the date of listing for *Groups 1 and 2* and the date of denial of listing in *Group 3*. Date of ICU admission will be also recorded.

4. Study Group, Control Group and Follow up

The study will collect data and samples from the whole cohort of consecutive patients who satisfy the inclusion criteria and do not present any of the exclusion criteria at study inclusion. Patients with decompensated cirrhosis and MELD > 20 without ACLF-2 or 3 (*Group 2*) at listing (study inclusion) will serve as controls. The study design is resumed in Figure 1.
The inclusion visit will collect clinical and laboratory data (D) and biological samples (S).

Group 1: This group will include all patients with ACLF-2 or 3 who are listed for liver transplantation (LT). Each visit (day 3 [D3], day 7 [D7)], day 14 [D14], day 21 [D21], day 28 [28] and then every month [M2, M3…]) will collect D until liver transplantation and visit D7 will collect S. An extra visit with D/S will be performed the day of delisting if the patient is definitively delisted. After delisting, the patient will follow the visit schedule until M12.

Group 2: This group will include all patients with decompensated cirrhosis and MELD > 20 without ACLF-2 or 3 who are listed for liver transplantation (LT). Each visit (day 28 [D28] and then every month [M2, M3…]) will collect D until liver transplantation. An extra visit with D/S will be performed the day of delisting if the patient is definitively delisted. After delisting, the patient will follow the visit schedule until M12. An extra visit with D/S will be performed if the patient develops ACLF 2-3 while listed. Thereafter, schedule visits will follow those described for *Group 1* (day 3 [D3], day 7 [D7)], day 14 [D14], day 21 [D21], day 28 [28] and then every month [M2, M3…]) until LT.

The day of liver transplantation: D/S of the recipient will be collected the day of LT before the procedure and numerous clinical data will be collected on the donor and graft characteristics and the surgery procedure.

After liver transplantation: Each visit (day 1 [D1], day 3 [D3], day 7 [D7]), month 1 [M1], month 3 [M3], will collect D and visits D3, D7, M1, M3 will collect S. Visits at month 6 [M6] and month 12 [M12] will collect D. Dates of ICU and hospital discharge will be recorded.

Group 3: This group will include the 500 first patients with ACLF-2 or 3 admitted to hospital that are excluded for liver transplantation (LT). Reason(s) for LT denial should be recorded. Each visit (day 3 [D3], day 7 [D7)], day 14 [D14], day 21 [D21], day 28 [28], month 3 [M3], month 6 [M6] and month 12 [M12] will collect D and visits D7 and M3 will collect S.

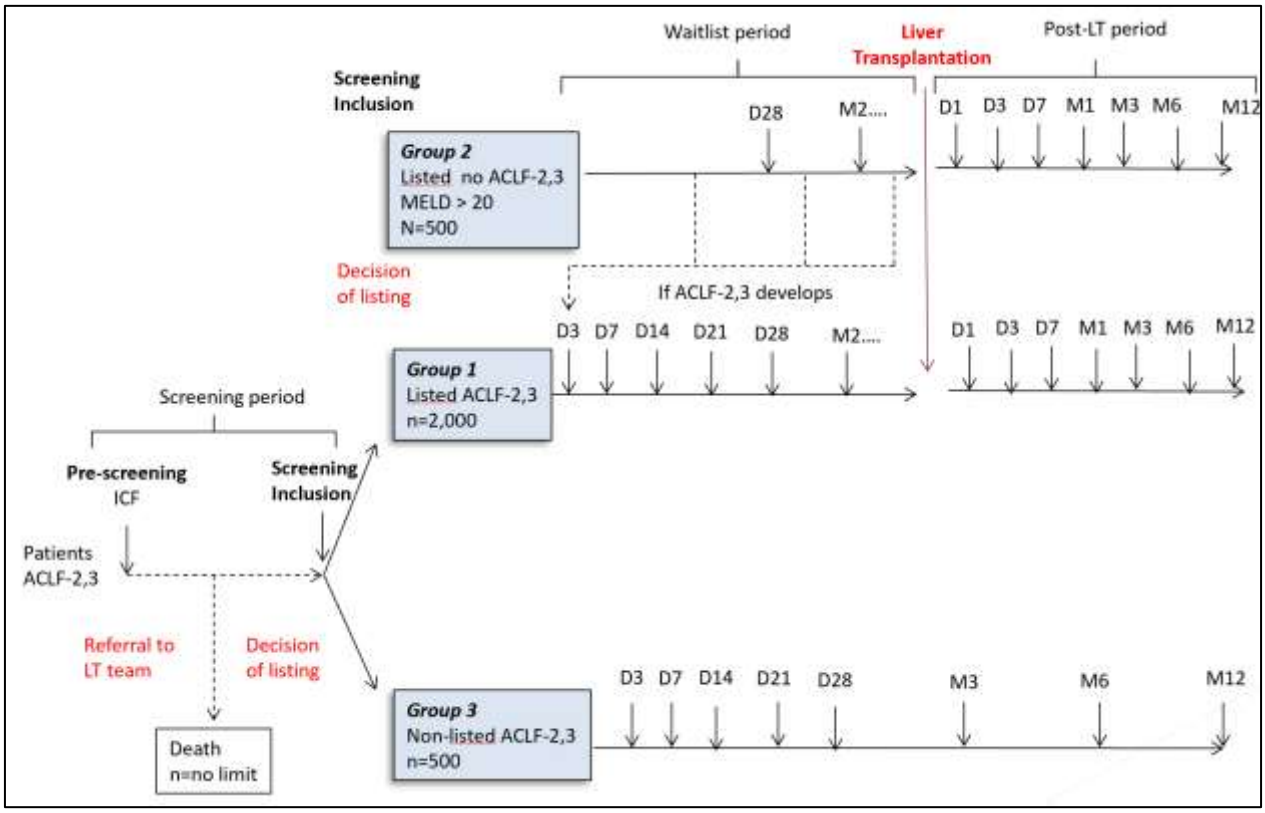

Figure 1. Design of the CHANCE study. ICF, informed consent form; LT, liver transplantation; D, day; M, month.

IV. Inclusion and exclusion criteria

Inclusion criteria

1. Male or female subject ≥18 years of age.

2. Subjects with diagnosis of liver cirrhosis (based on classical clinical, laboratory, endoscopic, and ultrasonographic features or on histology).

3. Subjects who have been hospitalized for acute decompensation of liver cirrhosis and referred to the transplant team:
   - *Group 1:* patients listed for liver transplantation with ACLF-2 or 3 at the time of listing or developing ACLF 2-3 while on the waiting list.
   - *Group 2:* patients listed for liver transplantation with decompensated cirrhosis without ACLF-2 or 3 and poor liver function (MELD>20) at the time of listing.
   - *Group 3:* patients having ACLF-2 or 3 are assessed for inclusion in the waiting list, but are finally not listed for liver transplantation.

4. Patients (or trusted person, family member or close relation if the patient is unable to express consent) who have been informed and signed their informed consent.

Exclusion criteria

A subject meeting any of the following exclusion criteria is NOT eligible for participation in the study.

1. Acute or subacute liver failure without underlying cirrhosis.
2. Patients with hepatocellular carcinoma outside Milan criteria or other active neoplasia.
3. Subjects listed for transplantation other than liver or liver-kidney transplant.
4. Subjects with previous liver transplantation.

5. Vulnerable population (person under temporary or permanent guardianship or deprived of liberty by a judicial decision).

6. Pregnant and/or breastfeeding woman

7. Patients with relevant known comorbidities that could impact the prognosis:

    a. Subjects with very severe hepatopulmonary syndrome (with PaO_2 < 50 mmHg on FiO_2 21%) or moderate to severe portopulmonary hypertension (non-reversible mPAP ≥ 35 mmHg or PVR ≥ 500 dyn.s.cm^{-5} or 6.25 WU).

    b. Subjects with severe (grade IV) pulmonary disease (Global Obstructive Lung Disease [GOLD]).

    c. Subjects with chronic kidney disease requiring hemodialysis

    d. Subjects with severe heart disease (NYHA class III and IV)

    e. Subjects with a known infection with human immunodeficiency virus (HIV)

    f. Subjects with severe neurological or psychiatric disorders

8. Patients who cannot provide prior informed consent and when there is documented evidence that the patient has no legal surrogate decision maker and it appears unlikely that the patient will regain consciousness or sufficient ability to provide delayed informed consent.

9. Physician and team not committed to provide intensive care if needed.

All patients meeting the inclusion criteria will be entered in a screening log. If the patient is not enrolled, the screening log will include information explaining why enrolment did not occur.

V. Primary and secondary endpoints

Primary endpoint

1-year graft and patient survival rates after liver transplantation

Secondary endpoints

Pre-Liver transplantation

Waiting time (from the listing to LT, days)

Mortality rate on the waiting list

Delisting rate on the waiting list

Clinical course (resolution/improvement/stabilization/worsening) of ACLF on the waiting list (evolution of grades, number of organ failures)

Incidence of ACLF development in patients listed without ACLF

Resources utilization on the waiting list

Post-Liver transplantation

3-month and 6-month graft and patient survival rates after liver transplantation

Hospital length of stay after LT (days)

ICU length of stay after LT (days)

3-month and 1-year health-related quality of life (HRQoL) after LT (by Chronic Liver Disease Questionnaire [CLDQ] and EQ-5D)

3-month, 6-month and 1-year rate of dependency of renal replacement therapy or kidney transplant

Resources utilization of LT and post-LT follow-up

VI. Data collection

Study Coordinators at each site will visit each intensive care department and liver transplant wards daily to identify potential candidates for enrollment. Permission to approach patients/families will be requested from attending physicians. All consecutive patients with ACLF-2 or 3 will be entered on a pre-screening log. When a decision of registration or not on the waiting list for LT will be made, all patients meeting the inclusion criteria will be entered on a screening log. If the patient is not enrolled, the screening log will include information explaining why enrollment did not occur (exclusion criteria or other).

Data collection (Q/D) will be performed via electronic Case Report Form of the study, issued in a separate document. In general the data acquisition will include information on:

- **Health trajectory (Q):** Demographics, previous history of decompensation, precipitating factors, medication, comorbidities including the use of predefined questionnaire **(Q)**
- **Clinical and laboratory data (D):** Important laboratory values and clinical features in order to calculate major scores including nutritional scores and HRQoL.

Besides crucial clinical data, the other major goal of the study is to collect **biological samples (S)** in this critical pre, peri and post-LT period. The following materials are required: peripheral serum, plasma (EDTA, citrate and Li-heparin), whole blood in Tempus tube (RNA), buffy coat (DNA), PBMC (only in designated centers), urine, liver and saliva. The list of samples to be collected can be found in the chapter IX of this protocol. The **Table 1** resumes the data and sample collections.

Data and sample collection

	Pre-screening	Screening	Inclusion	Day 3 D3	Day 7 D7	Day 14 D14	Day 21 D21	Day 28 D28	Month 2-x M2-x (#)	LT	Day 1 D1	Day 3 D3	Day 7 D7	Month 1 M1	Month 3 M3	Month 6 M6	Month 12 M12
				Pre-LT follow-up							Post-LT follow-up						
Group1	B	Q/D	D/S	D	D/S	D	D	D	D	D/S	D/S	D/S	D/S	D/S	D/S	D	D
Group2 (&)	B	Q/D	D/S					D	D	D/S	D/S	D/S	D/S	D/S	D/S	D	D
Group3	B	Q/D	D/S	D	D/S	D	D	D	D/S*								

Group 1: patients listed with ACLF-2 or 3
Group 2: patients listed with decompensated cirrhosis and MELD>20 without ACLF-2 or 3
Group 3: patients not listed with ACLF-2 or 3
Not included: patients not listed with ACLF-2 or 3 not admitted to ICU or admitted to ICU when the recruitment of Group 3 is finished (>500)

B: basic clinical data (etiology of cirrhosis, organ failures)
Q: questionnaire about medical trajectory and patient history
D: clinical and laboratory data
S: biological samples
a visit will be performed each month until LT for Group 1 and 2 & if ACLF-2 or 3 occures, the scheldule of visits will become similar to Group 1
* Data will be mandatory at month 3, 6 and 12. Sample at month 3

Table 1. Data and samples collection of the CHANCE study

VII. Statistical analysis

1. Study size

Those patients who satisfy the inclusion criteria and do not present any of the exclusion criteria will be enrolled for the study.

In the UNOS registry, around 30% of patients listed for LT with ACLF-3 were delisted or died on the waiting list [7]. Moreover, about 20% of patients listed with ACLF-3 experienced an improvement of their grade of ACLF (from 3 to 0-2) on the waiting list [22]. In retrospective studies, the 1-year post-LT mortality for patients with ACLF-3 was about 20% [6].

Based on these data, the CHANCE study will enroll 2,000 listed patients with ACLF-2 or 3 (*Group 1*). Consequently, we expect about 600 patients who will be delisted or die on the waiting list and about 280 patients who will improve their ACLF grade to 0-1. Therefore, we estimate that 1,120 patients will receive a liver transplant and 220 among them will die during the 1-year follow-up after LT. This sample sizes will allow us to comprehensively assess factors predictive of wait-list death and death after transplantation.

Patients from *Group 3* (n=500, patients with ACLF-2 or 3 not listed for LT) will serve as controls to assess the natural history of the syndrome despite organ supports and quantify the survival benefit of LT in ACLF 2 or 3 (comparison *Group 1* vs. *Group 3*).

Patients from *Group 2* (n=500, patients with decompensated cirrhosis and MELD > 20 without ACLF-2 or 3 listed for LT) will serve as other controls to quantify the impact of severe ACLF on the wait-list outcomes (comparison *Group 1* vs. *Group 2*). Some patients in the *Group 2* will develop ACLF-2 or 3 on the waiting list and then they will be shift to the follow-up of the *Group 1*. Based on UNOS registry, we could estimate the probability of this shift around 10% [22]. The primary objective of the CHANCE study is to compare 1-year survival rates after LT in patients with ACLF-2 or 3 at the time of LT with patients with decompensated cirrhosis without ACLF at the time of LT. Therefore, we could estimate that among included patients in different groups, 1,220 patients will have ACLF-2 or 3 at the time of LT and 680 patients will have no ACLF or ACLF-1 at the time of LT. This sample sizes will allow us to comprehensively assess the impact of severe ACLF on mortality after LT.

To ensure a maximum of 30% for LDLT in the CHANCE study, patients with ACLF-2 or 3 with a plan of LDLT will be not included in the study when the number of LDLT inside the study for

Group 1 will reach 300. Similarly, patients without ACLF-2 or 3 and MELD > 20 with a plan of LDLT will be not included in the study when the number of LDLT inside the study for *Group 2* will reach 150.

To ensure equilibrium of transplanted patients with ACLF-2 at the time of LT and those with ACLF-3 (maximum 50 vs. 50%), patients with ACLF-2 at the time of listing will be not included in the study when the number of transplanted patients with ACLF-2 will reach 600.

2. Descriptive analyses

A descriptive analysis will be carried out for all the information recorded at study inclusion visit (general data, precipitating events, admission-related data [type, place, cause], clinical data, laboratory data, scores, treatments), for changes from baseline in clinical, lab and score variables at each study time-point and for main follow-up data (admission to ICU, discharge, patient's evolution, patients' survival rates. These descriptive analyses may also be stratified by specific sub-groups of patients (etiology, precipitating factors, etc…). Categorical variables will be summarized by means of observed frequencies and percentages. Ordinal variables or continuous variables not normally-distributed will be summarized by the median of the distribution and ranges of values (quartiles or minimum and maximum values). For all continuous variables the mean, Standard Deviation and 95% Confidence Intervals will be used. Kaplan-Meier survival curves will be used to describe the time-to-event in specific groups of patients.

3. Analysis of the main objectives of the study

Primary objective: Survival results of LT in patients with severe ACLF

We will compare the 1-year graft and patient survival rates after LT in patients with ACLF-2 or 3 at the time of LT with patients with decompensated cirrhosis without ACLF-2 or 3 at the time of LT and transplant-free survival of patients with ACLF-2 or 3 not listed for LT using Kaplan-Meier survival curves and Log-rank tests. The cumulated incidence functions (CIFs) will be estimated by Fine-Gray methods and a Fine-Gray survival model for competing-risks will be used to assess risk factors and select the best subset of predictors.

Main secondary objectives:

Group comparisons will be made using Student's t-test, the Mann-Whitney test, the Chi-squared test, or Fisher's exact test, as appropriate. In order to identify factors associated with the occurrence of death, univariate and multivariate regression analyses will be performed using CIFs and Fine-Gray survival model for competing-risks. All changes from baseline clinical and QoL parameters will be also analysed by means of ANOVA models adjusted by those patients's characteristics showing significant imbalances between study groups. All tests will be two-tailed and a p value of less than 0.05 will be considered to be statistically significant.

VIII. Data management

Data management will be carried out and coordinated by the EF-CLIF Data Management Centre. The online data entry system developed for this study will allow the detection of incorrect or missing data in the database and the notification of the corresponding queries to study centers. Study investigators will be requested to carry out the indicated data corrections and modifications through the online application and all changes will be tracked by the system.

All queries arising during the data management process will be communicated by email (and phone contact, if required) to the investigator(s) in charge of the study at each site, who will solve them by correcting the entered data through the online application. Patients' personal data (name, address, phone number, etc.) will not be collected and in no case will be available to any investigator participating in the study, except for the main investigator at each site. Patients will be identified in all study databases by means of a unique code, which will also be used to identify all the samples collected for a specific patient. A listing including the correspondence between this study ID code and each patient's personal data will be kept by the site PI in a secure and safe place and not shared with anyone else. It will be used only if the investigator needs to retrieve a specific patient's personal data for the retirement of an informed consent or for important reasons involving that patient's health.

IX. Sample collection

1. Sample collection overview

The exploratory objectives of the CHANCE study Ancillary studies aimed to develop new biomarkers to predict the prognosis on the waiting list and after LT for patients with decompensated cirrhosis with or without ACLF-2 or 3, to investigate the impact of LT on systemic disturbances (inflammation, leukocyte dysfunction, metabolic alterations, dysbiosis) observed in ACLF and to explore the mechanisms of liver and extrahepatic organ recovery after LT in patients with ACLF-2 or 3 and determinants of this recovery. Proper collection of biological samples is critical for this process. The following samples will be systematically collected: serum, plasma (EDTA, citrate and Li-heparin), whole blood in Tempus tube (RNA), buffy coat (DNA), urine, liver and saliva. Also, in some designated centers, peripheral blood mononuclear cells (PBMCs) will be isolated.

All centers will receive a package containing the necessary tubes and the processing instructions (separation, aliquoting, freezing, registration and shipment). Detailed SOPs are specified in Appendix 2.

Samples of all patients will be kept on-site at -80º C and then will be sent at the end of the recruitment phase to the Coordinating Center in each of the geographical areas. Once all the samples are centralized in each of the Coordinating Centers they will be shipped to the EFClif Biorepository located in Biomat SA, Barcelona, Spain (Benjamín Santos, Senior Quality Quality Control, Email: archivodemuestrasbiologicas@grifols.com) for centralized storage (see more details in Biobanking information). EFClif has signed an agreement with Biomat SA in order to store the CHANCE Collection in their premises.

2. Blood, circulating cells and fluids

Serum (SOP-1): Collect venous blood in 1 SST tube (5 ml). Tube should be inverted five times, allowed 30 minutes clotting time at room temperature, and centrifuged* for 10 minutes at 1000-1300 RCF (g) in a swing bucket centrifuge. Transfer the collected serum into Wilmut plates in aliquots of 500 µl and store at -80ºC.

**Tubes should be centrifuged no longer than 2 hours after collection.*

Plasma (SOP-3, SOP-4, SOP-9): Collect venous blood in 3 EDTA (4 mL each), 2 citrate (4.5 mL each) and 1 Li-Heparin (4 ml each) tubes. Tubes should be centrifuged for 10 minutes at room temperature at 1000-1300 RCF (g) in a swing bucket centrifuge. Transfer the supernatant into Wilmut plates (aliquots of 500 µl) and store at -80ºC. Keep the EDTA tubes for PBMC Isolation.

DNA samples (SOP-2, SOP-3): Collect the buffy coats (the white thin cell fraction (~0.5 ml) just above de red cell cushion using a Pasteur plastic pipette) from EDTA and citrate tubes, transfer to Wilmut plates and store at -80ºC.

RNA samples (SOP-5): Collect ~3 mL of blood directly into Tempus™ Blood RNA Tube. Shake the tube vigorously for 10-20 sec (favoring cell lysis) and store at -80ºC as soon as possible.

Urine (SOP-6): Collect a half-full urine container to collect at least half full container and transfer urine aliquots (0.5 ml each) at the indicated vials in the Wilmut plate and store at -80ªC.

Saliva (SOP-7): Ask the patient to let saliva collect in the mouth for at least 1 minute and then drool it into the labeled cryotube. Repeat this process multiple times in order to collect at least 2 mL of saliva. Store at -80ªC as soon as possible.

Whole blood for PBMC isolation (SOP-9): After plasma extraction from the 2 10ml EDTA blood collection tubes, dilute the pelleted blood with DPBS -/- until reaching the original volume of blood. Mix the tubes by inversion very slow and carefully 5 times. Fill two pre-filled 12 ml LEUCOSEP tubes touching the side of the tube with the Pasteur pipette, keeping the tube vertical. Centrifuge both tubes at 1000 rcf (x g) for 10' at room temperature, in a swinging bucket rotor with break off. After centrifugation collect the layer above the porous barrier with a Pasteur pipette carefully in a 15 ml falcon tube (one for each LEUCOSEP tube). Wash the enriched cell fraction (lymphocytes / PBMCs) with 10 ml of DPBS and centrifugate for 10 minutes at 330 x g at room temperature. During centrifugation provide 500 ul freshly mixed FCS-DMSO 20% in each of the 3 labelled cryovials. After the centrifugation discard the supernatant and resuspend cell pellet in 1.5ml pure FCS. Slowly add 0.5ml of cell suspension to the 3 cryotube aliquots filled with FCS-DMSO 20% *(end concentration of DMSO in cell suspension is 10%).* Place cryotubes in Mr. Frosty filled with isopropanol for 24h in -80°C freezer. After 1day transfer cells to normal box and leave in -80°C freezer and avoid thawing samples!

Biopsy from livers of the recipient (SOP-8): Perform a liver sampling by a 18G Tru-cut® biopsy at the opening of the abdomen before any other procedure and put one half of the sample in formaldehyde for classical histology assessment and one half in Allprotect® tissue reagent (Qiagen) for immediate stabilization of DNA, RNA and proteins (approximately 100 µL reagent

per 10 mg of tissue). The stabilized tissue is stored at 15-25°C for up to 3 days and incubated overnight at 2-8°C. Then remove the tissue from the reagent and transfer it to -80°C for storage.

X. Human right issues and informed consent

1. Informed consent procedure

The informed consent will be obtained from all patients, or their legally authorized representative (surrogate), prior to participation in this study. Informed consent requires that the patient or patient's surrogate understand the details of the study and agree, without coercion, to participation in the study. In order to obtain informed consent, the following information shall be provided to each patient or patient's surrogate:

- The name of the study
- The name of the Principal Investigator
- The name of the Local Principal Investigator (Study Coordinator)
- An explanation that the study involves research
- An explanation of the study
- An explanation that additional follow-up by telephone or mail will occur over a period of one year post-recruitment or post-liver transplantation.
- A description that participation in the study will require additional biological material, but these materials will be collected within the routine examinations.
- A description that the patient's study number (allocated to the centers) will be used to identify records of medical care and to track the patient's survival after hospital discharge.
- A description that all records will be kept confidential, but that records may be examined by representatives of the Consortium.
- An explanation of whom to contact for answers to questions about the research and about research subjects' rights.
- An explanation of whom to contact in the event of research-related injury.

- A statement that participation in the study is voluntary and that a decision not to participate or to withdraw from the study after initially agreeing to participate will involve no penalty, loss of benefits or reduction in access to medical care.
- A statement that there will be no cost for the treatments provided as part of this study.
- A statement that there will be no payment for participation in this study.

Merely obtaining signature consent from the patient, or the patient's surrogate, does not constitute informed consent. However, the use of a standardized consent form aids in assuring that subjects/surrogates receive adequate and consistent information about the trial and have consented to participate.

The Study Coordinator at each site will introduce and explain the study to the patient (or the patient's surrogate) and present him/her with the detailed consent form and supplementary material to read and review. Subsequently, the Study Coordinator (or a designated physician) will review and discuss the study with the patient/surrogate and answer any questions that the patient/surrogate might have. The investigator will sign and date the consent form on the day the meeting with the patient/surrogate occurred.

The general intent of the study will be delineated. It will be explained to the patient/surrogate that no experimental drugs will be utilized in this study. The patient/surrogate will be informed that the patient's study number (allocated to the centers) will be recorded in the research records as a unique patient identifier. The patient/surrogate will also be informed that, at the data-coordinating center, any personal identifying information will be kept in a data-file separate from the files containing his/her other study data.

The informed consent process will be documented in a detailed progress note prior to study participation, i.e., prior to any procedure associated with risk or discomfort performed for study purposes rather than for patient care. In addition, the patient/surrogate will sign the informed consent in the presence of an independent witness not associated with the study. It must be ensured that the patient/surrogate understands every aspect of the study, including its risks and benefits, prior to signing the informed consent. The consent of the patient to participate in the study will be recorded on the study consent form. The original will be placed in the patient's medical record. Copies of the signed consent firm will be provided to the patient, the Research Office at the participating site (if required by the Institutional Review Board (IRB)) and will also be placed in the patient's study file.

2. **Surrogate consent**

The patients eligible for this study may be unable to provide informed consent due to hepatic encephalopathy or pharmacologic sedation. For this reason it is anticipated that for many patients, informed consent will be obtained from the patient's legally authorized representative (surrogate).

Patients with impaired decision-making capacity constitute a vulnerable population for research studies and require special protection. The following details each of the criteria and the corresponding justification for inclusion of patients with impaired decision making in this study:

(1) The investigator must demonstrate to the IRB that there is a compelling reason to include incompetent individuals or persons with impaired decision-making capacity as subjects. Incompetent persons or persons with impaired decision making capacity must not be subjects in research simply because they are readily available. Patients with critical illness are generally incapable of providing informed consent. Decision making capacity is generally present in less than 10 percent of critically ill patients due to impaired cerebral function from their underlying illness or from sedative medications that are part of the standard of care. Restricting studies of critically ill patients with ACLF to the small minority of patients with intact decision-making capacity would severely compromise the generalizability of the study results by limiting the study to a patient population not representative of the spectrum of critically ill patients with cirrhosis.

(2) Voluntary participation. Although incompetent to provide informed consent, some persons may resist participating in a research protocol approved by their representatives. Under no circumstances may subjects be forced or coerced to participate.

(3) Well-informed representatives. Procedures have been devised to assure that participant's representatives are well informed regarding their roles and obligations to protect incompetent subjects or persons with impaired decision making capacity. Health care agents and next-of kin or guardians must be given descriptions of both proposed research studies and the obligations of the person's representatives. They must be told that their obligation is to try to determine what the subject would do if competent, or if the subject's wishes cannot be determined, what they think is in the incompetent person's best interest. Surrogate decision-makers will be fully informed of the risks and benefits associated with participation in this study. They will be instructed that as a surrogate decision-maker, their obligation is to provide substituted judgment for the patient, based on their determination of what the patient would have done if

they were able to express their opinion. If they do not know what the patient would have decided, they are to provide or refuse consent on the basis of what they believe is in the patient's best interest. Identification of the patient's legally authorized representative for surrogate consent will be in accordance with prevailing state law. In order to obtain surrogate consent, two physicians will determine and document in the medical record that the patient lacks decision-making capacity and that there is little or no likelihood that the patient will regain decision-making capacity within the time-frame required for enrollment in this study. Patients regaining decision-making capacity will be notified of their participation in the study and formal re-consent for continued participation will be obtained. For patients who do not regain decision-making capacity in the second visit, the individual providing surrogate consent will be contacted for the third visit to determine if the patient is able to provide consent and so on. If the patient is able to provide consent, the patient will be contacted and re- consent will be obtained by telephone and/or mail.

XI. Study organization and administration

The Core Group will administer the study and oversee its organization.

1. Regional Coordinators

Following the EF-Clif experience, the nomination of Regional Coordinators and Assistants is instrumental to enhance the performance of the sites and accomplish the study goals.

The Regional Coordinator (RC) is the main link between the CHANCE Principal Investigators located in a specific Geographical Area and the CHANCE Management Team, which is constituted by the Executive Group of the study (Scientific Coordinator, Principal Investigator, Co-PIs) (for clinical topics) and the EF Clif (for Data Management and logistical issues).

The RC is responsible for maintaining a close and effective relationship with the CHANCE Principal Investigators of the sites that depend on him/her, and for the implementation of the clinical and work policies, procedures and standards established in the Study Protocol and the EF-Clif to ensure the correct practices in the sites.

The RC also coordinates the CHANCE Principal Investigators in the region and oversees and act as a liaison between them and the EF-Clif.

He/she is also responsible for ensuring project logistics, readiness, quality and delivery of the laboratory kits and the logistics, readiness, quality and sample preparation to export to the EF Clif biobank.

2. Monitoring bodies

The groups charged with monitoring the various aspects of the study will be the **Executive Group**, the **Steering Committee**, the **Core Group** and the **Study Group**.

The Scientific Coordinator, the **Principal Investigator** and the **Co-PIs** form the **Executive Group**, which is the management and decision-making body for the scientific aspects of the study and will monitor the performance of participating medical centers and the quality of data collected.

The **Executive Group** will meet prior to the beginning of patient intake, one month later, and every 3 months thereafter.

The **Core Group** (the Scientific Coordinator, the Principal Investigator, the Co-PIs, the Scientific Board and the EF-Clif General Manager) must oversee and approve the work performed by the **Executive Group** and must grant permission before any study data may be used for presentation or publication.

The **Steering Committee** is constituted by the Scientific Coordinator, the Principal Investigator, the Co-PIs, the Scientific Board and the Regional Coordinators.

The **Study Group**, which consists of all participating investigators and study coordinators, will meet every twelve months (at the occasion of the EASL or AASLD Annual Meetings) to discuss the progress of the study and any problems encountered during the conduct of the trial.

3. **Monitoring patient intake and probation**

The **Executive Group** will monitor the intake rate and operational aspects of the study. If recruitment is not proceeding at an appropriate rate, the **Executive Group** will scrutinize the reasons for patient exclusions. Based on this information, the **Executive Group** may choose to add additional centers, or to make minor modifications to the inclusion/exclusion criteria.

4. **Monitoring medical center performance**

Strict adherence to the protocol will be expected of every participating center and will be monitored by the **Regional Coordinators**, the **Core Group** and the **Study Group**.

XII. Publication policy

The first authors of the core CHANCE study will be the PIs, and senior experts from the EF-Clif will co-review the manuscripts.

Authors will include all 1 to 5 Investigators per center (depending on the proportion of valid patients) and the Core Group. Authors will receive the manuscript for review and will sign the authorship form.

In addition, the names of physicians who actively contributed to the study should be reported at the end of manuscript, with recognition of their authorship (i.e., sorting of the manuscript by PubMed by inputting their name). Other study group members (e.g., statistical assistants and members of the monitoring bodies) will be listed separately in the Appendix.

The publications deriving from the ancillary studies must include the participants who contributed with samples for the respective project, and the PI of the respective ancillary studies would serve and be listed as principal author for the manuscripts from the respective study.

The presentation or publication of any data collected by participating investigators is under the direct control of the Core Group. This is true whether the publication or presentation is concerned with the results of the principal undertaking or is associated with the study in some other way. No individual participating investigator has any inherent right to perform analyses or interpretations or to make public presentations or seek publication of any of the data (including those obtained in her/his own center) other than under the auspices and with the approval of the Core Group.

XIII. Qualifications of participating centers

Importantly, the success of this study majorly depends on the quality of samples and data collected, **strict adherence to the study protocol and its requirements is expected from each participating investigator**.

- Fulfillment of minimal requirements:
  - A liver transplant program with a significant volume (at least 40 liver transplants per year in the last 3 years and expertise in LT for very sick cirrhotic patients (high MELD score, critically ill…)
  - A team of hepatologists or equivalent physicians (gastroenterologists, internist and or intensive care physicians) involved in the care of patients with liver disease, 24 hours per day and 7 days per week.
  - An ICU/ITU embedded in the Liver Program or closely linked to it (having the experience in the management of critically ill patients with liver diseases). In any case, the attending physician must be familiar with liver disease management.
  - Research nurse or research fellow,
  - TRAINED person (with expertise in sampling biological material) responsible for the sample handling, shipping, form filling, freezers and refrigerators, sample treatment,
  - Availability of -80ºC freezer,
  - Availability of lab near the blood extraction room, containing a centrifuge with a swinging bucket rotor (recommended refrigerated) and basic lab instruments

Since biological samples are essential for ancillary studies, centers that have facility and experience to process samples are encouraged to participate in the study. Participation will be dependent upon approval of the protocol by the IRB at each site. All site personnel involved with the conduct of this study must be certified in Good Clinical Practices (GCP) and human subjects' protection training.

Finally, centers should be aware of the fact that the study will be performed in great part with their help.

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XV. Appendices

1. EASL-CLIF definition of ACLF and grades

The CLIF Consortium-organ failure scoring system (CLIF-C OFs)			
Organ/system	Subscore=1	Subscore=2	Subscore=3
Liver (total bilirubin, mg/dL)	< 6	≥ 6 - < 12	≥ 12
Kidney (creatinine, mg/dL)	< 2	≥ 2 - < 3.5	≥ 3.5 or RRT
Brain (West-Haven grade HE)	0	1 - 2	3 - 4
Coagulation (INR)	< 2	≥ 2 - < 2.5	≥ 2.5
Circulation (MAP, mmHg)	≥ 70	< 70	vasopressors
Lung PaO_2/FiO_2 or SpO_2/FiO_2	 > 300 or > 357	 ≤ 300 - > 200 or ≤ 357 - > 214	 ≤ 200 or ≤ 214

The shaded area describes criteria used to define organ failures.

Grade of ACLF

- ACLF grade 1 (ACLF-1):
- Patients with single kidney failure
- Patients with non-renal organ failure plus renal dysfunction (creatinine 1.5 – 1.9 mg/dL) and/or brain dysfunction (grade 1 – 2 HE).
- ACLF-2: patients with two organ failures
- ACLF-3: patients with three or more organ failures

CLIF, chronic liver failure; RRT, renal replacement therapy; HE, hepatic encephalopathy; INR, international normalized ratio; MAP, mean arterial pressure; FiO_2, fraction of inspired oxygen; PaO_2, partial pressure of arterial oxygen; SpO_2, pulse oximetric saturation.

2. Sample processing SOPs

0. Blood Collection-venipuncture (Version 1_November 2020)
1. SST Serum Separation Tube (Version 1_November 2020)
2. EDTA Buffy Coat Collection (Version 1_November 2020)
3. CITRATE Plasma and Buffy Coat Tube (Version 1_November 2020)
4. Li-HEPARIN Plasma Tube (Version 1_November 2020)
5. Tempus Blood RNA Collection Tube (Version 1_November 2020)
6. Urine Collection Tube (Version 1_November 2020)
7. Saliva Collection (Version 1_November 2020)
8. Liver Biopsy Collection (Version 1_November 2020)
9. EDTA Plasma and PBMCs Isolation (Version 1_November 2020)