Study Code: CRECOOLT 3.0

Impact of a risk stratification tool on the outcome of liver transplant recipients colonized with carbapenem resistant Enterobacteriaceae: an observational study

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PROTOCOL SIGNATURE PAGE

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Maddalena Giannella	Signature	12/09/2022 date
Matteo Rinaldi	Signature	12/09/2022 date

DECLARATION OF THE INVESTIGATOR:

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I declare that I have read the protocol and I agree to conduct this clinical study in accordance with all the requirements of the protocol and according to the Guidelines of Good Clinical Practice and the principles of the Declaration of Helsinki.

Maddalena Giannella (Principal Investigator)	Signature	12/09/2022 date

CONTENT INDEX

1 STUDY-SPECIFIC ABBREVIATIONS AND TERMS

OLT	orthotopic liver transplantation
CRE	carbapenem resistant Enterobacterales
SOT	solid organ transplant
HR	hazard ratio
SOFA	Sequential organ failure assessment
KIDGO	Kidney Disease: Improving Global Outcomes
AKI	Acute kidney failure
RRT	Renal replacent therapy
CMV	Cytomegalovirus
MV	Mechanical ventilation

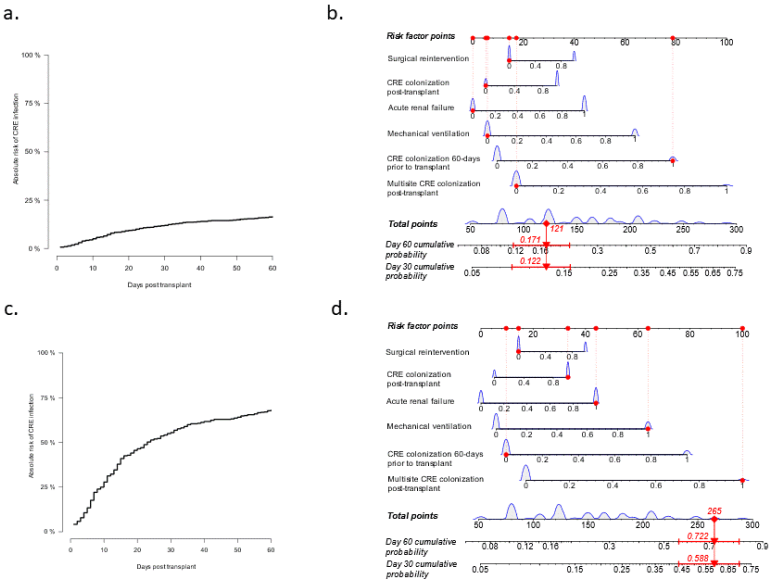
2 Introduction

2.1 Background and rationale

Patients undergoing orthotopic liver transplantation (OLT) are more susceptible to acquire colonization with multidrug resistant Gram-negative bacteria, in particular with carbapenem resistant Enterobacterales (CRE), than non-solid organ transplant (SOT) recipients or patients with other types of SOT (1). CRE colonization, either acquired before either emerged after OLT, has been associated with highest risk of developing CRE infection after OLT (2,3) with a dramatic impact on patient survival (4). For these reasons, along with the improvement of infection control and antimicrobial stewardship policies, preventive strategies targeted to patients colonized with CRE have been suggested (5,6). However, several uncertainties have to be considered concerning the optimal timing for intervention, the correct patient stratification, and the choice of drugs. Regarding the timing for intervention, surgical prophylaxis is regarded as an option. However, it should be considered that of the overall burden of CRE carriage in OLT recipients, pre-transplant acquisition accounted for lower than one third of isolations in a large multicenter retrospective study (7). Furthermore, even when CRE carriage is present at transplantation, the colonization status is frequently recognized in the immediate post-operative period by results of rectal swabs done at surgery. Finally, current data about the efficacy of targeted prophylaxis in patients colonized with CRE are very limited and showed controversial results (8,9). Thus, in the majority of cases the unique window for preventive strategies is represented by the post-transplant course. This requires an active screening policy as well as the possibility of stratifying colonized patients according to their risk of developing CRE infection, and eventually to die, in order to target specific interventions in those patients who could most benefit, and to optimize essential resources as the new drugs. Indeed, since the availability of new drugs such as ceftazidime/avibactam, meropenem/vaborbactam and cefiderocol, the therapeutic management and outcome of patients colonized and/or infected with CRE has changed in both general population and SOT recipients (10). New drugs are associated with higher rates of clinical cure and patient survival, as well as with lower toxicity, compared with old regimens (11). However, rates of microbiological failure around 10%, consisting in persistent positive cultures or relapsing infections associated with development of further resistance,

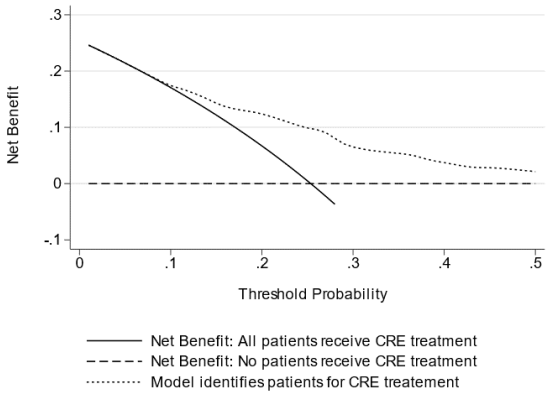
have been reported raising concerns about the need of optimizing the use of new drugs to avoid the loss of their efficacy.

To this end, we have recently carried out a large multicenter observational study aimed at building a prediction model to stratify patients according to their risk of developing CRE infection after OLT (7). The study cohort consisted of 800 OLT recipients colonized with CRE, 25% were colonized at OLT, and 75% acquired colonization within two weeks after OLT. Infection rate was of 30% and was similar between the two groups as well as the infection severity. All-cause 6-month mortality rate among patients who developed CRE infection was 58% vs. 20% among carries who did not develop infection (p<0.001). Almost all infections occurred within 60 days after transplant. Thus, we derived, and internally validated, a prediction tool able to stratify the risk of CRE infection at 30 and 60 days after OLT (CRECOOLT score) based on six variables easily to monitor such as CRE colonization detected in the 60 days prior to transplant, CRE colonization detected post-transplant, multisite colonization, need of prolonged (≥48 hours) mechanical ventilation, acute renal failure, and reintervention. Examples of the cumulative incidence of CRE infection and nomogram-derived prediction for a low-risk (panels a, b) and a high-risk (panels c, d) patient are illustrated in score was made available as app to be used at bed site.

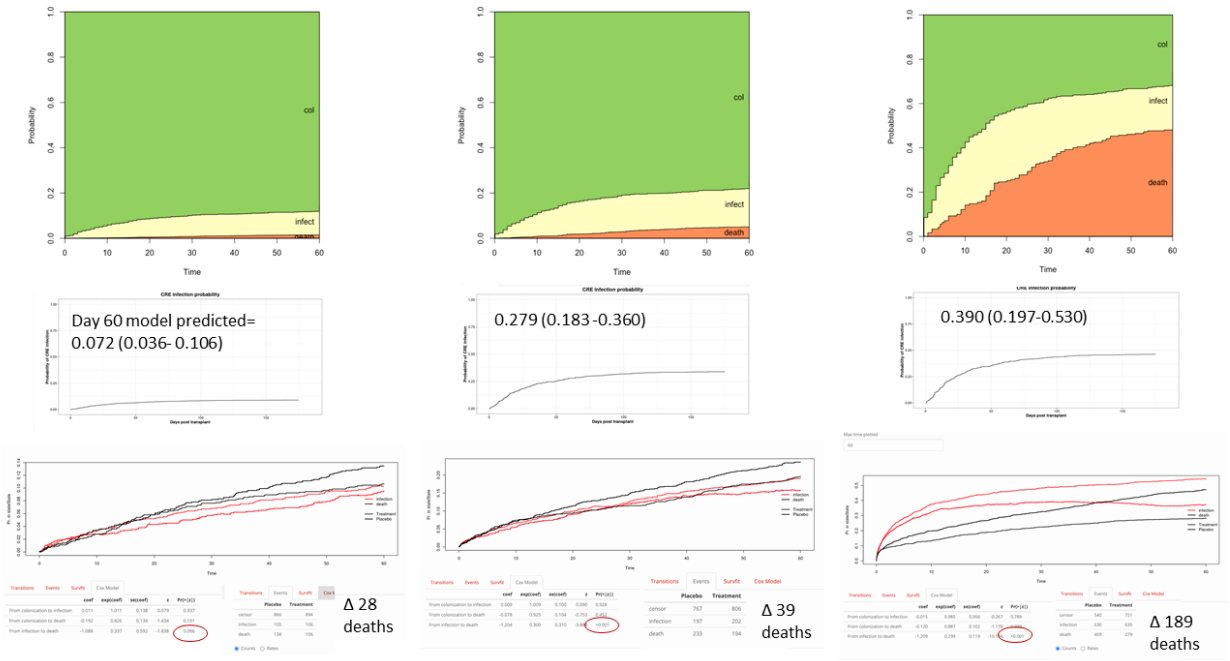


We explored the potential clinical utility of our model using a decision-curve analysis to examine the “net benefit” of applying the prediction model across a range of CRE infection threshold probabilities (12). A theoretical risk-model guided strategy (i.e. empiric administration of CRE-active antibiotics) was compared against two default strategies- “treat

all" and "treat none" suggesting that the model-directed intervention would show net benefit over default strategies when the overall CRE infection risk exceeded 10%.



Finally, we have explored the risk of death using the same variables included in the CRECOOLT score by a multi-state model obtaining three risk groups: low, intermedium and high (unsubmitted data). We have then estimated the potential impact of using ceftazidime-avibactam in each of such groups finding a significant protective role (HR 0.32) only in patients with intermedium or high risk of dying.



2.2 Importance of the study and its clinical relevance

Although CRE infection after OLT have a dramatic impact on patient survival and several implementations have been proposed (i.e. preventive strategies or targeted surgical prophylaxis), a standardized approach in colonized patients is still missing. We recently developed and internally validated a bed-side score to stratify the risk of CRE infection (7). We deem such score could be useful to select patients at high of infection as well as of poor outcome supporting clinicians in the therapeutic management in such difficult setting. With the present study we aimed at investigating the impact on outcome of the systematic evaluation of the risk score in OLT recipients colonized with CRE.

2.3 Primary objective

The primary objective is to investigate the impact on all-cause 90-day mortality in OLT recipients colonized with CRE using the CRECOOLT score for the systematic evaluation of CRE infection risk.

2.4 Secondary objectives

Secondary objectives include:

- To analyse days of therapy with anti-CRE antibiotic regimens in patients with and without systematic evaluation of CRE infection risk, according to clinical practices.
- To evaluate rates of documented CRE infections and their relapses with selection of further resistance in patients with and without systematic evaluation of CRE infection risk.
- To evaluate the length of hospital, ICU stay and rates of hospital readmission in patients with and without systematic evaluation of CRE infection risk.

2.5 Primary endpoint

Primary endpoint will be all-cause mortality assessed at 90 days after OLT.

2.6 Secondary endpoints

Secondary endpoints will be assessed from OLT to 180 days and include:

- Overall days of CRE antibiotics;
- Rates of CRE infection;
- Rates of CRE infection relapse;
- Rates of CRE infection relapse with resistance to previously used antibiotics;
- Hospital and ICU lengths of stay;
- Hospital readmission for infectious complications.

3 Investigational plan

3.1 Type of study

Cross sectional study..... ☐
Retrospective Case-control study .....☐
Retrospective cohort study ..... ☐
Prospective cohort study..... ☐
Retrospective and prospective cohort study..**X**
Descriptive pilot/feasibility study ..... ☐
Qualitative study ..... ☐

3.2 Study design

All consecutive adult (≥18 years) patients undergoing OLT and found to be colonized with CRE within 60 days prior to or after transplantation.

Retrospective period will be from January 2018 to December 2021. This time period has been established considering the introduction in the clinical use of new drugs active against CRE (i.e. ceftazidime-avibactam), to include both pre-COVID-19 and pandemic periods, and it is before developing the CRE-COOLT score.

Prospective period will be carried out over 18-month period, starting soon after the approval by Ethic Committees. During this period, all patients undergoing OLT will be prospectively evaluated for study participation. In eligible patients who will accept to participate the CRECOOLT score will be systematically calculated once week, or at the occurrence of complications, until 60 days after OLT.

The therapeutic management of all patients, during both retrospective and prospective periods, will be established by the attending physicians according with routine practice and not dictated by study protocol. Standard antibiotic prophylaxis according to local protocol will be performed during both study periods. Targeted (anti-CRE) antibiotic prophylaxis will be accepted, but for a duration no longer than 48 hours (see exclusion criteria).

The minimum follow-up period for all enrolled patients, during both retrospective and prospective periods, will be of 180-days after OLT. This follow-up duration has been established considering that OLT recipients colonized with CRE usually develop CRE infection within 60 days after OLT, indeed the CRE-COOLT score has been developed and proposed to be used during this time period (7). Thus, primary outcome (all-cause mortality) will be evaluated at 90 days after OLT, while secondary outcomes (especially relapse of CRE infections and emergence of further resistance) will be evaluated at 180 days (see endpoints).

Total duration of the study: 24 months (18 recruitment / data collection +6 follow-up).

Start of the study: soon after the approval by Ethic Committees and "*nulla osta"* of General Manager.

Definitions

CRE is defined as any *Enterobacterales* displaying *in vitro* non-susceptibility to any of the carbapenems according to the criteria (Clinical and Laboratory Standards Institute or European Committee on Antimicrobial Susceptibility Testing) adopted at the participating center during the study period. EUCAST criteria (13).

Colonization status is defined as isolation of CRE from rectal swab or other samples, other than blood cultures or sterile fluids, (e.g., urine, respiratory samples, superficial skin samples) in absence of symptoms and signs of infection. Multisite colonization is defined as concomitant CRE isolation from more than one of such samples.

CRE infection is defined according to Center for Disease Control criteria (14). CRE infection relapse is defined as new symptoms and positive cultures yielding CRE in patients with prior documented clinical and microbiological response to anti-CRE antibiotic course.

Study variables

The following variables will be collected, according to normal clinical practice: demographic data (age and sex); comorbidities according to Charlson index; underlying liver disease, and severity of liver disease according to Model for End stage Liver Disease (MELD) at inclusion in waiting list and at OLT.

For graft characteristics donor age, cold ischemia time, combined transplant, culture results of organ donor and preservation fluid will be collected.

Intraoperative variables will include: antibiotic prophylaxis, biliary anastomosis, bleeding with need of massive transfusion (≥40 units of cellular blood products), prolonged intervention (≥ 8 hours).

Post-OLT complications will include: re-intervention, Acute Kidney Injury (AKI) according to KDIGO criteria (15), renal replacement therapy (RRT), prolonged (≥48 hours) mechanical ventilation (MV), graft dysfunction (primary or secondary), biopsy-proven rejection, re-transplantation, and CMV DNAemia >100,000 copies/ml.

Management of CRE colonization will be recorded according to the following categories: no strategy adopted, pre-emptive strategy according to the risk stratification using the CRECOOLT tool, standard empirical/targeted treatment upon to clinical deterioration. All drugs used for these purposes will be recorded with relative administration schedules and onset/end dates.

For CRE infection: clinical severity according to SOFA and septic shock criteria on the day of positive clinical sample collection (infection onset) will be recorded, infection source and presence of bloodstream infection, appropriateness of empirical therapy (defined as administration of at least one *in vitro* active drug within 24 hours from infection onset), targeted antibiotics with relative administration schedules and onset/end dates, source control, documentation of and time to microbiological clearance, clinical response at 7 days, and CRE infection relapse with susceptibility pattern of recurrent isolate.

For outcome, lengths of hospital and ICU stay after OLT, number of re-hospitalization, all-cause mortality.

3.3 Mono or multicenter study

Monocentric ..... ☐
National multicenter ..... ☐
International multicenter ..... **X**

All the transplant programs belonging to the CRECOOLT study group (7) will be invited to participate.

3.3.1 Inclusion Criteria

- Signature of the informed consent
- Age ≥ 18 years
- CRE colonization within 60 days prior to or after transplantation

3.3.2 Exclusion Criteria

- Patients receiving targeted antibiotic prophylaxis (against CRE) for a period longer than 48 hours
- Patients receiving graft from a donor with cultures yielding a carbapenem-resistant Gram negative bacteria

3.3.3 Population size of the study and statistical power

Our hypothesis is that the systematic use of the CRE-COOLT score at the bedside of OLT recipients colonized with CRE may improve antibiotic use and outcome of patients. According with prior literature data, the post-OLT all-cause mortality rate in CRE carriers is around 30%, ranging from 20 to 58% in non-infected and CRE infected patients, respectively (7). Our hypothesis is to observe in the prospective period a reduction of overall mortality of about 15%, thus accepting a power of at least 80% and an alpha error of 5%, we calculated a sample size of 120 patients in both study period (retrospective/prospective).

Descriptive statistics will be obtained for all the variables assessed in the study population. Mean and standard deviation will be used for normally distributed continuous variables, median and interquartile ranges for skewed distributions, proportions for categorical variables. Comparison between retrospective and prospective periods will be done, differences will be tested with parametric or nonparametric tests for quantitative variables according with their distribution, and with Pearson's Chi-square or Fisher's exact test, as appropriate, for categorical variables.

To assess the impact of a systematic use of CRE-COOLT score on all-cause 90-day mortality, univariate and multivariate Cox regression analysis will be carried out. Statistical significance will be represented by a p value of <0.05.

3.4 Grants

Are there any funding for the study?

No ..... ☐
Yes, with internal grants..... ☐
Yes, by institutional third parties ..... ☐
Yes, from private third parties X

The study was submitted to Pfizer Global Medical Grants and received a positive evaluation (Pfizer Grant 75038507). Access to funds is subject to the submission of the final version of the protocol with the relative favorable opinion of the Ethics Committee on the Pfizer Global Medical Grants system, no later than 10/30/2022, and to an agreement between our center of affiliation (Department of Medical and Surgical Sciences of the University of Bologna) and Pfizer to be reached within the next 3 months.

4 Data management

Pseudonymized data will be collected using a pre-established electronic case report form (eCRF) and managed using REDCap capture tool hosted by Alma Mater University of Bologna (16). Data sources will be clinical charts and hospital electronic records.

During all the study period, the principal investigator will inform investigators about the status of patient enrolment, follow-up, and data collection by newsletter periodically.

At the end of the enrolment and follow-up period, the principal investigator will be responsible for reviewing all collected data for integrity and accuracy. Queries will be generated and sent to the local investigators. The database will be locked after fulfilment of all the queries.

5 Administrative procedures and declarations

5.1 Informed consent and consent to the processing of personal data

The study protocol, any protocol amendment, informed consent, consent to the processing of personal data and any other information for patients must be approved by the Ethics Committee.

To participate in the study, each patient must provide written informed consent as well as consent to the processing of their personal data.

5.1.1 Methods of acquiring informed consent and consent to the processing of personal data

Informed consent will be obtained by local investigators in collaboration with the attending physicians. For the retrospective cohort, it will be obtained during the standard scheduled follow-up visits. For the prospective cohort, informed consent will be administered to the patients during the evaluation visit for enrolment. Only patients who will provide informed consent will be enrolled.

5.1.2 Cases for which the acquisition of consent to the processing of personal data is not required

For the retrospective cohort, if patients will be unable to give the consent (deceased or lost at follow-up), data will be collected, in order to avoid a significant reduction in the sample size generating potential bias on results according to Italian regulatory dispositions (art. 110, comma 1 d.lgs. 196/2003 and in accordance with the Provision containing the requirements relating to the processing of particular categories of data, pursuant to art. 21, paragraph 1 of Legislative Decree 10 August 2018, n. 101 issued by the Guarantor Authority for the protection of personal data (provv. n.146/2019). It will be mandatory to provide information about the study to all enrolled patients if reachable in a second time (Reg. UE 2016/679).

5.2 Study – specific insurance

Considering the nature of the study, a specific insurance is considered unnecessary.

5.3 Amendments to the protocol and changes to the conduct of the study

If during the enrolment period, the principal and local investigators will agree that any deviation from the original protocol is needed to reach the predefined sample size and/or to avoid bias in the assessment of outcomes, the protocol will be revised and an amendment will be submitted to Ethic Committees.

5.4 Conclusion of the study

After the lock of database, preliminary results will be shared with all the investigators in order to plan publication and other dissemination activities. These results will be used to communicate to local Ethic Committees the conclusion of the study.

5.5 Publication policy

Preliminary results will be revised by all the investigators in order to refine statical analysis, and to plan publication and other dissemination activities (presentation of results at International Congresses of Infectious Diseases and Transplant Societies).

For authorship, Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals (ICMJE Recommendations) will be followed. The ICMJE recommends that authorship be based on the following criteria:

- Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
- Drafting the work or revising it critically for important intellectual content; AND
- Final approval of the version to be published; AND
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

According to the rules of good scientific practice, the principal investigator can claim the position of last author if he/she is not already first author. In the case of several research project leaders, they should agree on the last authorship among themselves at an early stage; in this context, shared first and last authorships should also be considered. As a rule, the "corresponding author" should be the research project leader with primary

responsibility. The middle group of co-authors will be included according to contribution to the manuscript as per the ICMJE recommendations. The CRECOOLT working group will be included as co-author in all manuscripts and communications. In the CRECOOLT group all the investigators and the attending physicians who contributed to patient enrolment and data acquisition should be acknowledged.

5.6 Documentation archive

The principal investigator is responsible for the archiviation and conservation of the essential documents of the study, before, during and after the study conduction, according to the Italian law and good clinical practice.

Patient data will be gathered pseudo-anonymously, included subjects will be identified by a number/code. Principal and local investigators will keep the original data of the patient and his personal informed consent in a safe place.

5.7 Inspections/checks

If a regolatory authority will request an ispection, the principal investigator will soon inform the Ethic Committee.

5.8 Contact persons

Contacts (telephone numbers and emails) of the involved physicians are reported in the Investigator Folder for the local center.

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