Clinical Infectious Diseases

Development of a Risk Prediction Model for Carbapenem-Resistant Enterobacteriaceae Infection after Liver Transplantation: A Multinational Cohort Study

--Manuscript Draft--

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Full Title:	Development of a Risk Prediction Model for Carbapenem-Resistant Enterobacteriaceae Infection after Liver Transplantation: A Multinational Cohort Study
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Abstract:	Background. Patients colonized with carbapenem resistant Enterobacteriaceae (CRE) are at higher risk of developing CRE infection after liver transplantation (LT) with associated high morbidity and mortality. Prediction model for CRE infection after LT among carriers could be useful to target preventive strategies. Methods. Multinational multicenter cohort study of consecutive adult patients underwent LT and colonized with CRE before or after LT, from January 2010 to December 2017. Risk factors for CRE infection were analyzed by univariate analysis and by Fine-Gray sub-distribution hazard model, with death as competing event. A nomogram to predict 30- and 60-day CRE infection risk was created. Results. 840 LT recipients found to be colonized with CRE before (n=203) or after (n=637) LT were enrolled. CRE infection was diagnosed in 250 (29.7%) patients within 19 (IQR 9-42) days after LT. Pre-and post-LT colonization, multisite post-LT colonization, prolonged mechanical ventilation, acute renal injury, and surgical re-intervention were retained in the prediction model. Median 30 and 60-day predicted risk was 15% (IQR 11-24%) and 21% (IQR 15-33%), respectively. Discrimination and prediction accuracy for CRE infection was acceptable on derivation (AUC 74.6, Brier index 16.3) and bootstrapped validation dataset (AUC 73.9, Brier index 16.6). Decision-curve analysis suggested net benefit of model-directed intervention over default strategies (treat all, treat none) when CRE infection probability exceeded 10%. The risk prediction model is freely available as mobile application at https://idbologna.shinyapps.io/CREPostOLTPredictionModel/ . Conclusions. Our clinical prediction tool could enable better targeting interventions for CRE infection after transplant.
Response to Reviewers:	Ajit P. Limaye, M.D., Special Section Editor Clinical Infectious Diseases email: cid.editorialoffice@idsociety.org Bologna, December 2020 Dear Dr. Limaye: Many thanks for your comments on our manuscript CID-105646 entitled "Development of a Risk Prediction Model for Carbapenem-Resistant Enterobacteriaceae Infection after Liver Transplantation: A Multinational Cohort Study". We have carefully considered every comment and suggestion raised by the reviewers and changed our manuscript accordingly. We attach the answers to all the items raised and two copies of the revised manuscript, one of them with all the changes clearly marked in yellow. REVIEWER COMMENTS Reviewer #1: Giannella and colleagues present a multinational cohort study from which a risk prediction model for post-transplant CRE infection was developed. This is a topic

of significant importance given the morbidity and mortality associated with CRE infections in liver and other solid organ transplant recipients. Further, while CRE colonization has been associated with CRE infections following liver transplantation, there is a paucity of data which helps stratify the risk for infection among those colonized patients. Overall, this is a high-yield manuscript of interest to the transplant community. The authors do highlight limitations of the study and certainly future studies would be required to confirm these findings.
AUTHORS' RESPONSE: Thanks for the kind comment.
One minor point would be the following: All patients were screened for rectal CRE carriage at pre-defined time points. However, screening for CRE colonization at other sites was not uniformly performed. While it would make sense that patients with multi-site colonization are at higher risk for invasive infection, it would be important to highlight how this may impact the results.
AUTHORS' RESPONSE: We agree with reviewer, we have commented this limitation in the discussion (Page 13, line 318-322).

Reviewer #2: Dr. Giannella and colleagues performed a multinational cohort study including 840 liver transplant (LT) recipients identified as colonized, either pre-operatively or post-operatively, with carbapenem resistant Enterobacteriaceae (CRE). They developed a clinical prediction tool for identifying those at highest right of post-operative CRE infection. Of the 840 CRE colonized patients, 203 (24.2%) were identified pre-transplant and 637 (75.8%) acquired CRE colonization post-transplant. CRE infection occurred in 250 (29.7%) of cases overall. On the predictive model, they identified pre- and post-LT CRE colonization, multisite colonization, KPC strain, induction with anti-thymocyte globulin, prolonged mechanical ventilation, acute renal injury, and surgical re-intervention as predictive factors for post-transplant CRE infection. The manuscript is well written and potentially interesting. The amount and quality of the data collected is difficult to beat. However, I have some concerns about the selection of variables and the applicability of the proposed predictive model. Therefore, I have included some suggestions to try to improve the overall quality of the manuscript.
AUTHORS' RESPONSE: Thanks for the kind comment and for the suggestions provided.

Major comments:
1. There were a number of notable between-group differences for those colonized pre-transplant as opposed to post-transplant, including higher rates of moderate-severe renal disease, more frequent prior transplantation, higher MELD scores, higher rates of hepato-renal syndrome, higher rates of grade III/refractory ascites, more frequent prior GI bleeding, higher rates of hepatic encephalopathy, more frequent preceding ICU admission, more frequent acute-on-chronic liver failure, more frequent occurrence of any infection, more frequent choledochojejunostomy, higher rates of prolonged surgery, or more frequent use of maintenance steroids in the pre-transplant CRE colonization groups. Despite that, the observed CRE infection rate was similar overall between pre- and post-liver transplant colonized patients. One would predict an increased CRE infection rate in the pre-transplant colonized group. The lack of observed difference in CRE rates may be explained by the observation that 25.1% of patients colonized pre-transplant with CRE received targeted perioperative prophylaxis. Thus, it would be of interest to know if the rate of CRE infection differed between those who did and did not receive CRE-targeted prophylaxis. Despite this, I believe that most transplant centers would target antimicrobial prophylaxis to cover CRE in known colonized patients undergoing liver transplantation.
AUTHORS' RESPONSE: Among the 203 patients colonized with CRE at OLT, 51 received targeted perioperative prophylaxis, the rate of CRE infection was 31.4% and 33.6% among patients exposed and unexposed to targeted perioperative prophylaxis (p=0.90). No further data about drugs and timing of targeted prophylaxis were collected. We have added this information in the text (Page 11, line 260-262).
Regarding the last sentence. We agree with the need/opportunity to intervene at transplantation with targeted prophylaxis in patients known to be colonized with CRE. However, the effectiveness of targeted prophylaxis and which agents should be used are not well established. Even in pre-OLT carriers, the colonization data is often only acquired in the immediate post-operative period. Furthermore, most patients are found to be colonized with CRE during the first weeks after OLT. Finally, as shown in previous studies including unselected OLT recipients, both colonization at OLT and

colonization after OLT were the strongest predictors of CRE infection (Freire et al Transplantation 2017, Giannella et al Clin Microbiol Infect 2019). For these reasons, we believe that an individualized risk stratification for colonized patients, either pre or post OLT, could help better targeting preventive strategies not only in the perioperative but also in the post-operative period.

2. Regarding the predictive model, some changes in the selection of variables may result in a more robust and comprehensive model:
- Screening for CRE colonization at sites other than rectal swab was performed according to clinical judgment and local policy. Multisite CRE colonization may have been diagnosed in more critically-ill patients with more access to respiratory samples. In addition, its inclusion runs the risk of "double-counting" colonization as a risk factor. To build an efficient predictive model, this variable would probably lead to biases in identifying patients at risk in the clinical setting.
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- The inclusion of KPC-producing isolates in the multivariate model is also a bit unusual given that there was no statistically significant difference between the group not developing CRE infection and those developing CRE infection (70.8% vs. 72.8%, p=0.61) in the presence of KPC being the identified genotypic strain of CRE present. Regardless, including colonization in general, multisite colonization, and then colonization with a KPC-producing isolate seems run the risk of counting a similar variable repeatedly and over-inflating an individual's risk of CRE infection.
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- With all the previous considerations, I would like to suggest a new predictive model with additional justification of the reasons to exclude some variables:
• There is no data collected on the incidence of CRE infection in non-colonized liver-transplant patients. Therefore, the predictive model will predict the risk of CRE infections in CRE colonized patients but will not be useful to predict the risk in non-colonized patients. Probably, the risk of CRE infection in non-colonized patients is low, but, from the present study data there is no possibility in knowing that risk.
AUTHORS' RESPONSE: We have addressed this issue in the discussion (Page 14, line 334-336).
•Reintervention should be included and will reflect a more complicated course of liver transplantation. Re-laparotomy may facilitate CRE infection in colonized patients. Also, could predict the length of ICU stay.
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•Mechanical ventilation for more than 48 h in the preceding 2 weeks: I believe this variable is important as it may predict the risk of CRE pneumonia. In the proposed online calculator, mechanical ventilation is treated as a binary variable without any defined criteria. As the evaluation of the score in the clinical practice would be a dynamic process in the follow up of patients at risk, it would be difficult to justify that prolonged ventilation 40 days ago may predict the risk of CRE pneumonia itself. There are alternative variables that may reflect the more complicated course of liver transplant (AKI, re-operation). Therefore, when evaluating the risk of CRE infection, the impact of mechanical ventilation on the risk of CRE infection should be limited in time. As this variable will be time-dependent, the outcome (CRE infection) should be recalculated according to
AUTHOR RESPONSE: We agree with this comment, unfortunately we collected such variable only as binary variable asking for the need of prolonged (>48 h) MV before the development of CRE infection. We have addressed this limitation in the discussion (Page 14, line 325-327).
•Acute kidney injury: This is one of the most important predictors of post-liver transplant infection and should be maintained. Contrary to mechanical ventilation suggestion, AKI at any point post-transplant would reflect post-transplant complications

and should not be limited in time.
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•Regarding the transplant timeline of CRE colonization, without considering multisite colonization as this variable can be biased by more intensive screening in ICU patients, it looks like pre-transplant CRE colonization has more impact on CRE infections than colonization post-transplant. Therefore, I would suggest including only pre-transplant colonization by CRE.
AUTHORS' RESPONSE: We have appreciated this comment; eventually we have leaved in the model pre- and post-transplant colonization and multisite colonization after LT for the reasons explained below.
•Would it be possible to evaluate the robustness and predictive power of a model including these variables: Reoperation, mechanical ventilation for more than 48 h in the preceding 2 weeks, acute kidney injury and pre-transplant CRE colonization? If this proposed model predicts well the occurrence of CRE infection, then would potentially be applicable to other transplant programs with high prevalence of CRE. In addition, it should be clearly stated that the model will only work in patients with proven CRE colonization. I suspect the selection of some variables may have decreased the predictive power of the model.
AUTHOR'S RESPONSE: We have re-performed the analysis following the suggestions of the reviewer. The table below shows indices of the model discrimination (Harrell's C statistic), accuracy (Brier Index) as well as improvements in the prediction accuracy versus a null model that contains only CRE colonization prior to transplant. As seen below, the more parsimonious model proposed by the reviewer displayed poorer discrimination and accuracy for identifying patients who develop CRE infection within 60 days post-transplant.
ModelC-Statistic (AUC)
(higher number is better)Brier decomposition score
(lower scores are better)Improvements in prediction accuracy vs. null model -CRE col pre 60d)
(higher percentages are better)
Full model:
1.ATG
2.Reintervention
3.Mechanical ventilation
4.Acute renal failure
5.KPC carbapenem.
6.CRE col pre 60d
7.Multisite colon. Pre trans
8.CRE col post 60d
9.Multisite colon post trans75.1
(71.3-78.9)16.1
(6.6-25.6)14.8%
Reviewer suggested model:
1.Reintervention
2.Mechanical ventilation
3.Acute renal failure
4.CRE col pre 60d69.2
[65.2-73.2)17.4
(7.6-27.2)8.21%

However, we agree with the reviewer that a simpler model may be possible. Therefore, using a base model of CRE colonization within 60 days prior to transplant, we examined the degree to which including each variable from our original model improved model performance. Our analysis, in part, supported the reviewer's suggestion that ATG, KPC carbapenemase, and multisite colonization prior to transplantation could be removed from model without marked changes in prediction accuracy.
Addition of predictor to base model (CRE col pre 60d)C-Statistic, AUC (95%)Improvements in prediction accuracy vs. null model
(CRE col pre 60d) (higher percentages are better)
ATG53.8 (50.5-57.2)0.71%
Reintervention54.5 (51.0-58.0)2.63%
Mechanical ventilation59.7 (55.5-63.8)7.45%

Acute renal failure61.7 (57.5-65.8)3.63%
KPC carbapenemase53.9 (49.7-58.1)0.72%
Multisite col. pre trans53.9 (50.5-57.2)0.62%
CRE colonisation post-transplant57.6 (54.1-61.0)1.71%
Multisite colon post-transplant62.6 (58.7-66.5)6.85%

However, colonization documented post-transplant and colonization at additional sites (an indicator of higher inoculum even though not systematically assessed in all patients) were both associated with improved model performance. Therefore, we have propose a revised model that addresses the reviewer's suggestion as best as possible:
Table 3. Final multivariable analysis for predicting risk of CRE infection after liver transplantation
VariableSubhazard ratio (95% CI)β-coefficientP value
Reintervention1.37 (1.04-1.80)0.30 (0.02-0.57)0.02
Prolonged mechanical ventilation2.01 (1.49-2.71)0.70 (0.40-0.99)<0.0001
Acute renal injury1.69 (1.26-2.27)0.52 (0.23-0.82)0.001
CRE colonisation 60 days prior to transplant2.17 (1.33-3.53)0.78 (0.29-1.26)0.002
CRE colonization within 60 days after transplant1.41 (0.91-2.19)0.35 (-0.09-0.78)0.12
Multisite colonization within 60 days after transplant2.87 (1.98-4.20)1.06 (0.68-1.43)<0.0001
Abbreviations: CI confidence interval, CRE carbapenem-resistant Enterobacteriaceae, KPC Klebsiella pneumoniae carbapenemase-producing.
The discrimination and calibration of this revised simpler model (6 variables) is nearly identical the originally proposed model (9 variables) for predicting CRE infection within 60 days post-transplant as shown in the figure below of the model fit for the (a) derivation dataset; and (b) Bootstrapped-resampled dataset.

Therefore, we have revised all of the corresponding tables, figures and nomogram (in the manuscript and online app) to reflect the new simpler model.

Minor comments:
- Anti-thymoglobulin is used throughout and perhaps should be changed to anti-thymocyte globulin.
AUTHORS' RESPONSE: We have done as suggested.
- BLBLI acronym on page 5 should be spelled out on first use.
AUTHORS' RESPONSE: We have added the spelling.
- Line 16 on page 7 should perhaps replace "revise" with "review".
AUTHORS' RESPONSE: We have done as suggested.
- Page 10, last paragraph: "Considering the competing risks of death or CRE infection…" CRE infection should be removed as it is not acting as a competing event when evaluating the risk of CRE infection.
AUTHORS' RESPONSE: We have done as suggested.
- Figure 1 on page 24 has "participating" misspelled.
AUTHORS' RESPONSE: We corrected the error.
Should you or any of the reviewers wish any further clarification, please, do not hesitate to contact us. We hope that our manuscript may be now found suitable for publication in your prestigious journal.
Looking forward to hearing from you
Best regards,
Maddalena Giannella

Editor-in-Chief of Clinical Infectious Diseases

December, 2020

Dear Editor,

We have electronically re-submitted the revised manuscript CID-105646 entitled: “Development of a Risk Prediction Model for Carbapenem-Resistant Enterobacteriaceae Infection after Liver Transplantation: A Multinational Cohort Study**”**. We have carefully considered every comment and suggestion raised by the reviewers and changed our manuscript accordingly.

We attach a response letter with the answers to all the items raised by the reviewers and two copies of the revised manuscript, one of them with all the changes clearly marked in yellow.

Should you or any of the reviewers wish any further clarification, please, do not hesitate to contact us. We hope that our manuscript may be now found suitable for publication in your prestigious journal.

Looking forward to hearing from you

Best regards,

The corresponding authors

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Ajit P. Limaye, M.D., Special Section Editor
Clinical Infectious Diseases
email: cid.editorialoffice@idsociety.org

Bologna, December 2020

Dear Dr. Limaye:

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REVIEWER COMMENTS

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One minor point would be the following: All patients were screened for rectal CRE carriage at pre-defined time points. However, screening for CRE colonization at other sites was not uniformly performed. While it would make sense that patients with multi-site colonization are at higher risk for invasive infection, it would be important to highlight how this may impact the results.

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prophylaxis and which agents should be used are not well established. Even in pre-OLT carriers, the colonization data is often only acquired in the immediate post-operative period. Furthermore, most patients are found to be colonized with CRE during the first weeks after OLT. Finally, as shown in previous studies including unselected OLT recipients, both colonization at OLT and colonization after OLT were the strongest predictors of CRE infection (Freire et al Transplantation 2017, Giannella et al Clin Microbiol Infect 2019). For these reasons, we believe that an individualized risk stratification for colonized patients, either pre or post OLT, could help better targeting preventive strategies not only in the perioperative but also in the post-operative period.

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AUTHOR'S RESPONSE: We have re-performed the analysis following the suggestions of the reviewer. The table below shows indices of the model discrimination (Harrell's C statistic), accuracy (Brier Index) as well as improvements in the prediction accuracy versus a null model that contains only CRE colonization prior to transplant. As seen below, the more parsimonious model proposed by the reviewer displayed poorer discrimination and accuracy for identifying patients who develop CRE infection within 60 days post-transplant.

Model	**C-Statistic (AUC)** (higher number is better)	**Brier decomposition score** (lower scores are better)	**Improvements in prediction accuracy vs. null model -CRE col pre 60d)** (higher percentages are better)
Full model: 1. ATG 2. Reintervention 3. Mechanical ventilation 4. Acute renal failure 5. KPC carbapenem. 6. CRE col pre 60d 7. Multisite colon. Pre trans 8. CRE col post 60d 9. Multisite colon post trans	75.1 (71.3-78.9)	16.1 (6.6-25.6)	14.8%
Reviewer suggested model: 1. Reintervention 2. Mechanical ventilation 3. Acute renal failure 4. CRE col pre 60d	69.2 [65.2-73.2)	17.4 (7.6-27.2)	8.21%

However, we agree with the reviewer that a simpler model may be possible. Therefore, using a base model of CRE colonization within 60 days prior to transplant, we examined the degree to which including each variable from our original model improved model performance. Our analysis, in part, supported the reviewer's suggestion that ATG, KPC carbapenemase, and multisite colonization prior to transplantation could be removed from model without marked changes in prediction accuracy.

Addition of predictor to base model (CRE col pre 60d)	C-Statistic, AUC (95%)	Improvements in prediction accuracy vs. null model (CRE col pre 60d) (higher percentages are better)
ATG	53.8 (50.5-57.2)	0.71%
Reintervention	54.5 (51.0-58.0)	2.63%
Mechanical ventilation	59.7 (55.5-63.8)	7.45%
Acute renal failure	61.7 (57.5-65.8)	3.63%
KPC carbapenemase	53.9 (49.7-58.1)	0.72%
Multisite col. pre trans	53.9 (50.5-57.2)	0.62%
CRE colonisation post-transplant	57.6 (54.1-61.0)	1.71%
Multisite colon post-transplant	62.6 (58.7-66.5)	6.85%

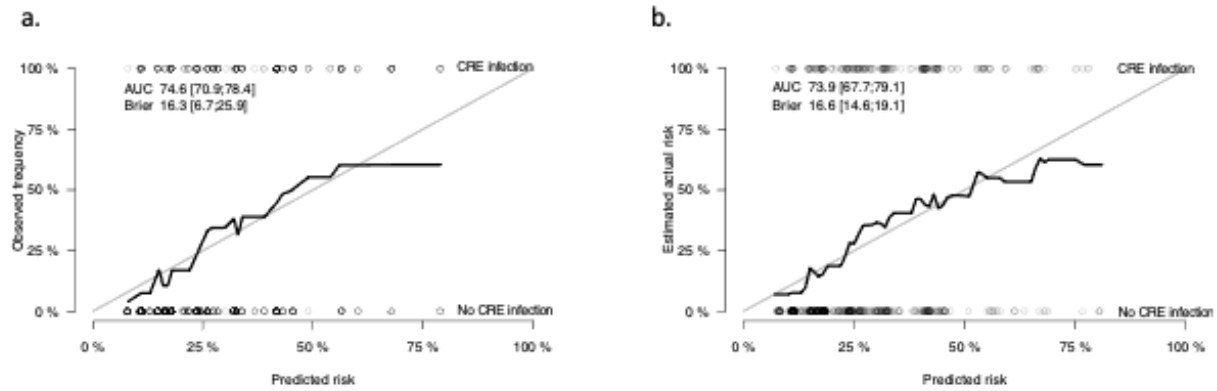
However, colonization documented post-transplant and colonization at additional sites (an indicator of higher inoculum even though not systematically assessed in all patients) were both associated with improved model performance. Therefore, we have propose a revised model that addresses the reviewer's suggestion as best as possible:

Table 3. Final multivariable analysis for predicting risk of CRE infection after liver transplantation

Variable	Subhazard ratio (95% CI)	β-coefficient	P value
Reintervention	1.37 (1.04-1.80)	0.30 (0.02-0.57)	0.02
Prolonged mechanical ventilation	2.01 (1.49-2.71)	0.70 (0.40-0.99)	<0.0001
Acute renal injury	1.69 (1.26-2.27)	0.52 (0.23-0.82)	0.001
CRE colonisation 60 days prior to transplant	2.17 (1.33-3.53)	0.78 (0.29-1.26)	0.002
CRE colonization within 60 days after transplant	1.41 (0.91-2.19)	0.35 (-0.09-0.78)	0.12
Multisite colonization within 60 days after transplant	2.87 (1.98-4.20)	1.06 (0.68-1.43)	<0.0001

Abbreviations: CI confidence interval, CRE carbapenem-resistant Enterobacteriaceae, KPC Klebsiella pneumoniae carbapenemase-producing.

The discrimination and calibration of this revised simpler model (6 variables) is nearly identical the originally proposed model (9 variables) for predicting CRE infection within 60 days post-transplant as shown in the figure below of the model fit for the (a) derivation dataset; and (b) Bootstrapped-resampled dataset.



Therefore, we have revised all of the corresponding tables, figures and nomogram (in the manuscript and online app) to reflect the new simpler model.

Minor comments:

- Anti-thymoglobulin is used throughout and perhaps should be changed to anti-thymocyte globulin.

AUTHORS' RESPONSE: We have done as suggested.

- BLBLI acronym on page 5 should be spelled out on first use.

AUTHORS' RESPONSE: We have added the spelling.

- Line 16 on page 7 should perhaps replace "revise" with "review".

AUTHORS' RESPONSE: We have done as suggested.

- Page 10, last paragraph: "Considering the competing risks of death or CRE infection…" CRE infection should be removed as it is not acting as a competing event when evaluating the risk of CRE infection.

AUTHORS' RESPONSE: We have done as suggested.

- Figure 1 on page 24 has "participating" misspelled.

AUTHORS' RESPONSE: We corrected the error.

Should you or any of the reviewers wish any further clarification, please, do not hesitate to contact us. We hope that our manuscript may be now found suitable for publication in your prestigious journal.

Looking forward to hearing from you

Best regards,

Maddalena Giannella

CID-105646-unmarked

Development of a Risk Prediction Model for Carbapenem-Resistant Enterobacteriaceae Infection after Liver Transplantation: A Multinational Cohort Study

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40-word summary: One third of CRE carriers develops infection after LT with dramatic impact on survival. Our prediction tool could enable better targeting of early interventions for CRE infection after LT as opposed to universal prophylaxis or treatment in high-prevalence centers.

ABSTRACT

Background. Patients colonized with carbapenem resistant Enterobacteriaceae (CRE) are at higher risk of developing CRE infection after liver transplantation (LT) with associated high morbidity and mortality. Prediction model for CRE infection after LT among carriers could be useful to target preventive strategies.

Methods. Multinational multicenter cohort study of consecutive adult patients underwent LT and colonized with CRE before or after LT, from January 2010 to December 2017. Risk factors for CRE infection were analyzed by univariate analysis and by Fine-Gray sub-distribution hazard model, with death as competing event. A nomogram to predict 30- and 60-day CRE infection risk was created.

Results. 840 LT recipients found to be colonized with CRE before (n=203) or after (n=637) LT were enrolled. CRE infection was diagnosed in 250 (29.7%) patients within 19 (IQR 9-42) days after LT. Pre- and post-LT colonization, multisite post-LT colonization, prolonged mechanical ventilation, acute renal injury, and surgical re-intervention were retained in the prediction model. Median 30 and 60-day predicted risk was 15% (IQR 11-24%) and 21% (IQR 15-33%), respectively. Discrimination and prediction accuracy for CRE infection was acceptable on derivation (AUC 74.6, Brier index 16.3) and bootstrapped validation dataset (AUC 73.9, Brier index 16.6). Decision-curve analysis suggested net benefit of model-directed intervention over default strategies (treat all, treat none) when CRE infection probability exceeded 10%. The risk prediction model is freely available as mobile application at https://idbologna.shinyapps.io/CREPostOLTPredictionModel/.

Conclusions. Our clinical prediction tool could enable better targeting interventions for CRE infection after transplant.

INTRODUCTION

The emergence and spread of carbapenem resistant *Enterobacteriaceae* (CRE) over the last two decades threatens the safety of patients undergoing life-saving surgical procedures or necessary immunosuppressive treatments [1]. Solid organ transplant (SOT) recipients, in particular those undergoing liver transplantation (LT), are at increased risk of colonization with CRE due to repeated contacts with health care system, exposure to broad spectrum antibiotics, complexity of surgery, and altered immune status [2]. Compared with other settings (e.g., geriatric units, long-term care facilities) the incidence of infection per CRE colonization days is higher in SOT recipients [3], and associated with poorer survival [4]. In a prospective multicentre study of 887 SOT recipients in Italy, mortality was more than 10 times higher in patients with positive cultures for CRE [5].

Knowledge of CRE infection risk factors is essential to develop targeted preventive strategies. Risk factors for CRE infection after LT were analysed in two large single-centre cohort studies, from Brazil and Italy, including 386 and 553 patients, respectively [6, 7]. In both studies CRE carriage, either detected before or after transplant, was found to be the strongest predictor of subsequent CRE infection. Based on these findings, some investigators have proposed that preventive strategies should be implemented in all CRE carriers undergoing LT [8]. However, a more accurate prediction of which carriers are at higher risk could help to target preventive measures or timely appropriate antibiotic therapy in patients at lower risk (i.e. < 10%) of CRE infection.

With this objective in mind, we performed a multicentre, multinational cohort study of patients undergoing LT colonized with CRE before or after transplantation, with the aim of developing a multivariable prediction model for CRE infection within 30 and 60 days of LT.

MATERIAL AND METHODS

Study design

We performed a multicentre multinational retrospective cohort study. The enrolment period was between January 2010 to December 2017, or from the onset of systematic CRE colonization screening in the centre to December 2017 (see supplementary Table 1). Follow-up was of 180 days after LT. Data were accrued from October 2019 to June 2020. Data sources were clinical charts and hospital electronic records, they were de-identified before entry into a standardized electronic case report form (eCRF), and managed using REDCap capture tool hosted by Alma Mater University of Bologna [9]. Collected data were periodically checked for accuracy by an investigator of the coordinating centre (MR). Queries for incongruous or missing data were submitted to investigators to ensure high quality and completeness. The study was first approved by Institutional Review Board (IRB) of the promoting centre (n. 155/2019/Oss/AOUBo on March 20, 2019), then by IRB of all participating centres.

Setting

Fifteen hospitals performing LT participated in the study: seven from Italy (Bologna, Modena, Turin, Padua, Palermo, Milan, and Udine); four from Brazil (2 in São Paulo, 1 in Fortaleza and 1 in Rio de Janeiro); two from Spain (Madrid and Majadahonda); one from United States (Miami); and one from Israel (Petah-Tikva) (see Supplementary Table 1 and Figure 1). An active surveillance screening for CRE colonization was required by the study protocol. All centres performed systematic screening of CRE carriage by rectal swab (RS), at inclusion in waiting list, at LT and weekly after LT until hospital discharge. Screening for CRE colonization at other sites was performed according to clinical judgment and local policy.

Study population

All consecutive adult (≥18 years) patients who underwent LT during the study period and were found to be colonized with CRE were enrolled. Patients were included only once at the time of the first CRE carriage detection. CRE was defined as any *Enterobacteriaceae* displaying *in vitro* non-susceptibility to any of the carbapenems according to the criteria (CLSI or EUCAST) adopted at the participating

centre during the study period. The colonization status was defined as isolation of CRE from RS 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 was defined when CRE was concomitantly isolated from more than one of such samples. Patients found to be colonized with CRE by samples obtained at inclusion in the waiting list, during the period in waiting list, or at the time of LT were considered as CRE carriers pre-LT, whereas those found to be colonized within 180 days after LT were considered as post-LT CRE carriers.

Predictors of CRE infection after LT

The primary endpoint was the time to development of CRE infection, diagnosed within 180 days after LT, according to Center for Disease Control criteria [10]. The assessment of CRE infection was made by the local investigator and revised by an investigator of the promoting centre (MR), in case of no agreement a third blinded investigator of the promoting centre (MG) was asked to review the case for establishing the final diagnosis. Infection severity was determined using SOFA score and septic shock criteria [11].

Candidate prediction variables included: 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 LT. Complications occurred within 90 days before LT were recorded and included: admission to intensive care unit (ICU); grade 3 and/or refractory ascites [12]; hepato-renal syndrome (HRS) [12]; gastrointestinal bleeding; hepatic encephalopathy [13]; development of acute-on-chronic liver failure (ACLF) [14]; any infection, candidemia, and *Clostridioides difficile* infection. For graft characteristics donor age, cold ischemia time, and combined transplant were collected. Intraoperative variables included: antibiotic prophylaxis, biliary anastomosis, bleeding with need of massive transfusion (≥40 units of cellular blood products), prolonged intervention (≥ 8 hours). Complications occurred from LT to the diagnosis of CRE infection or death or 180 days (whichever occurred first) were recorded and

included: re-intervention, acute kidney injury (AKI) according to KIDGO 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 [16]. Management of CRE colonization was recorded according to the following categories: no strategy adopted, anti-CRE surgical prophylaxis, decolonization with non-absorbable antibiotics, faecal microbiota transplantation, pre-emptive strategy (defined as early start of anti-CRE coverage upon to clinical deterioration). For outcome, duration of the hospital stay after LT, number of re-admissions within 180-days after LT, and all-cause 180-days mortality were recorded.

Sample size

Sample size required for the clinical prediction model was estimated for a time-to-event outcome using the pmsampsize package in R (version 4.02, Core Team (2020). **R**: A language and environment for statistical computing. **R** Foundation for Statistical Computing, Vienna, Austria) using the methods proposed by Riley et al. [17]. We estimated that 850 patients with 213 outcome events (event rate 0.25 by day 180) would be sufficient to evaluate 20 candidate predictors (>10 events per candidate predictor) with an estimated 60-day event rate of 0.17.

Missing data

A complete-case analysis was performed.

Statistical analysis methods

Categorical variables were expressed as absolute numbers and their relative frequencies. Continuous variables were expressed as mean ± standard deviation (SD) if normally distributed, or as median and interquartile range (IQR) if non-normally distributed. We used the Fine-Gray sub-distribution hazard model to develop a clinical prognostic index for CRE infection over the first 180 days after LT with death as competing event.

Although colonization status has been modelled in previous studies as a time-varying covariate [18], inclusion of the time-dependent covariates limits the ability of the Fine-Gray sub-distribution hazard model to make inferences about the effects of covariates on the cumulative incidence function (CIF) in the presence of competing risks, which is an underlying motivation for our model. Furthermore, simulation studies have shown that a sub-distribution approach for time-dependent covariates often produces misleading results [19], and is not currently recommended [20]. Therefore, we focused on colonization status as a risk factor only in the 60-days prior to and 60 days following OLT to minimize time-dependent effects.

Using a base model of CRE colonization within 60 days prior to transplant, we examined the degree to which including each other variable from our original model improved its performance. Additional candidate variables were selected *a priori* based on statistical significance in univariate analysis, published data and clinical experience and whether they improved prediction accuracy of the baseline model. Only variables available for assessment during the risk period were included. We assumed that a predictive model for CRE infection would have the most clinical utility for accurate prediction of CRE infection in the first 30 and 60 days post OLT, as the risk appeared to plateau after this period. Therefore we used C-index (AUC) to assess the discrimination of the model with censored CRE infection status, and calibration plots of day-60 observed versus expected absolute risks with the Brier score to assess the accuracy of predictions. To control for centre effect, the analysis was also performed for each participating centre. Model discrimination and calibration measures were calculated using 100-fold cross validation bootstrap resampling to avoid bias. A decision curve was prepared to examine potential model utility across a range of CRE infection probabilities (0-60%) at 60 days post LT. All analyses were performed using R version 4.02 with riskRegression, prodlim, ggplot2, survival, and rmda packages. The final regression model was visualized by preparing a nomogram to predict 30-day and 60-day risk using methods described by Zhang et al [21] and the regplot package. An online prediction tool (Shiny App) was prepared using the DynNom package in R. Results were reported according to TRIPOD guidelines [22].

RESULTS

Overall, 840 LT recipients colonized with CRE before or after transplantation were enrolled, with 250 episodes of CRE infection post LT (29.7%) during 104,596 patient days of follow-up. Distribution of patients and of CRE infections across participating centres is shown in Figure 1. The characteristics of study population are shown in Table 1. Median age was 55 (IQR 46-62) years, male sex (65.4%). Viral hepatitis was the primary indication for LT in 376/840 subjects (44.8%), followed by alcohol 207/840 (24.6%). Over one-quarter 247/840 (29.4%) of patients had hepatocellular carcinoma (HCC). MELD at inclusion in waiting list and at LT was 19 (IQR 14-25) and 23 (IQR 16-30), respectively. CRE carriage was detected before or at LT in 203 (24.2%) patients, the median time from detection to LT was 6 (IQR 0-32) days. The remaining 637 (75.8%) patients were found to be colonized with CRE within a median of 13 (IQR 7-28) days after LT. Comparison of pre- and post-LT carriers showed several differences (see Table 1). Specifically, pre-LT carriers presented with more severe liver disease and complicated clinical course before transplantation. CRE infection rate among pre- and post-LT carriers was 33% and 28.7%, and the median time to infection following LT was 9 (IQR 4-22) and 23 (IQR 13-52) days, respectively (see Supplementary Figure 1). The median time from detection of CRE carriage to the diagnosis of CRE infection was shorter for post-LT carriers (8 vs. 19 days, $p<0.001$). Among the 203 pre-OLT carriers, 51 received targeted perioperative prophylaxis, the rate of CRE infection was 31.4% and 33.6% among those exposed and unexposed to targeted prophylaxis ($p=0.90$), respectively. Post-LT CRE infections in pre-LT carriers presented with higher severity according to median SOFA and septic shock rates than in post-LT carriers. However, there was no difference in clinical outcome (all-cause 180 days mortality 56.7% vs. 58.5%, $p=0.46$), data shown in Supplementary Table 2.

To screen risk factors for CRE infection, we performed univariate analysis of patients with and without CRE infection as shown in Table 2. Considering the competing risks of death, a Fine-Gray

model was used to perform regression of risk factors to estimate their association with the cumulative incidence of CRE infection up to 180 days post LT. Baseline cumulative incidence of death versus CRE infection over 180 following OLT is shown in Figure 2. The following variables were retained in the final model: pre-LT CRE colonization, post-LT CRE colonization, multisite post-LT colonization, prolonged MV, AKI, and surgical re-intervention (Table 3). The fitted regression risk model was then rendered as a nomogram (Figure 3). Model-predicted cumulative probabilities of developing CRE infection at day 30 and 60 post LT are shown in Figure 4. The median 30 and 60-day predicted risk was 15% (IQR 11-24%) and 21% (IQR 15-33%), respectively. Nearly one-quarter (23.5%) of patients at day 30, and 3% of patients at day 60 had a cumulative predicted risk of CRE infection ≤10%. The model showed acceptable 60-day discrimination and prediction accuracy for CRE infection when assessed against the derivation AUC 74.6 (95% 70.9-78.4), Brier index 16.3 (95%CI 6.7-25.9) and bootstrapped validation dataset AUC 73.9 (95%CI 67.7-79.1), Brier index 16.6 (95%CI 14.6-19.1) (Figure 5). Discrimination and calibration were also evaluated by centre and were consistent with the overall performance, except for higher variability and lower AUCs in centres with lower number of recruited patients (see Supplementary Figure 2). The score was designed to be used in the immediate peri-transplant period, ideally from the day of transplantation up to 2-3 weeks after transplantation. During this period, the patient should be frequently evaluated for carriage status, multisite colonization, prolonged MV, AKI, and/or re-intervention. According with the presence of such variables the cumulative risk of CRE infection within 30 and 60 days after LT can be predicted (see examples in Figure 6 and mobile application at https://idbologna.shinyapps.io/CREPostOLTPredictionModel/, instructions for using app are further provided as supplementary material).

Finally, we explored the potential clinical utility of our model using a decision-curve analysis (DCA) to examine the "net benefit" of applying the prediction model across a range of CRE infection threshold probabilities [23]. A theoretical risk-model guided strategy (i.e. empiric administration of CRE-active antibiotics) is compared against two default strategies- "treat all" and "treat none." A model is only

298 useful at a given disease threshold if it has a higher net benefit than treat all or treat none. Our
299 analysis suggested that the model-directed interventions for CRE post LT would show net benefit
300 over default strategies when the overall CRE infection threshold probability exceeded 10% (Fig. 7).

301

302 **DISCUSSION**

303 Assessing a large multinational cohort of LT recipients colonized with CRE (n=840) we have found
304 that one third developed CRE infection after LT. This rate was similar among pre- and post-LT CRE
305 carriers. Most infection episodes occurred within 2 months after LT. The time to infection was earlier
306 for pre-LT CRE carriers, and the clinical severity was higher in this group. All-cause 6-month mortality
307 among carriers was as high as 30%, with similar rates between pre- and post-LT CRE carriers.
308 However, it reached 58% among patients who developed CRE infection.

309 We confirmed that the CRE carriage status is strongly associated with the risk of CRE infection after
310 LT, either if detected before or after LT [6, 7]. However, we also confirmed our previous hypothesis
311 that, given a probability of CRE infection higher than 10%, a strategy of treating all CRE carriers could
312 be inferior to a targeted strategy in terms of efficacy, as well as safety, considering potential adverse
313 events of anti-CRE drugs and the risk for further selection pressure. A longitudinal risk stratified
314 approach, could be useful for several reasons. In general, LT is not an elective surgery, thus it could
315 be difficult to implement a strategy before transplantation other than in a minority of patients
316 known to be colonized at the time of inclusion or while on waiting list. Another opportunity for
317 intervention could be surgical prophylaxis. However, data about colonization is frequently available
318 only during the immediate post-operative period. Finally, in most patients CRE colonization becomes
319 evident in the first weeks after LT, typically with a complicated post-operative course, which also
320 increases the risk of infection.

Along with pre- and post-LT colonization status, multisite colonization after LT was a predictor of infection. The relationship between multisite colonization with infection development has been already observed in other settings [3]. It is indicative of high colonization burden leading to an increased risk of CRE infection [24]. Indeed, its inclusion in our prognostic model improved model performance. Nevertheless, multisite colonization was not systematically screened in this study. Therefore, we cannot rule out that colonization from different sites may be weighted differently in the model if this risk factor had been systematically screened, as patients with a more complicated course may have been cultured more frequently.

The other complications included in our score are widely described as factors involved in the increase risk of infection after LT [25]. AKI has been also associated with microbiome dysbiosis that could favour prolonged CRE carriage and infection [26]. As for MV, it is worth mentioning that we considered such variable, as a qualitative data, from LT to CRE infection, death or end of follow-up. A more detailed information about timing and duration of MV may improve the model performance.

Our study has several limitations. First, the retrospective design could have limited identification of all CRE carriers. However, the presence of a local prospective registry of CRE carriers and/or good collaboration with local microbiology laboratories reduced this bias. Heterogeneity in local assays and criteria for identification and determination of CRE could exist. However, obtained data are in line with international literature. The majority of involved centres were high endemic CRE hospitals, with KPC as main mechanism of CRE. Thus, our findings could not apply to transplant centres with low prevalence of CRE, or with KPC alternative main mechanisms among CRE. Finally, we analysed the CRE infection risk only in colonized patients, thus the performance of our prediction tool should be confirmed in unselected LT patients.

To conclude, one third of CRE carriers develop infection after LT with a dramatic impact on patient survival. Effective preventive or pre-emptive strategies are needed. Our clinical prediction tool could enable better targeting of early interventions for CRE infection after transplant as opposed to

346 universal prophylaxis or treatment in high-prevalence centres. Further studies are needed to further
347 validate the model and establish which strategy would be more effective, in terms of adverse events
348 and collateral resistance impact and, ultimately patient survival after transplantation.

349

350

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CONFLICT OF INTEREST

Authors have no conflict of interest related to the present study.

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439 **Table 1.** Characteristics of study population according to the timing of CRE carriage detection

	Total N=840 (%)	**pre-OLT carrier N=203 (%)**	**post-OLT carrier N=637 (%)**	**P**
Demographic data				
Age (years) [median (IQR)]	55 (46-62)	53 (42-60)	56 (47-62)	0.005
Sex, male	549 (65.4)	132 (65)	417 (65.5)	0.9
Comorbidities				
Myocardial infarction	23 (2.7)	5 (2.5)	18 (2.8)	0.827
Congestive heart failure	29 (3.5)	7 (3.4)	22 (3.5)	0.9
Peripheral vascular disease	33 (3.9)	6 (3)	27 (4.2)	0.4
Cerebrovascular disease	22 (2.6)	8 (3.9)	14 (2.2)	0.18
COPD	46 (5.5)	6 (3)	40 (6.3)	0.07
Connective tissue disease	9 (1.1)	3 (1.5)	6 (0.9)	0.38
Peptic ulcer disease	62 (7.4)	20 (9.9)	42 (6.6)	0.09
Diabetes without organ damage	147 (17.5)	38 (18.7)	109 (17.1)	0.3
Moderate/severe renal disease	82 (9.8)	28 (13.8)	54 (8.5)	0.03
Diabetes with organ damage	76 (9)	15 (7.4)	61 (9.6)	0.2
Any tumor within 5 years	271 (32.3)	45 (22.2)	226 (35.5)	0.001
Moderate/severe liver disease	820 (97.6)	199 (98)	621 (97.5)	0.79
Charlson index [median (IQR)]	5 (4-7)	5 (3-6)	5 (4-7)	0.004
Underlying liver disease				
Viral hepatitis	376 (44.8)	74 (36.5)	302 (47.4)	0.006
Alcohol	207 (24.6)	55 (27.1)	152 (23.9)	0.2
Metabolic disease	69 (8.2)	12 (5.9)	57 (8.9)	0.1
Autoimmune disease	41 (4.9)	12 (5.9)	29 (4.6)	0.45
Fulminant hepatitis	52 (6.2)	7 (3.4)	45 (7.1)	0.04
Hepatocellular carcinoma	247 (29.4)	37 (18.2)	210 (33)	<0.001
Prior transplant	39 (4.6)	20 (9.9)	19 (3)	<0.001
MELD at waiting list inclusion (median, IQR)	19 (14-25)	21 (16-28)	19 (14-24)	<0.001
MELD at OLT (median, IQR)	23 (16-30)	27 (21-32)	22 (15-29)	<0.001
Variables relative to the last 90 days before OLT				
Hepato-renal syndrome	235 (28)	88 (43.3)	147 (23.1)	<0.001
Ascites grade III/refractory	385 (45.8)	108 (53.2)	277 (43.5)	0.01
GI bleeding	138 (16.4)	43 (21.2)	95 (14.9)	0.025
Encephalopathy	343 (40.8)	96 (47.3)	247 (38.8)	0.02
ICU admission	161 (19.2)	70 (34.5)	91 (14.3)	<0.001
ACLF	98 (11.7)	44 (21.7)	54 (8.5)	<0.001
Any infection	326 (38.8)	137 (67.5)	189 (29.7)	<0.001
Intraoperative variables				
Combined transplant	45 (5.4)	12 (5.9)	33 (5.2)	0.7
Donor age	51 (36-65)	50 (35-65)	51 (36-65)	0.4
Cold ischemia time (hours) (median, IQR)	7 (6-8)	7 (6-8)	7 (6-8)	0.62
Biliary anastomosis				
Duct to duct without Kehr	382 (45.5)	84 (41.4)	298 (46.8)	0.19
Duct to duct with Kehr	347 (41.3)	76 (37.4)	271 (42.5)	0.20

Choledochojejunostomy	111 (13.2)	43 (21.2)	68 (10.7)	<0.001
Intraoperative bleeding	294 (35)	88 (43.3)	206 (32.3)	0.005
Prolonged surgery (>8 hours)	248 (29.5)	70 (34.5)	178 (27.9)	0.046
Induction regimen				
None	87 (10.4)	24 (11.8)	63 (9.9)	0.51
Bolus of steroids	701 (83.5)	171 (84.2)	530 (83.2)	0.75
Antithymocyte globulins	26 (3.1)	3 (1.5)	23 (3.6)	0.09
Basiliximab	167 (19.9)	37 (18.2)	130 (20.4)	0.55
Rituximab	14 (1.7)	0 (0)	14 (2.2)	0.02
Maintenance regimen				
Steroids	612 (72.9)	162 (79.8)	450 (70.6)	0.011
Cyclosporin	42 (5)	8 (3.9)	34 (5.3)	0.28
Tacrolimus	770 (91.7)	181 (89.2)	589 (92.5)	0.15
Micophenolate mophetil	287 (34.2)	70 (34.5)	217 (34.1)	0.9
Post-operative complications				
Acute renal injury	462 (55)	118 (58.1)	344 (54)	0.33
Renal replacement therapy	299 (35.6)	90 (44.3)	209 (32.8)	0.003
Mechanical ventilation ≥48 hours	266 (31.7)	64 (31.5)	202 (31.7)	0.96
PGNF	128 (15.2)	28 (13.8)	100 (15.7)	0.58
Re-intervention	322 (38.3)	74 (36.5)	248 (38.9)	0.56
Re-transplantation	79 (9.4)	11 (5.4)	68 (10.7)	0.025
Rejection	152 (18.1)	30 (14.8)	122 (19.2)	0.17
CMV treatment	225 (26.8)	46 (22.7)	179 (28.1)	0.15
Infections other than CRE	479 (57)	106 (52.2)	373 (58.6)	0.12
Clostridioides difficile infection	37 (4.4)	8 (3.9)	29 (4.6)	0.44
Candidemia	68 (8.1)	11 (5.4)	57 (8.9)	0.14
CRE carriage				
Time between OLT and carriage detection (days) (median, IQR)	9 (0.25-21)	-6 (-32-0)	13 (7-28)	<0.001
First positive sample				
Rectal swab	719 (85.6)	189 (93.1)	530 (83.2)	0.001
Respiratory sample	45 (5.4)	3 (1.5)	42 (6.6)	0.002
Urine	69 (8.2)	22 (10.8)	47 (7.4)	0.14
Multi-site colonization	113 (13.5)	26 (12.8)	87 (13.7)	0.81
Resistance mechanism				
KPC	600 (71.4)	149 (73.4)	451 (70.8)	0.53
VIM	7 (0.8)	3 (1.5)	4 (0.6)	0.23
OXA-48	17 (2)	6 (3)	11 (1.7)	0.21
Not tested	115 (13.7)	19 (9.4)	96 (15.1)	0.05
Management of CRE colonization				
No strategy adopted	521 (62)	85 (41.9)	436 (68.4)	<0.001
Target perioperative prophylaxis	51 (6.1)	51 (25.1)	0 (0)	<0.001
Decolonization	16 (1.9)	4 (2)	12 (1.9)	0.57
Pre-emptive strategy[1]	21 (2.5)	6 (3)	15 (2.4)	0.4
Outcome				
Length of ICU stay after OLT (days) (median, IQR)	7 (4-15)	7 (4-14)	7 (4-15)	0.38
Length of hospital stay after OLT (days) (median, IQR)	27 (16-50)	23 (14-37)	29 (17-54)	<0.001
All-cause 6-months mortality	263 (31.3)	69 (34)	194 (30.5)	0.39

Time from OLT to death (days) (median, IQR)	70 (29-145)	32 (10.5-77.5)	88.5 (43-162)	<0.001
Hospital re-admissions (median, IQR)	0 (0-1)	0 (0-1)	0 (0-1)	0.07

[1]Start of an anti-CRE treatment if complications occur

Abbreviations: IQR interquartile range, COPD chronic obstructive pulmonary disease, MELD Model End Stage Liver Disease, OLT orthotopic liver transplant, ICU intensive care unit, ACLF acute on chronic liver failure, PGNF primary graft non-function, CMV cytomegalovirus, CRE carbapenem-resistant *Enterobacteriaceae*, KPC *Klebsiella pneumoniae* carbapenemase-producing, SBP spontaneous bacterial peritonitis.

447 **Table 2.** Comparison of patients with and without CRE infection within 180 days after liver transplant

	Patients without CRE infection, n=590 (%)	**Patients with CRE infection, n=250 (%)**	***P***
Demographic data			
Age (years) [median (IQR)]	55 (46-61)	56 (47-62)	0.5
Sex, male	389 (65.9)	160 (64)	0.59
Comorbidities			
Myocardial infarction	17 (2.9)	6 (2.4)	0.7
Congestive heart failure	19 (3.2)	10 (4)	0.57
Peripheral vascular disease	27 (4.6)	6 (2.4)	0.14
Cerebrovascular disease	15 (2.5)	7 (2.8)	0.83
COPD	30 (5.1)	16 (6.4)	0.51
Connective tissue disease	6 (1)	3 (1.2)	0.81
Peptic ulcer disease	44 (7.5)	18 (7.2)	0.89
Diabetes without organ damage	103 (17.5)	44 (17.6)	0.96
Moderate/severe renal disease	45 (7.6)	37 (14.8)	0.001
Diabetes with organ damage	55 (9.3)	21 (8.4)	0.67
Any tumor within 5 years	192 (32.5)	79 (31.6)	0.79
Moderate/severe liver disease	576 (97.6)	244 (97.6)	0.98
Charlson index [median (IQR)]	5 (4-7)	5 (4-7)	0.56
Underlying liver disease			
Viral hepatitis	260 (44.1)	116 (46.4)	0.53
Alcohol	157 (26.6)	50 (20)	0.04
Metabolic disease	48 (8.1)	21 (8.4)	0.89
Autoimmune disease	32 (5.4)	9 (3.6)	0.26
Fulminant hepatitis	36 (6.1)	16 (6.4)	0.87
Hepatocellular carcinoma	174 (29.5)	73 (29.2)	0.93
Prior transplant	24 (4.1)	15 (6)	0.22
MELD at waiting list inclusion (median, IQR)	19 (14-24)	19 (14-26)	0.73
MELD at OLT (median, IQR)	22.5 (16-29)	25 (16-33)	0.02
Events prior 90 days to OLT			
Hepato-renal syndrome	150 (25.4)	85 (34)	0.01
Ascites grade III/refractory	278 (47.1)	107 (42.8)	0.25
Bleeding	89 (15.1)	49 (19.6)	0.11
Encephalopathy	245 (41.5)	98 (39.2)	0.53
ICU admission	110 (18.6)	51 (20.4)	0.55
ACLF	66 (11.2)	32 (12.8)	0.5
Infections	223 (37.8)	103 (41.2)	0.36
Intraoperative variables			
Combined transplant	25 (4.2)	20 (8)	0.03
Donor age	51 (35-65)	51 (36-67)	0.6
Cold ischemia time (hours) (median, IQR)	7 (6-8)	7 (5:50-8)	0.36
Choledochojejunostomy	71 (12)	40 (16)	0.22
Intraoperative bleeding	196 (33.2)	98 (39.2)	0.1
Prolonged surgery	162 (27.5)	86 (34.4)	0.04
Induction regimen			
Bolus of steroids	476 (80.7)	225 (90)	0.001

Antithymocyte globulins	12 (2)	14 (5.6)	0.006
Basiliximab/Daclizumab	113 (19.2)	54 (21.6)	0.42
Rituximab	6 (1)	8 (3.2)	0.02
Maintenance regimen			
Steroids	414 (70.2)	198 (79.2)	0.007
Cyclosporin	31 (5.3)	11 (4.4)	0.6
Tacrolimus	549 (93.1)	221 (88.4)	0.03
Micophenolate mophetil	215 (36.4)	72 (28.8)	0.03
Post-operative complications			
Acute renal injury	286 (48.5)	176 (70.4)	<0.001
Renal replacement therapy	173 (29.3)	126 (50.4)	<0.001
Mechanical ventilation >48 hours	135 (22.9)	131 (52.4)	<0.001
PGNF	74 (12.5)	54 (21.6)	0.001
Re-intervention	200 (33.9)	122 (48.8)	<0.001
Re-transplantation	43 (7.3)	36 (14.4)	0.001
Rejection	101 (17.1)	51 (20.4)	0.26
CMV DNAemia>100,000 copies/mL	156 (26.4)	69 (27.6)	0.73
Infections other than CRE	333 (56.4)	146 (58.4)	0.6
Clostridioides difficile infection	26 (4.4)	11 (4.4)	0.99
Candidemia	36 (6.1)	32 (12.8)	0.001
CRE carriage			
Pre-OLT carriage	136 (23.1)	67 (26.8)	0.25
Post-OLT carriage	454 (76.9)	183 (73.2)	0.25
Rectal swab	517 (87.6)	202 (80.8)	0.01
Respiratory sample	21 (3.6)	24 (9.6)	<0.001
Urine sample	50 (8.5)	19 (7.6)	0.67
Multisite colonization	54 (9.2)	59 (23.6)	<0.001
pre-OLT	17 (2.9)	9 (3.6)	<0.001
post-OLT	37 (6.3)	50 (20)	0.66
Genotype of the colonizing strain			
KPC	418 (70.8)	182 (72.8)	0.61
VIM	4 (0.7)	3 (1.2)	0.45
OXA-48	11 (1.9)	6 (2.4)	0.6
Not tested	76 (12.9)	39 (15.6)	0.3
Management of CRE colonization			
Target perioperative prophylaxis	35 (5.9)	17 (6.8)	0.6
Decolonization	12 (2)	4 (1.6)	0.7
Pre-emptive strategy[1]	11 (1.9)	10 (4)	0.07
Outcome			
Length of ICU stay after OLT (days) (median, IQR)	6 (3-10)	14 (5-29)	<0.001
Length of hospital stay after OLT (days) (median, IQR)	22 (15-40)	43 (24-76)	<0.001
All-cause 6-months mortality	118 (20)	145 (58)	<0.001
Hospital re-admissions (median, IQR)	0 (0-1)	0 (0-1)	<0.001

448 [1]Start of an anti-CRE treatment if complications occur.

449 Abbreviations: IQR interquartile range, COPD chronic obstructive pulmonary disease, MELD Model

450 End Stage Liver Disease, OLT orthotopic liver transplant, ICU intensive care unit, SBP spontaneous

bacterial peritonitis, ACLF acute on chronic liver failure, PGNF primary graft non-function, CMV cytomegalovirus, CRE carbapenem-resistant *Enterobacteriaceae*, KPC *Klebsiella pneumoniae* carbapenemase-producing.

454 **Table 3.** Final multivariable analysis for predicting risk of CRE infection after liver transplantation

Variable	Subhazard ratio (95% CI)	β-coefficient	P value
Reintervention	1.37 (1.04-1.80)	0.30 (0.02-0.57)	0.02
Prolonged mechanical ventilation	2.01 (1.49-2.71)	0.70 (0.40-0.99)	<0.0001
Acute renal injury	1.69 (1.26-2.27)	0.52 (0.23-0.82)	0.001
CRE colonisation 60 days prior to transplant	2.17 (1.33-3.53)	0.78 (0.29-1.26)	0.002
CRE colonization within 60 days after transplant	1.41 (0.91-2.19)	0.35 (-0.09-0.78)	0.12
Multisite colonization within 60 days after transplant	2.87 (1.98-4.20)	1.06 (0.68-1.43)	<0.0001

455

456 Abbreviations: CI confidence interval, CRE carbapenem-resistant Enterobacteriaceae.

457

Figure legends:

Figure 1. Study flow chart with detailed data about involved centers.

Figure 2. Cumulative incidence of CRE infection and death post-transplant. The cumulative incidence was derived from the estimated sub-distribution hazard function following regression with a Fine-Gray model.

Figure 3. Model-based nomogram for predicting 30 and 60-day cumulative risk of developing CRE infection. Points are summed for each risk factor.

Figure 4. Distribution of model-predicted (a) 30-day and (b) 60-day risk for CRE infection following LT.

Figure 5. Calibration curves for the (a) derivation dataset and (b) Bootstrap resampled validation dataset. The C-index (AUC) and Brier score are expressed as the point estimates and 95% CI. An AUC > 0.8 is considered to give good discrimnatory accuracy for a clinical prediction model. The Brier score is a measure of the accuracy of probabilistic predictions. The lower the Brier score is for a set of predictions, the better the predictions are calibrated. An ideal model has pairs of observed and predicted probabilites that like on the 45-degree angle line. Dotplots shown the distribution of predicted risk for patients who developed (top) and did not develop (bottom) CRE infection.

Figure 6. 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) LT patient. The blue lines with shading in the nomogram represent the distribution of individual risk factors (0=no, 1=yes) and the distribution of total assigned points on the bottom scale in the original derivation data set . Red points on the upper scale indicate assigned points per risk factor. The red arrowsat the bottom of the nomogram indicate the total calculated points for the patient and corresponding 30-day and 60 day predicted cumulative risk with 95% CI.

Figure 7. Decision-curve analysis of model-directed anti CRE therapy. Net benefit at 60 days represents true cases of CRE infection that would be treated with a CRE-active antibiotic regimen-patients without CRE infection unnecessarily receiving CRE-active therapy. The curves indicate that a model-directed antibiotic strategy should have a higher net benefit than default strategies (all patients receive CRE-active therapy or no patients receive CRE-active therapy) at CRE infection probabilities greater than 10-50 %.

Figure 1

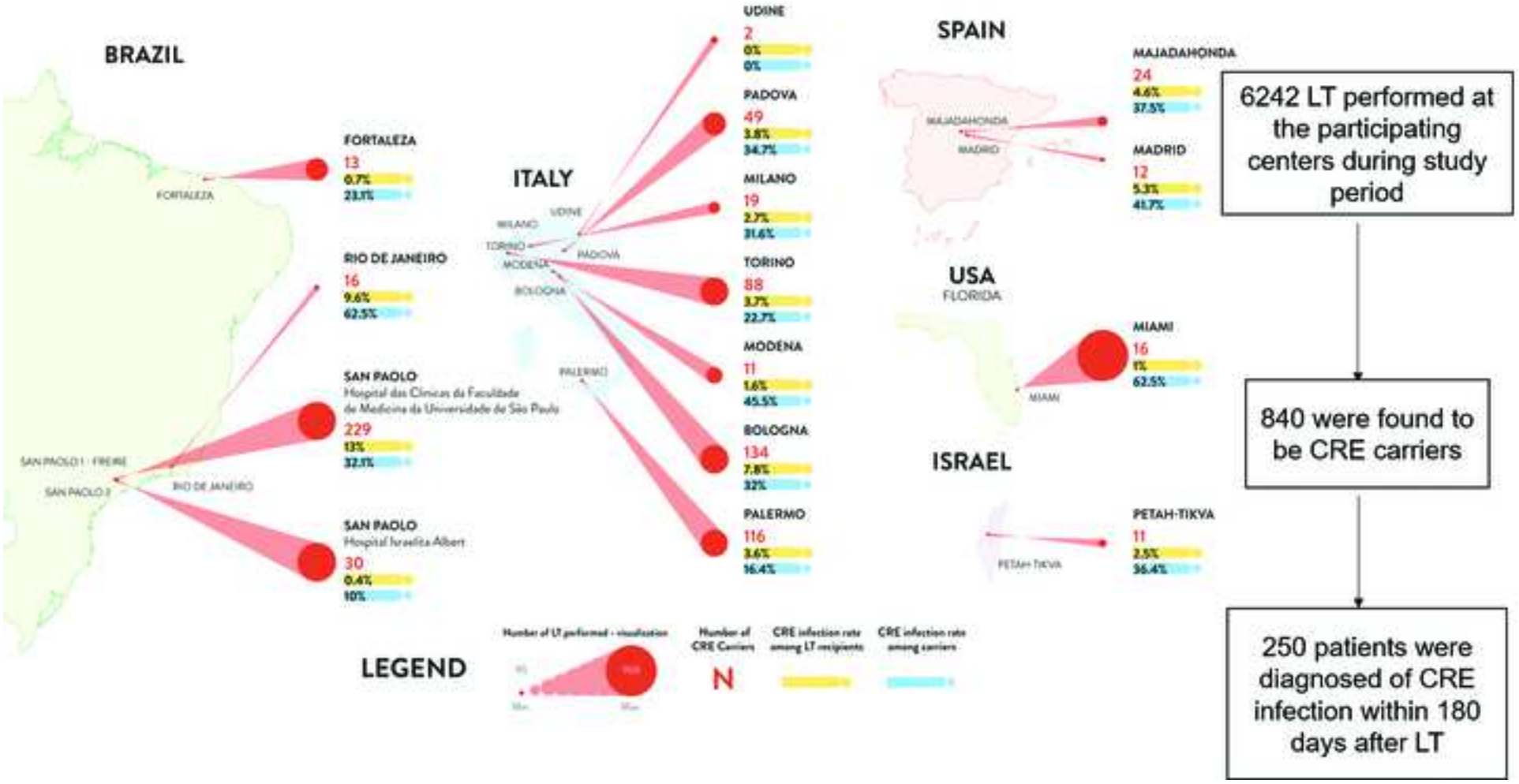


Figure 2

Click here to access/download;Figure (.tif and .eps files only);Figure 2.tif

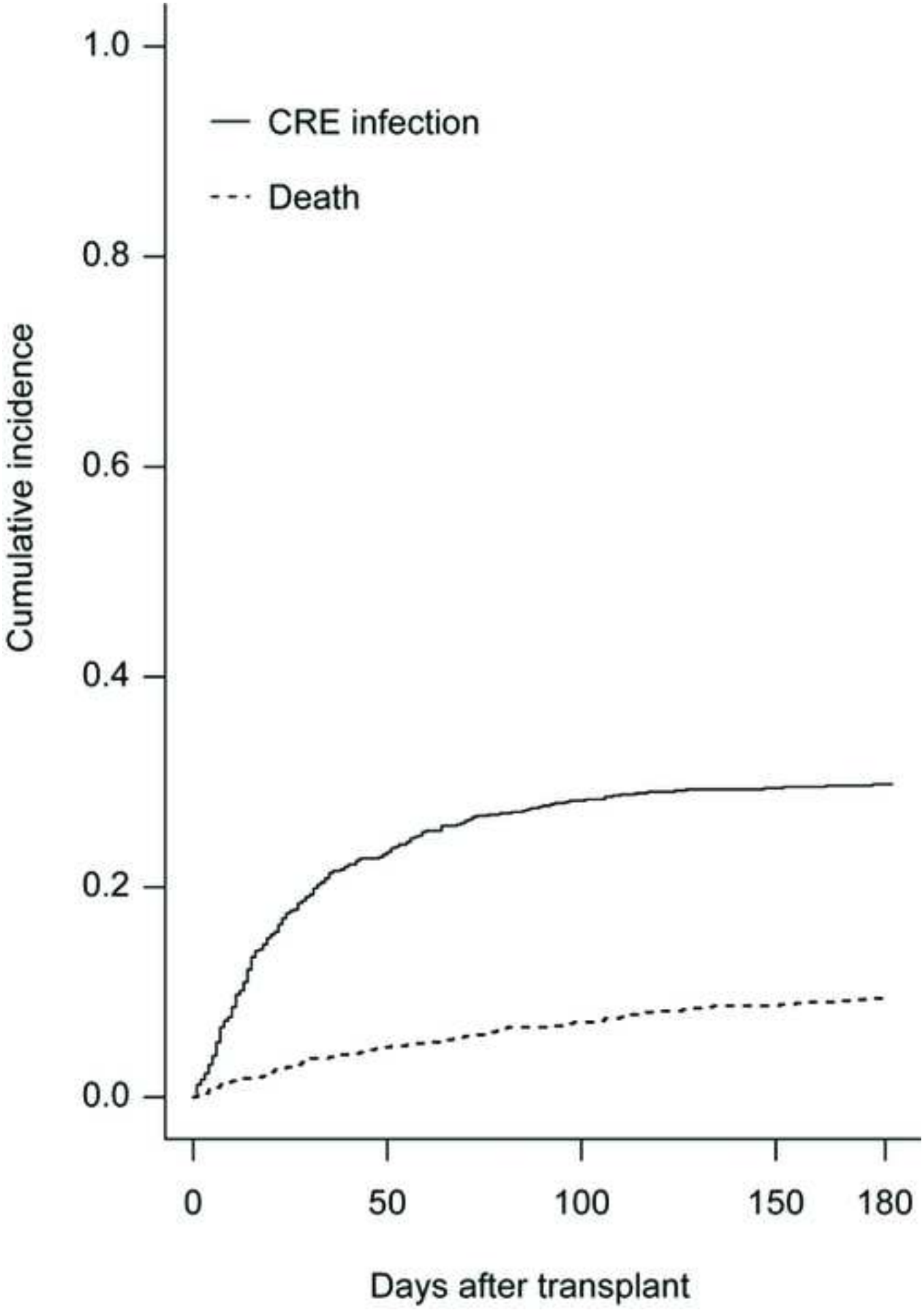


Figure 3

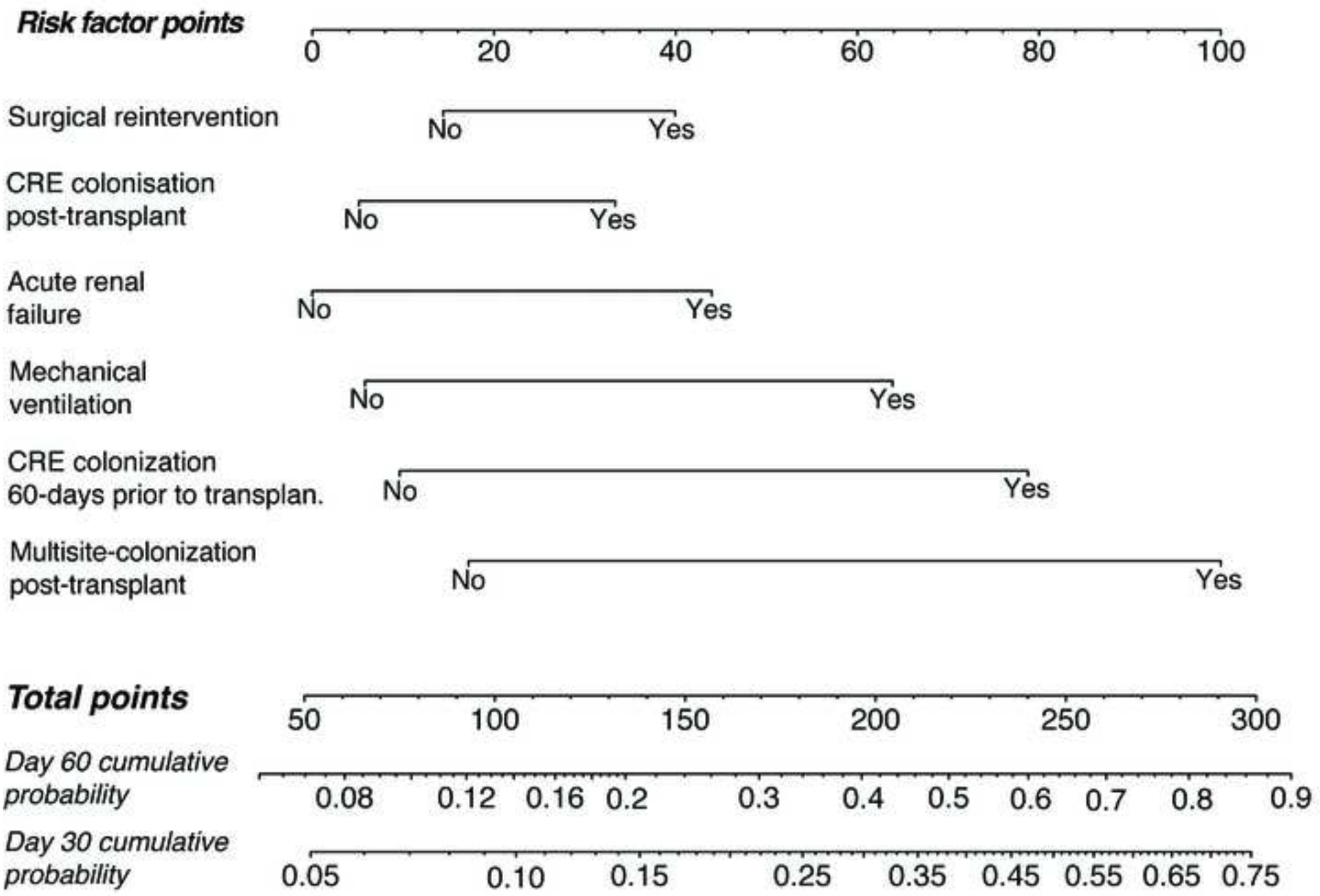


Figure 4

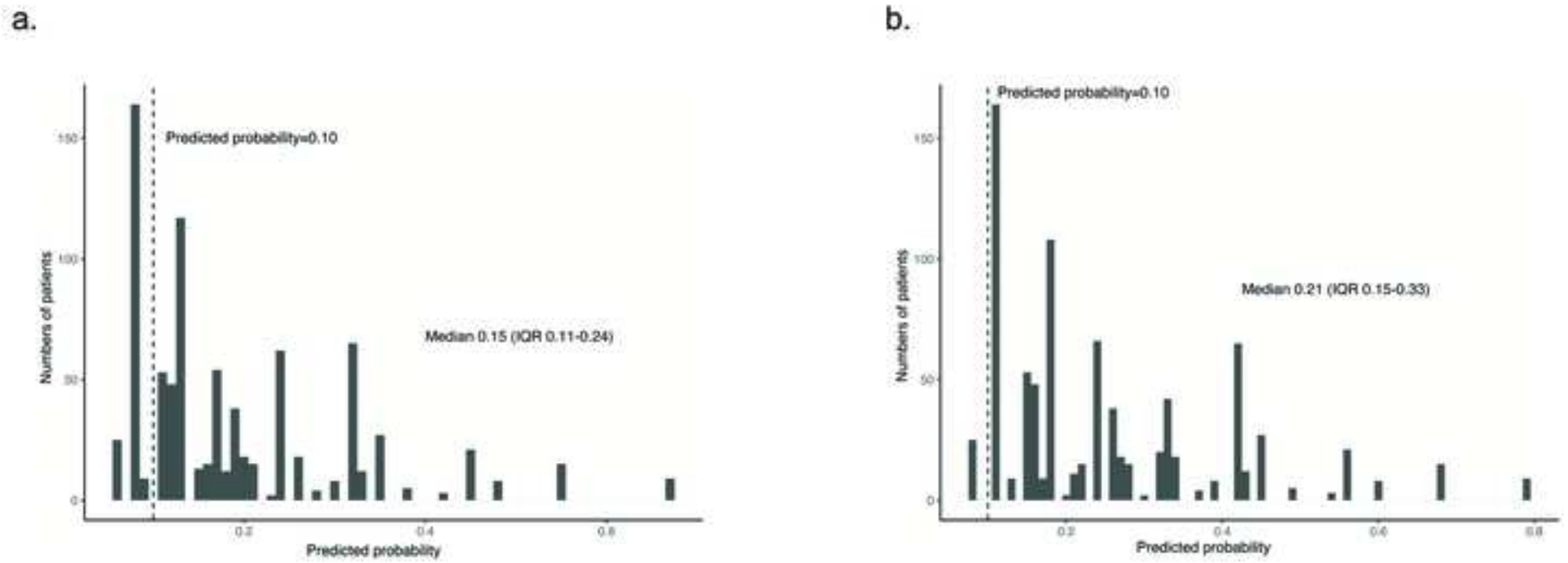


Figure 5

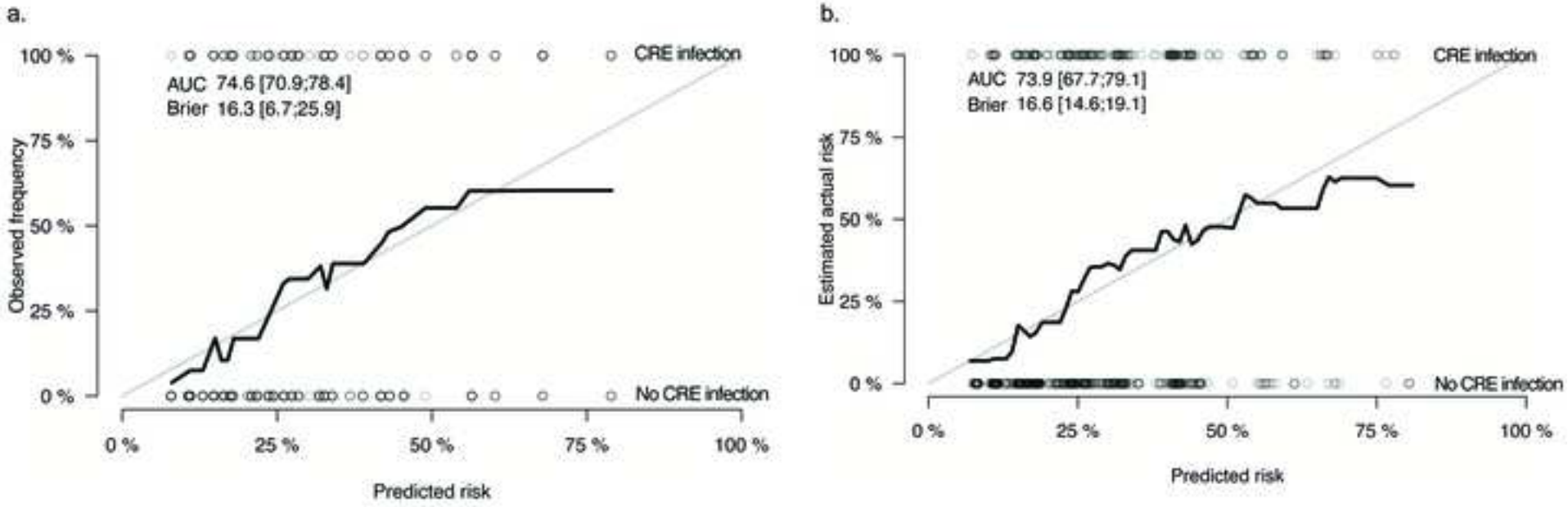


Figure 6

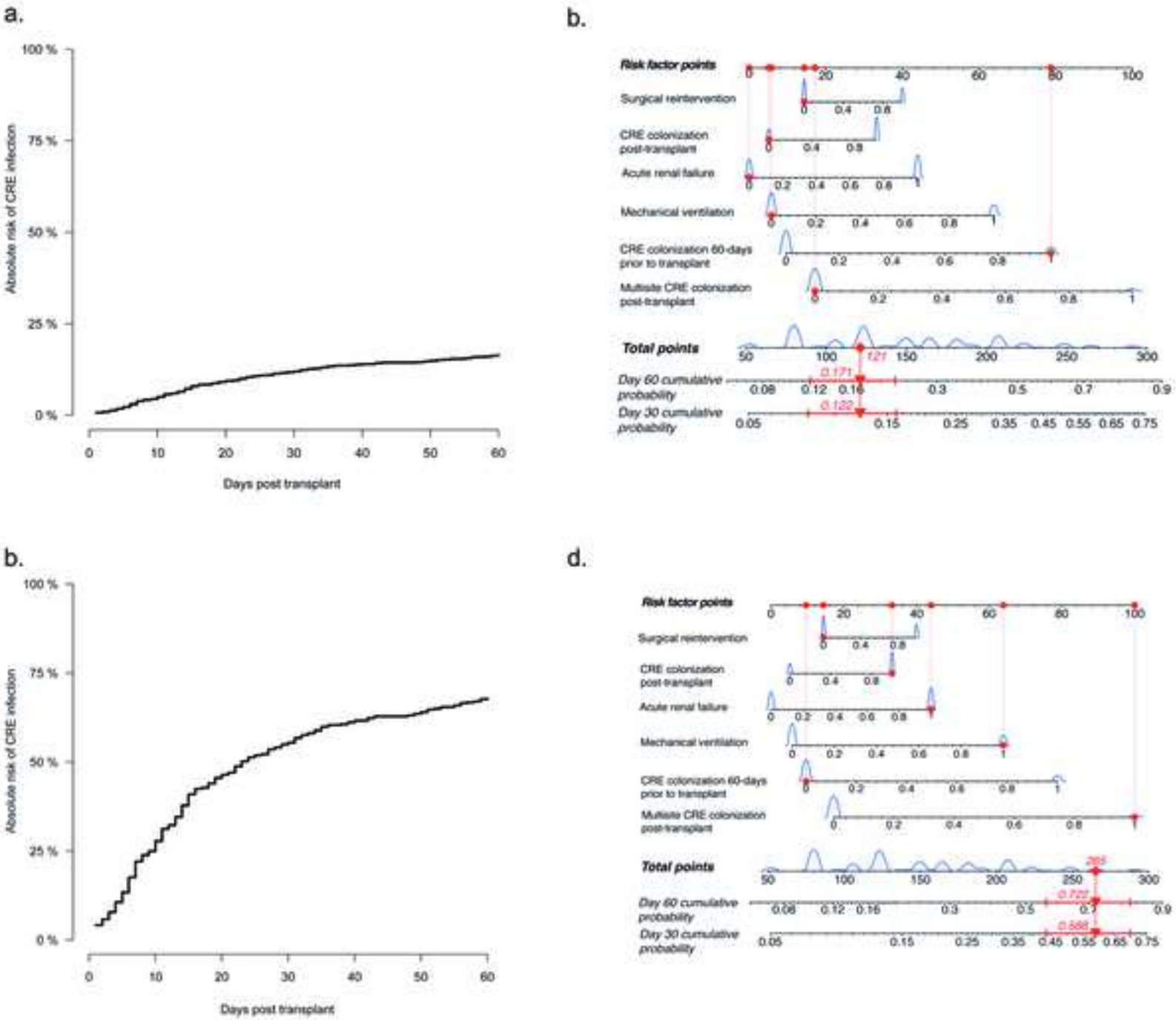
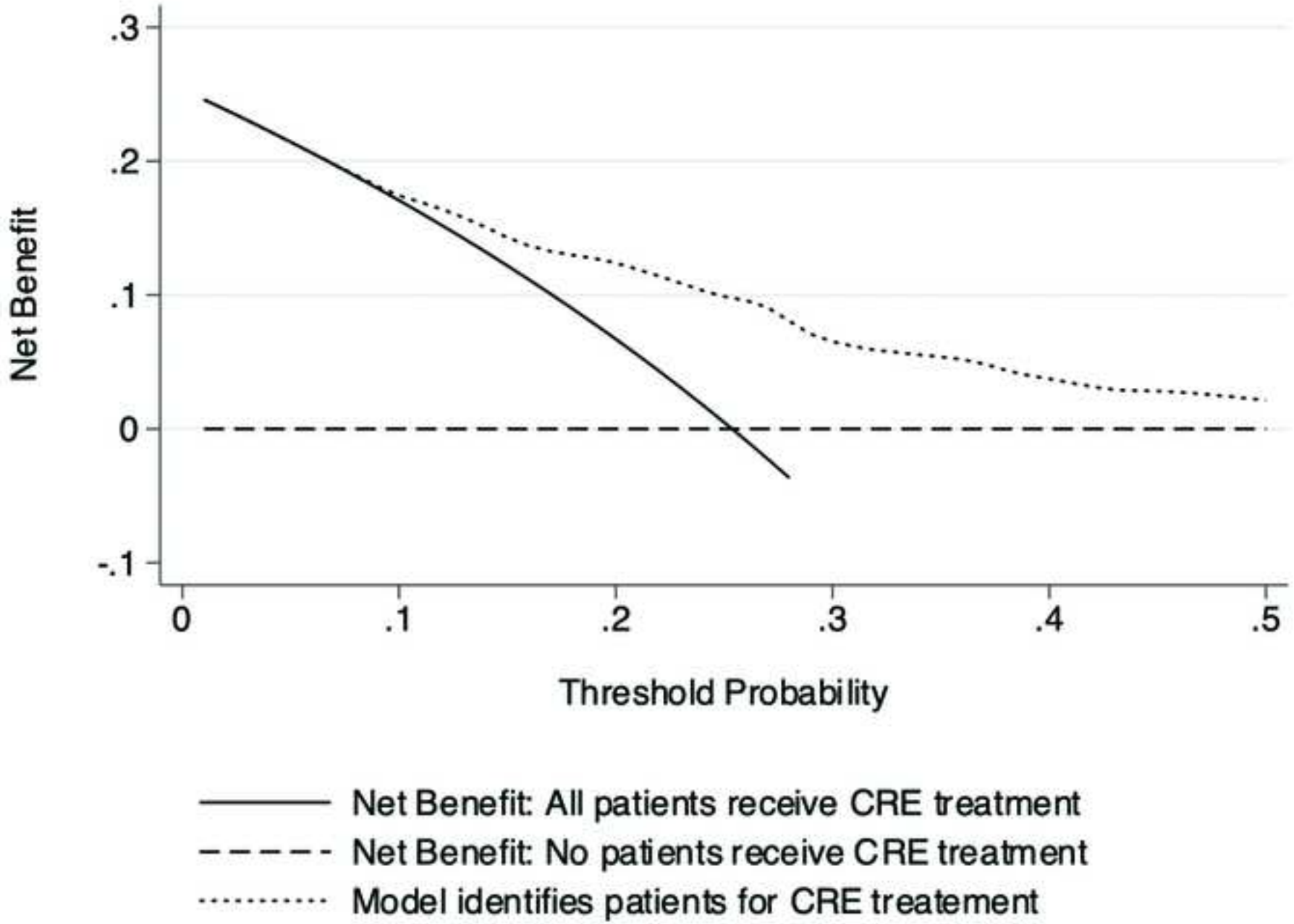


Figure 7



Supplementary Table 1: Details about participating centers

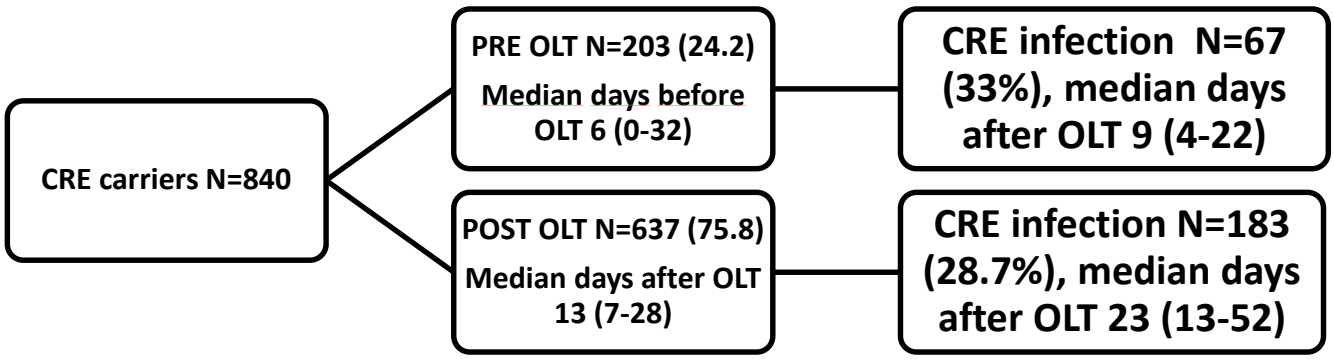
Center	Year of implementation systematic screening for CRE carriage before and after LT	Microbiology methods for CRE carriage screening	Standard regimen of antibiotic prophylaxis and its duration	Local policy for management of CRE carriers at LT	Local policy for management of CRE carriers after LT
Policlinico S. Orsola - Bologna	2010	Selective culture - molecular test	Amoxicillin/clavulanate 24 hours	No strategy	Antibiotic coverage for CRE only upon to the suspicion of infection
IRCCS ISMETT - Palermo	2013	Selective culture	Ampicillin + Cefotaxime 48 hours	No strategy	Antibiotic coverage for CRE only upon to the suspicion of infection
AOU Città della Salute e della Scienza - Torino	2014	Immune assay	Piperacillin/tazobactam 72 hours	Targeted anti-CRE prophylaxis in selected cases	Antibiotic coverage for CRE if complications occur
Multivisceral Transplant Unit - Padua	2013	Selective culture	III generation cephalosporin 24 hours	No strategy	Antibiotic coverage for CRE only upon to the suspicion of infection
Ca' Granda Ospedale Maggiore	2014	Selective culture	Piperacillin/tazobactam 24 hours	No strategy	Antibiotic coverage for CRE only upon to the suspicion of infection

Policlinico - Milano					
Azienda Ospedaliero-Universitaria - Modena	2012	Molecular test	Ampicillin+Cefotaxime 72 hours	Targeted anti-CRE prophylaxis IN selected cases	Antibiotic coverage for CRE in absence of symptoms if complications occur
Presidio Ospedaliero Universitario S. Maria della Misericordia - Udine	2012	Selective culture	Piperacillin/tazobactam > 72 hours	Targeted anti-CRE prophylaxis	Antibiotic coverage for CRE if complications occur
Hospital das Clinicas da Faculdade de Medicina da Universidade - São Paulo	2009	Selective culture	Ampicillin+Cefotaxime 48 hours	Targeted anti-CRE prophylaxis	No strategy
Hospital Israelita Albert - São Paulo	2010	Selective culture	Cefotaxime + Amoxicillin/clavulanate 48 hours	Targeted anti-CRE prophylaxis	No strategy

Quinta D'or Hospital – Rio de Janiero	2010	Selective culture	Ampicillin/sulbactam 24 hours	Targeted anti-CRE prophylaxis	Antibiotic coverage for CRE only upon to the suspicion of infection
Hospital Universitario Walter Cantidio - Fortaleza	2015	Selective culture	Cefotaxime + Ampicillin 24 hours	Targeted anti-CRE prophylaxis	No strategy
Hospital Universitario Puerta de Hierro - Majadahonda	2009	Selective culture	Cefotaxime + Amoxicillin/clavulanate 48 hours	No strategy	No strategy
Hospital General Universitario Gregorio Maranon - Madrid	2016	Selective culture	Cefazolin 48 hours	Targeted anti-CRE prophylaxis	Antibiotic coverage for CRE only upon to the suspicion of infection
Jackson Memorial Institute - Miami	2010	Selective culture	Ampicillin/sulbactam + Fluconazole 24 hours	Targeted anti-CRE prophylaxis	No strategy

Rabin Medical Center, Beilinson Hospital – Petah-Tikva	2013	Molecular test	Cefazolin 12 hours	No strategy	No strategy

Supplementary Figure 1. Distribution of CRE infections according to CRE carriage acquisition before or after OLT



Supplementary Table 2: Characteristics of CRE infections according to time of CRE carriage acquisition.

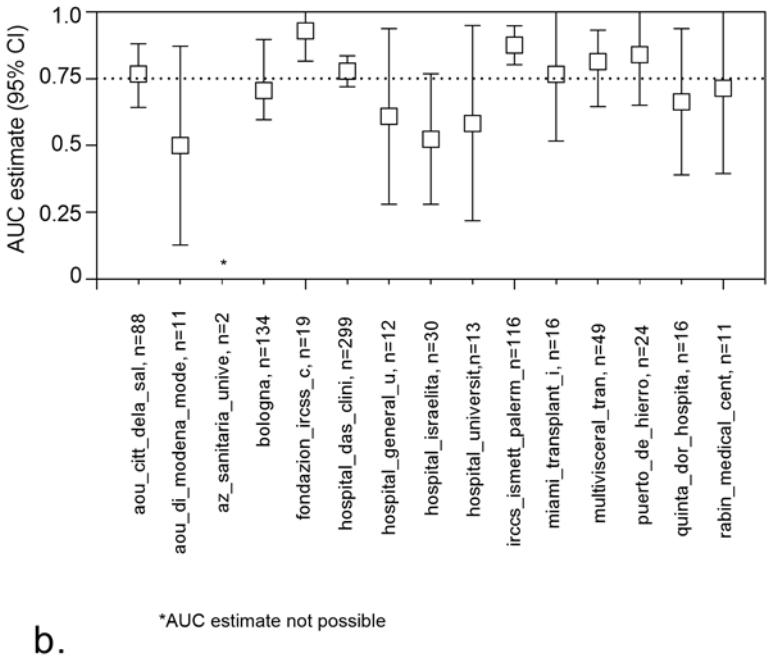
	Total N=250 (%)	CRE pre N=67 (%)	CRE post N=183 (%)	p
Time to infection				
from OLT (days) (median, IQR)	19 (9-42)	9 (4-22)	23 (13-52)	<0.001
from carriage detection (days) (median, IQR)	10 (3-25)	19 (8-56)	8 (3-18)	<0.001
Clinical severity				
SOFA score at infection onset (median, IQR)	8 (5-13)	11 (5-15)	6 (4-12)	0.005
Septic shock	96 (38.4)	35 (52.2)	61 (33.3)	0.005
Type of infection				
BSI	89 (35.6)	25 (37.3)	64 (35)	0.42
Primary	34/89 (38.2)	10 (40)	24 (37.5)	0.8
Secondary	37/89 (41.6)	11 (44)	26 (40.6)	0.77
Catheter-related	18/89 (20.2)	4 (16)	14 (21.9)	0.54
Pneumonia	60 (24)	16 (23.9)	44 (24)	0.97
IAI	56 (22.4)	9 (13.4)	47 (25.7)	0.026
UTI	22 (8.8)	4 (6)	18 (9.8)	0.25
SSI	65 (26)	23 (34.3)	42 (23)	0.07
Management of CRE infection				
Appropriate empiric therapy	149 (59.6)	45 (67.2)	104 (56.8)	0.09
Appropriate targeted therapy	216 (86.4)	59 (88.1)	157 (85.8)	0.41
Treatment duration (days) (median, IQR)	14 (7-20)	12 (5-22)	14 (7-19)	0.5
Source control	134 (53.6)	28 (68.3)	63 (67.7)	0.95
Time from CRE infection to source	2 (0-7)	3.5 (1-10)	1 (0-6)	0.028

control (days) (median, IQR)				
Outcome				
Length of ICU stay (days) (median, IQR)	14 (5-29)	14 (6-24)	13 (5-30)	0.98
Length of hospital stay after OLT (days) (median, IQR)	43 (24-76)	33 (19-49)	46 (26-85)	0.002
All-cause 6-months mortality	145 (58)	38 (56.7)	107 (58.5)	0.46
Hospital re-admissions (median, IQR)	0 (0-1)	0 (0-1)	0 (0-1)	0.49

Abbreviations: IQR interquartile range, OLT orthotopic liver transplant, SOFA sequential organ failure assessment, BSI bloodstream infection, IAI intra-abdominal infection, UTI urinary tract infection, SSI surgical site infection, CRE carbapenem-resistant *Enterobacteriaceae*, ICU intensive care unit.

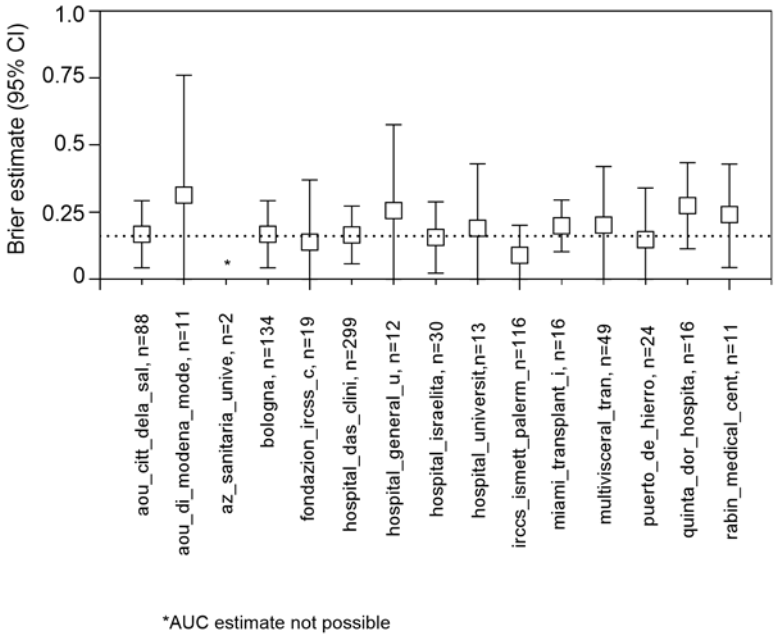
Supplemental Fig 2. Study-site specific CRE risk model (a) discrimination; and (b) prediction accuracy as indicated by c-index (AUC) and the Breir score. AUC> 0.8 indicate good discriminatory accuracy of the mode. are indicative of better model descimination. Lower Brier scores (range 0-1) are indicative of better prediction accurary

a.



*AUC estimate not possible

b.



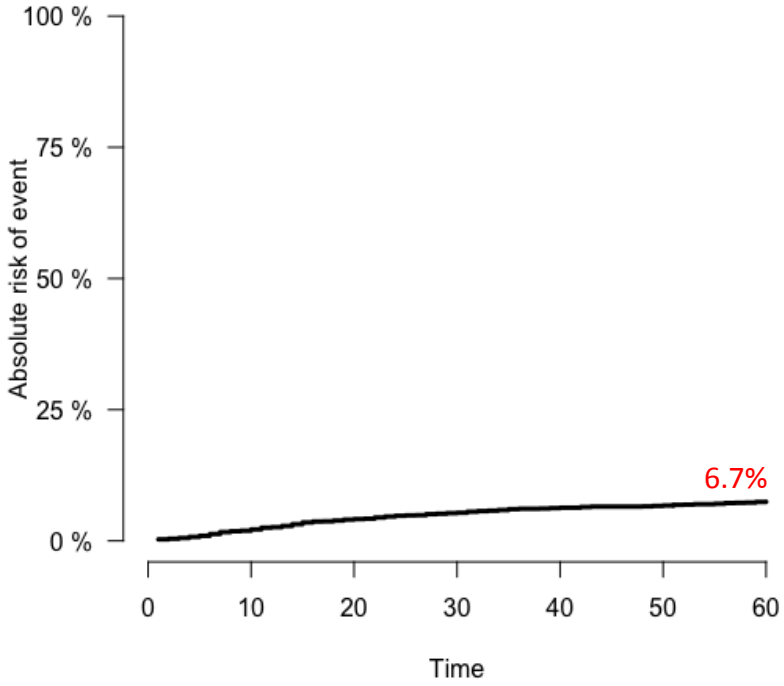
*AUC estimate not possible

Instructions for using app

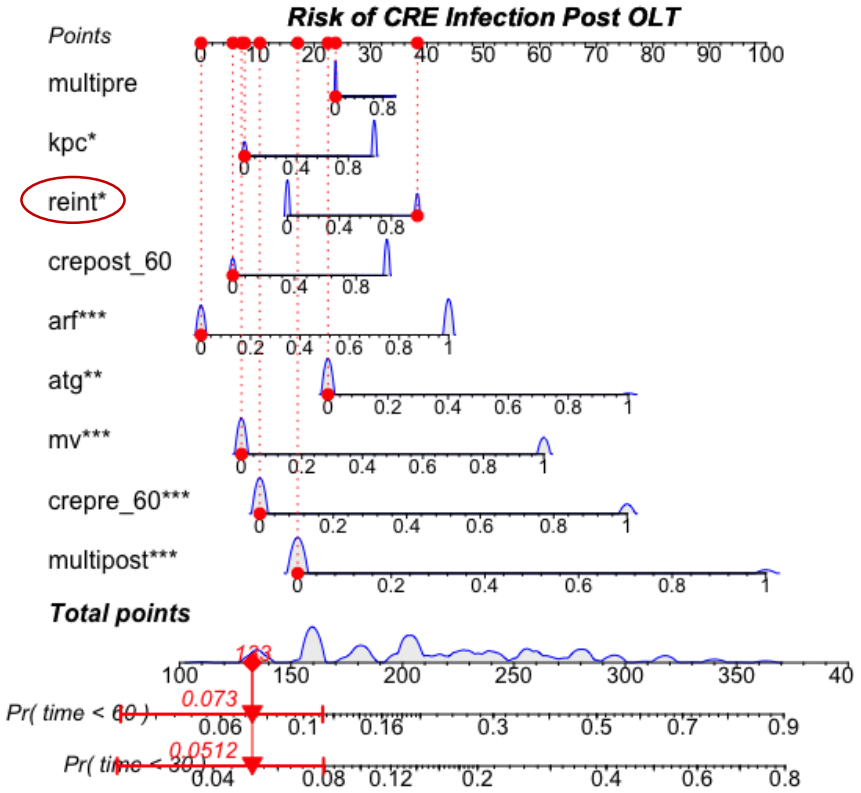
Click here to access/download;Supplemental data/Appendix-published online only;CREwebapp.pptx

Patient ID #22

Original Fine-gray model



Weighted Cox-regression



Type "0" when risk factor absent; type "1" when risk factor present

atg
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reint
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arf
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mv
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kpc
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crepre_60
0

multipre
0

crepost_60
0

multipost
0

Risk factors
Are entered in
As “0” or “1”

☑ Predicted Survival at this Follow Up:

time
60

Enter time for specific prediction

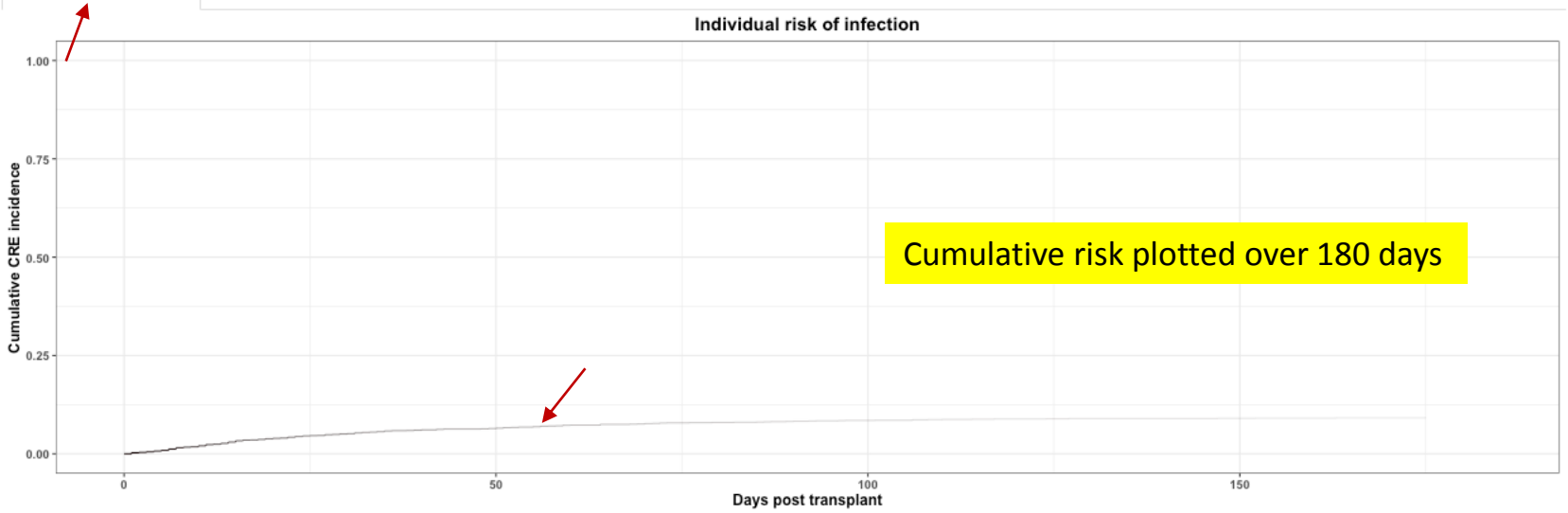
☑ Alpha blending (transparency)

Predict

Press Quit to exit the application

Quit

CRE cumulative risk | CRE risk at specified day | Numerical CRE risk summary | Model Summary



Cumulative risk plotted over 180 days

Type "0" when risk factor absent; type "1" when risk factor present

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Predicted Survival at this Follow Up:

time
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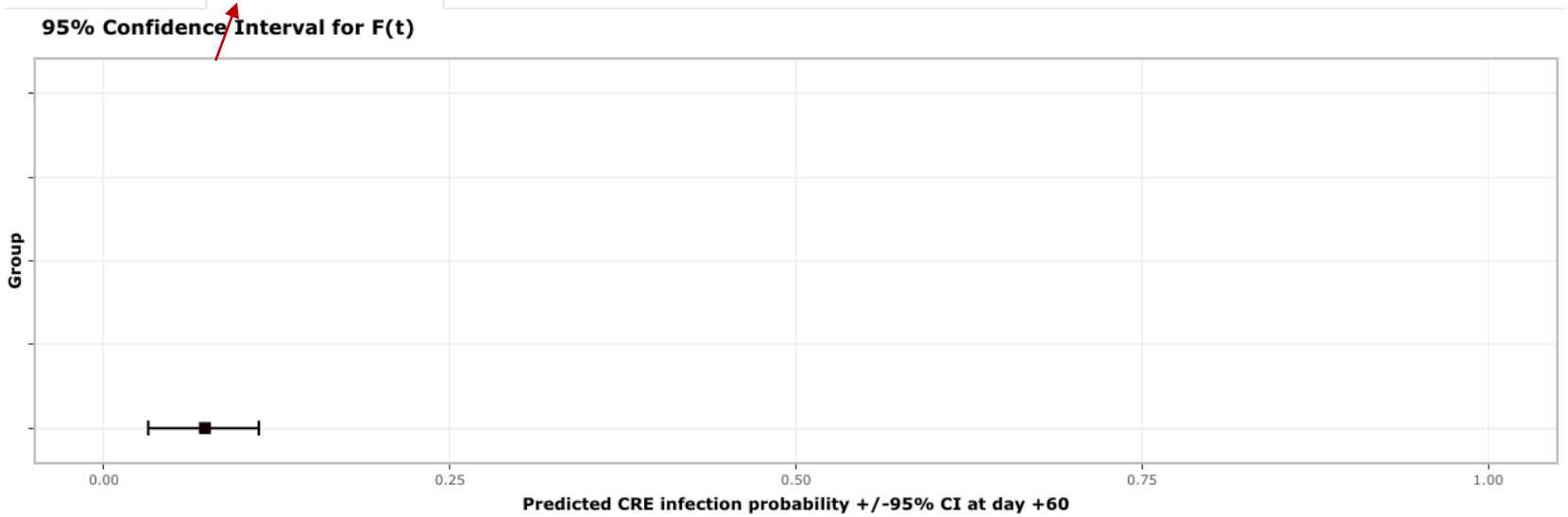
Alpha blending (transparency)

Predict

Press Quit to exit the application

Quit

CRE cumulative risk | CRE risk at specified day | Numerical CRE risk summary | Model Summary



Risk estimate plotted for Day 60
Note… the confidence intervals are affected by the number of patients with this combination of risk factor, thus the model provides the user an indication of uncertainty in prediction based on the specific combination of risk factors

Type "0" when risk factor absent; type "1" when risk factor present

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Predicted Survival at this Follow Up:

time
60

Alpha blending (transparency)

Predict

Press Quit to exit the application

Quit

CRE cumulative risk | CRE risk at specified day | Numerical CRE risk summary | Model Summary

time	atg	reint	arf	mv	kpc	crepre_60	multipre	crepost_60	multipost	Prediction	Lower.bound	Upper.bound
1	60	0	1	0	0	0	0	0	0	0.073	0.032	0.112

Numeric Risk prediction with 95% CI

Type "0" when risk factor absent; type "1" when risk factor present

atg
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arf
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mv
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kpc
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crepre_60
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multipre
0

crepost_60
0

multipost
0

Risk factors
Are entered in
As “0” or “1”

Predicted Survival at this Follow Up:

time
60

You can go back and change the time frame for prediction…

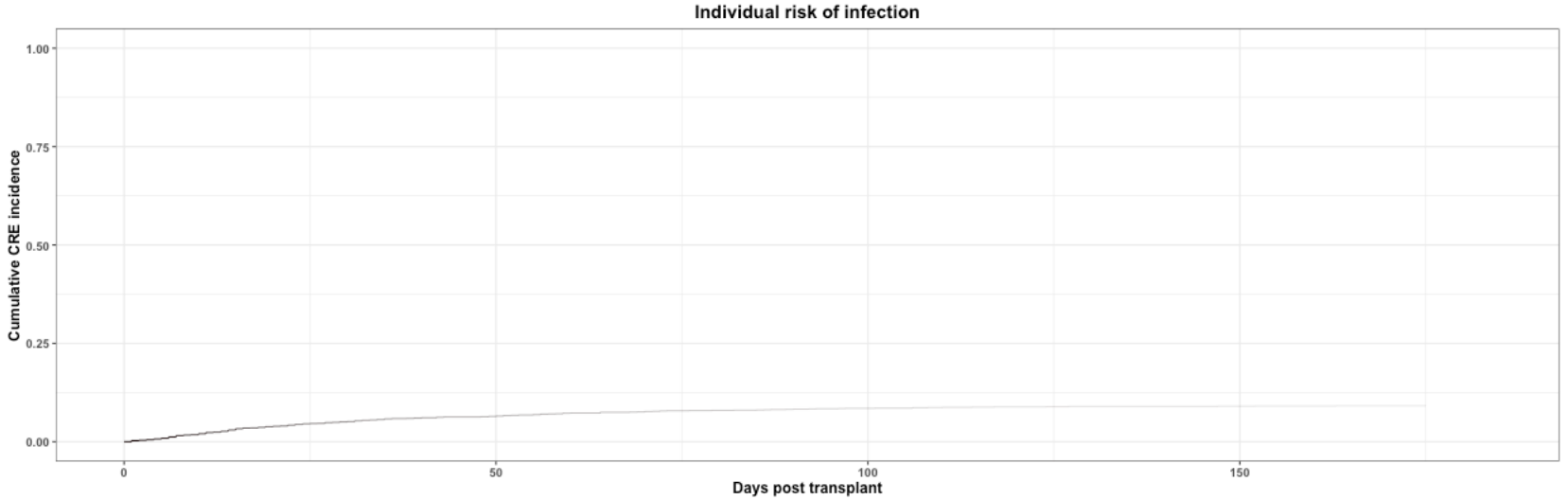
Alpha blending (transparency)

Predict

Press Quit to exit the application

Quit

CRE cumulative risk | CRE risk at specified day | Numerical CRE risk summary | Model Summary



Type "0" when risk factor absent; type "1" when risk factor present

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crepost_60
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multipost
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☑ Predicted Survival at this Follow Up:

time
60

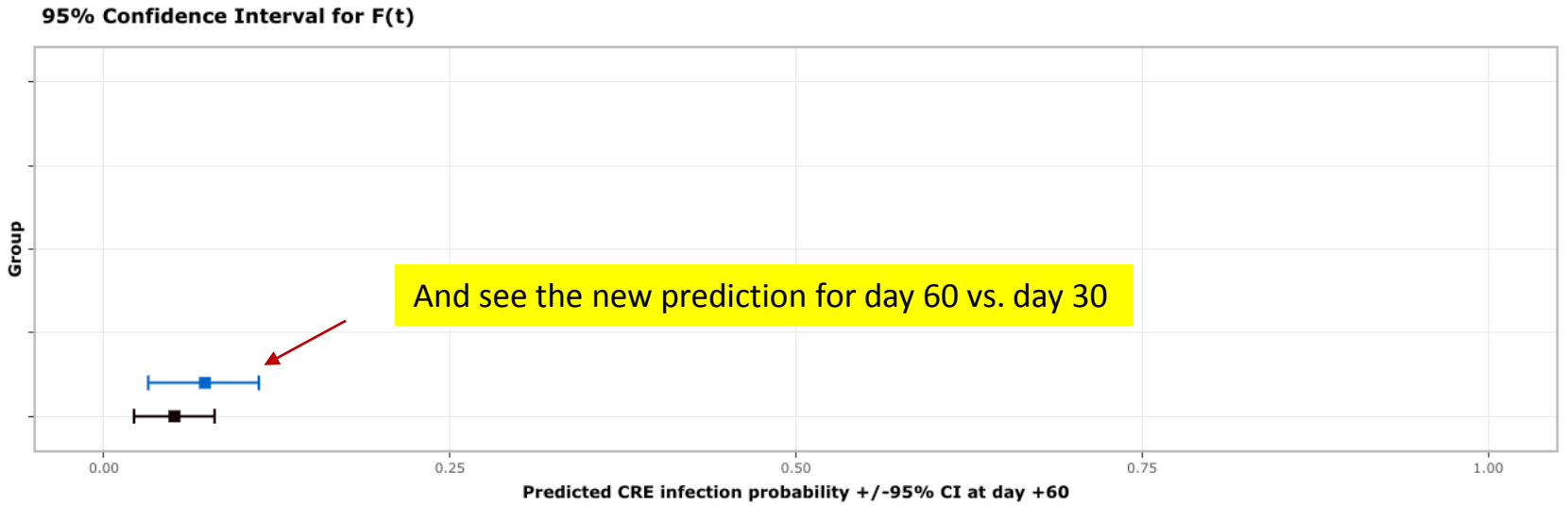
☑ Alpha blending (transparency)

Predict

Press Quit to exit the application

Quit

CRE cumulative risk | CRE risk at specified day | Numerical CRE risk summary | Model Summary



Type "0" when risk factor absent; type "1" when risk factor present

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arf
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kpc
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crepre_60
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multipre
0

crepost_60
1

multipost
0

☑ Predicted Survival at this Follow Up:

time
60

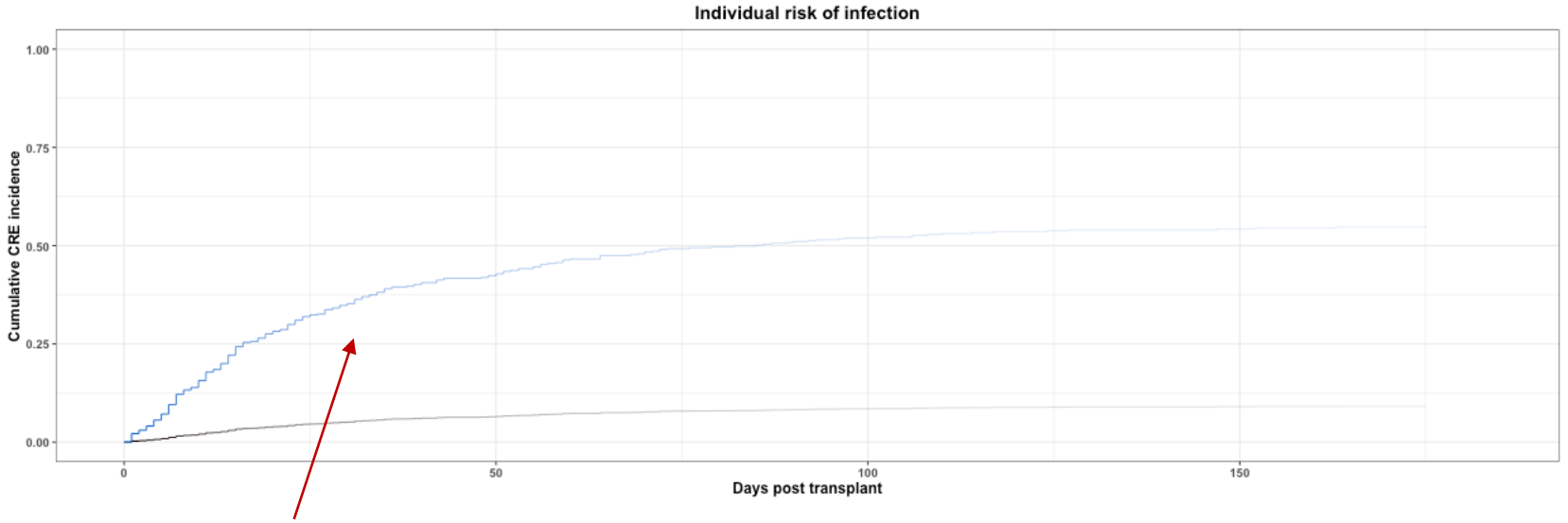
☑ Alpha blending (transparency)

Predict

Press Quit to exit the application

Quit

CRE cumulative risk | CRE risk at specified day | Numerical CRE risk summary | Model Summary



You can also add new risk factors, to see how it affects the prediction…

i..e this »low-risk patient» develops, ARF, goes on mechanical ventilation, and Becomes colonised post OLT with KPC

Type "0" when risk factor absent; type "1" when risk factor present

atg
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reint
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arf
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kpc
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crepre_60
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multipre
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crepost_60
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multipost
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☑ Predicted Survival at this Follow Up:

time
60

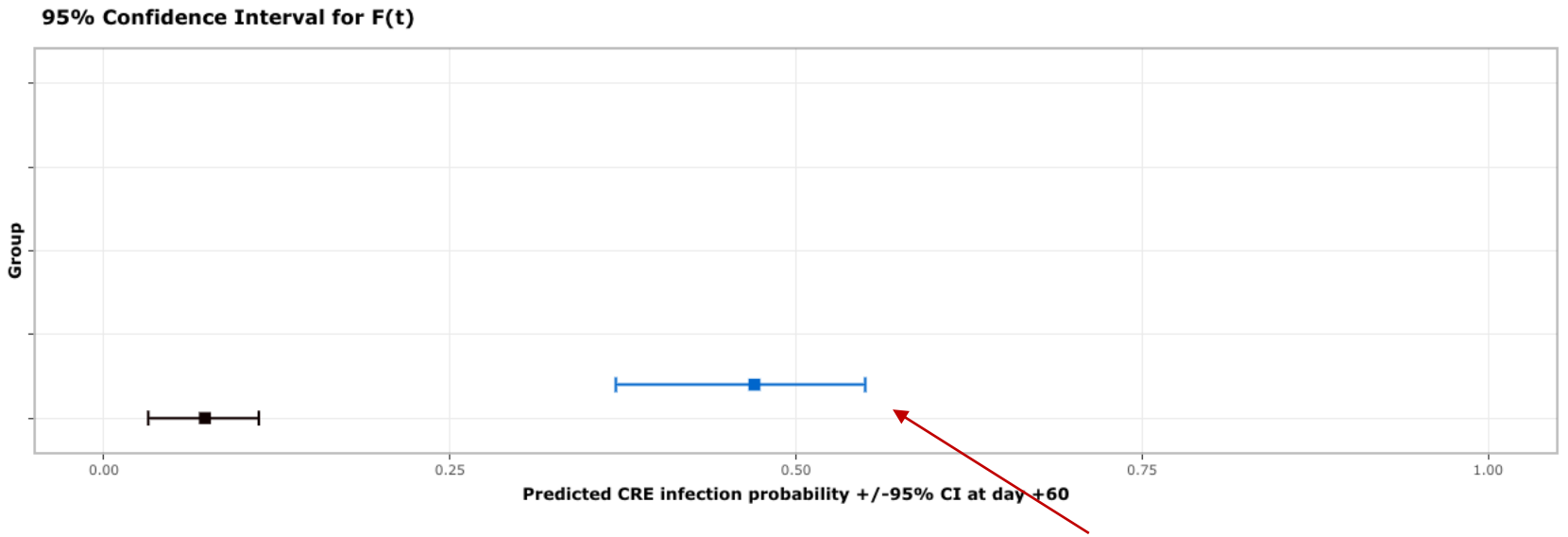
☑ Alpha blending (transparency)

Predict

Press Quit to exit the application

Quit

CRE cumulative risk | CRE risk at specified day | Numerical CRE risk summary | Model Summary



Comparing Day CRE prediction from previous single risk factor, and now with additional risk factors (note wider CI as fewer patients in the dataset had this combination of variables)

https://idbologna.shinyapps.io/CRE_POST_OLT/

CID-105646-marked

Development of a Risk Prediction Model for Carbapenem-Resistant Enterobacteriaceae Infection after Liver Transplantation: A Multinational Cohort Study

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Text word count: 2996 **Abstract word count:** 249

Running title: CRE infection risk in liver transplant

Key words: SOT, liver transplantation, CRE carriage, CRE infection

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40-word summary: One third of CRE carriers develops infection after LT with dramatic impact on survival. Our prediction tool could enable better targeting of early interventions for CRE infection after LT as opposed to universal prophylaxis or treatment in high-prevalence centers.

ABSTRACT

Background. Patients colonized with carbapenem resistant Enterobacteriaceae (CRE) are at higher risk of developing CRE infection after liver transplantation (LT) with associated high morbidity and mortality. Prediction model for CRE infection after LT among carriers could be useful to target preventive strategies.

Methods. Multinational multicenter cohort study of consecutive adult patients underwent LT and colonized with CRE before or after LT, from January 2010 to December 2017. Risk factors for CRE infection were analyzed by univariate analysis and by Fine-Gray sub-distribution hazard model, with death as competing event. A nomogram to predict 30- and 60-day CRE infection risk was created.

Results. 840 LT recipients found to be colonized with CRE before (n=203) or after (n=637) LT were enrolled. CRE infection was diagnosed in 250 (29.7%) patients within 19 (IQR 9-42) days after LT. Pre- and post-LT colonization, multisite post-LT colonization, prolonged mechanical ventilation, acute renal injury, and surgical re-intervention were retained in the prediction model. Median 30 and 60-day predicted risk was 15% (IQR 11-24%) and 21% (IQR 15-33%), respectively. Discrimination and prediction accuracy for CRE infection was acceptable on derivation (AUC 74.6, Brier index 16.3) and bootstrapped validation dataset (AUC 73.9, Brier index 16.6). Decision-curve analysis suggested net benefit of model-directed intervention over default strategies (treat all, treat none) when CRE infection probability exceeded 10%. The risk prediction model is freely available as mobile application at https://idbologna.shinyapps.io/CREPostOLTPredictionModel/.

Conclusions. Our clinical prediction tool could enable better targeting interventions for CRE infection after transplant.

INTRODUCTION

The emergence and spread of carbapenem resistant *Enterobacteriaceae* (CRE) over the last two decades threatens the safety of patients undergoing life-saving surgical procedures or necessary immunosuppressive treatments [1]. Solid organ transplant (SOT) recipients, in particular those undergoing liver transplantation (LT), are at increased risk of colonization with CRE due to repeated contacts with health care system, exposure to broad spectrum antibiotics, complexity of surgery, and altered immune status [2]. Compared with other settings (e.g., geriatric units, long-term care facilities) the incidence of infection per CRE colonization days is higher in SOT recipients [3], and associated with poorer survival [4]. In a prospective multicentre study of 887 SOT recipients in Italy, mortality was more than 10 times higher in patients with positive cultures for CRE [5].

Knowledge of CRE infection risk factors is essential to develop targeted preventive strategies. Risk factors for CRE infection after LT were analysed in two large single-centre cohort studies, from Brazil and Italy, including 386 and 553 patients, respectively [6, 7]. In both studies CRE carriage, either detected before or after transplant, was found to be the strongest predictor of subsequent CRE infection. Based on these findings, some investigators have proposed that preventive strategies should be implemented in all CRE carriers undergoing LT [8]. However, a more accurate prediction of which carriers are at higher risk could help to target preventive measures or timely appropriate antibiotic therapy in patients at lower risk (i.e. < 10%) of CRE infection.

With this objective in mind, we performed a multicentre, multinational cohort study of patients undergoing LT colonized with CRE before or after transplantation, with the aim of developing a multivariable prediction model for CRE infection within 30 and 60 days of LT.

MATERIAL AND METHODS

Study design

We performed a multicentre multinational retrospective cohort study. The enrolment period was between January 2010 to December 2017, or from the onset of systematic CRE colonization screening in the centre to December 2017 (see supplementary Table 1). Follow-up was of 180 days after LT. Data were accrued from October 2019 to June 2020. Data sources were clinical charts and hospital electronic records, they were de-identified before entry into a standardized electronic case report form (eCRF), and managed using REDCap capture tool hosted by Alma Mater University of Bologna [9]. Collected data were periodically checked for accuracy by an investigator of the coordinating centre (MR). Queries for incongruous or missing data were submitted to investigators to ensure high quality and completeness. The study was first approved by Institutional Review Board (IRB) of the promoting centre (n. 155/2019/Oss/AOUBo on March 20, 2019), then by IRB of all participating centres.

Setting

Fifteen hospitals performing LT participated in the study: seven from Italy (Bologna, Modena, Turin, Padua, Palermo, Milan, and Udine); four from Brazil (2 in São Paulo, 1 in Fortaleza and 1 in Rio de Janeiro); two from Spain (Madrid and Majadahonda); one from United States (Miami); and one from Israel (Petah-Tikva) (see Supplementary Table 1 and Figure 1). An active surveillance screening for CRE colonization was required by the study protocol. All centres performed systematic screening of CRE carriage by rectal swab (RS), at inclusion in waiting list, at LT and weekly after LT until hospital discharge. Screening for CRE colonization at other sites was performed according to clinical judgment and local policy.

Study population

All consecutive adult (≥18 years) patients who underwent LT during the study period and were found to be colonized with CRE were enrolled. Patients were included only once at the time of the first CRE carriage detection. CRE was defined as any *Enterobacteriaceae* displaying *in vitro* non-susceptibility to any of the carbapenems according to the criteria (CLSI or EUCAST) adopted at the participating

centre during the study period. The colonization status was defined as isolation of CRE from RS 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 was defined when CRE was concomitantly isolated from more than one of such samples. Patients found to be colonized with CRE by samples obtained at inclusion in the waiting list, during the period in waiting list, or at the time of LT were considered as CRE carriers pre-LT, whereas those found to be colonized within 180 days after LT were considered as post-LT CRE carriers.

Predictors of CRE infection after LT

The primary endpoint was the time to development of CRE infection, diagnosed within 180 days after LT, according to Center for Disease Control criteria [10]. The assessment of CRE infection was made by the local investigator and revised by an investigator of the promoting centre (MR), in case of no agreement a third blinded investigator of the promoting centre (MG) was asked to review the case for establishing the final diagnosis. Infection severity was determined using SOFA score and septic shock criteria [11].

Candidate prediction variables included: 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 LT. Complications occurred within 90 days before LT were recorded and included: admission to intensive care unit (ICU); grade 3 and/or refractory ascites [12]; hepato-renal syndrome (HRS) [12]; gastrointestinal bleeding; hepatic encephalopathy [13]; development of acute-on-chronic liver failure (ACLF) [14]; any infection, candidemia, and *Clostridioides difficile* infection. For graft characteristics donor age, cold ischemia time, and combined transplant were collected. Intraoperative variables included: antibiotic prophylaxis, biliary anastomosis, bleeding with need of massive transfusion (≥40 units of cellular blood products), prolonged intervention (≥ 8 hours). Complications occurred from LT to the diagnosis of CRE infection or death or 180 days (whichever occurred first) were recorded and

included: re-intervention, acute kidney injury (AKI) according to KIDGO 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 [16]. Management of CRE colonization was recorded according to the following categories: no strategy adopted, anti-CRE surgical prophylaxis, decolonization with non-absorbable antibiotics, faecal microbiota transplantation, pre-emptive strategy (defined as early start of anti-CRE coverage upon to clinical deterioration). For outcome, duration of the hospital stay after LT, number of re-admissions within 180-days after LT, and all-cause 180-days mortality were recorded.

Sample size

Sample size required for the clinical prediction model was estimated for a time-to-event outcome using the pmsampsize package in R (version 4.02, Core Team (2020). **R**: A language and environment for statistical computing. **R** Foundation for Statistical Computing, Vienna, Austria) using the methods proposed by Riley et al. [17]. We estimated that 850 patients with 213 outcome events (event rate 0.25 by day 180) would be sufficient to evaluate 20 candidate predictors (>10 events per candidate predictor) with an estimated 60-day event rate of 0.17.

Missing data

A complete-case analysis was performed.

Statistical analysis methods

Categorical variables were expressed as absolute numbers and their relative frequencies. Continuous variables were expressed as mean ± standard deviation (SD) if normally distributed, or as median and interquartile range (IQR) if non-normally distributed. We used the Fine-Gray sub-distribution hazard model to develop a clinical prognostic index for CRE infection over the first 180 days after LT with death as competing event.

Although colonization status has been modelled in previous studies as a time-varying covariate [18], inclusion of the time-dependent covariates limits the ability of the Fine-Gray sub-distribution hazard model to make inferences about the effects of covariates on the cumulative incidence function (CIF) in the presence of competing risks, which is an underlying motivation for our model. Furthermore, simulation studies have shown that a sub-distribution approach for time-dependent covariates often produces misleading results [19], and is not currently recommended [20]. Therefore, we focused on colonization status as a risk factor only in the 60-days prior to and 60 days following OLT to minimize time-dependent effects.

Using a base model of CRE colonization within 60 days prior to transplant, we examined the degree to which including each other variable from our original model improved its performance. Additional candidate variables were selected *a priori* based on statistical significance in univariate analysis, published data and clinical experience and whether they improved prediction accuracy of the baseline model. Only variables available for assessment during the risk period were included. We assumed that a predictive model for CRE infection would have the most clinical utility for accurate prediction of CRE infection in the first 30 and 60 days post OLT, as the risk appeared to plateau after this period. Therefore we used C-index (AUC) to assess the discrimination of the model with censored CRE infection status, and calibration plots of day-60 observed versus expected absolute risks with the Brier score to assess the accuracy of predictions. To control for centre effect, the analysis was also performed for each participating centre. Model discrimination and calibration measures were calculated using 100-fold cross validation bootstrap resampling to avoid bias. A decision curve was prepared to examine potential model utility across a range of CRE infection probabilities (0-60%) at 60 days post LT. All analyses were performed using R version 4.02 with riskRegression, prodlim, ggplot2, survival, and rmda packages. The final regression model was visualized by preparing a nomogram to predict 30-day and 60-day risk using methods described by Zhang et al [21] and the regplot package. An online prediction tool (Shiny App) was prepared using the DynNom package in R. Results were reported according to TRIPOD guidelines [22].

RESULTS

Overall, 840 LT recipients colonized with CRE before or after transplantation were enrolled, with 250 episodes of CRE infection post LT (29.7%) during 104,596 patient days of follow-up. Distribution of patients and of CRE infections across participating centres is shown in Figure 1. The characteristics of study population are shown in Table 1. Median age was 55 (IQR 46-62) years, male sex (65.4%). Viral hepatitis was the primary indication for LT in 376/840 subjects (44.8%), followed by alcohol 207/840 (24.6%). Over one-quarter 247/840 (29.4%) of patients had hepatocellular carcinoma (HCC). MELD at inclusion in waiting list and at LT was 19 (IQR 14-25) and 23 (IQR 16-30), respectively. CRE carriage was detected before or at LT in 203 (24.2%) patients, the median time from detection to LT was 6 (IQR 0-32) days. The remaining 637 (75.8%) patients were found to be colonized with CRE within a median of 13 (IQR 7-28) days after LT. Comparison of pre- and post-LT carriers showed several differences (see Table 1). Specifically, pre-LT carriers presented with more severe liver disease and complicated clinical course before transplantation. CRE infection rate among pre- and post-LT carriers was 33% and 28.7%, and the median time to infection following LT was 9 (IQR 4-22) and 23 (IQR 13-52) days, respectively (see Supplementary Figure 1). The median time from detection of CRE carriage to the diagnosis of CRE infection was shorter for post-LT carriers (8 vs. 19 days, $p<0.001$). Among the 203 pre-OLT carriers, 51 received targeted perioperative prophylaxis, the rate of CRE infection was 31.4% and 33.6% among those exposed and unexposed to targeted prophylaxis ($p=0.90$), respectively. Post-LT CRE infections in pre-LT carriers presented with higher severity according to median SOFA and septic shock rates than in post-LT carriers. However, there was no difference in clinical outcome (all-cause 180 days mortality 56.7% vs. 58.5%, $p=0.46$), data shown in Supplementary Table 2.

To screen risk factors for CRE infection, we performed univariate analysis of patients with and without CRE infection as shown in Table 2. Considering the competing risks of death, a Fine-Gray

model was used to perform regression of risk factors to estimate their association with the cumulative incidence of CRE infection up to 180 days post LT. Baseline cumulative incidence of death versus CRE infection over 180 following OLT is shown in Figure 2. The following variables were retained in the final model: pre-LT CRE colonization, post-LT CRE colonization, multisite post-LT colonization, prolonged MV, AKI, and surgical re-intervention (Table 3). The fitted regression risk model was then rendered as a nomogram (Figure 3). Model-predicted cumulative probabilities of developing CRE infection at day 30 and 60 post LT are shown in Figure 4. The median 30 and 60-day predicted risk was 15% (IQR 11-24%) and 21% (IQR 15-33%), respectively. Nearly one-quarter (23.5%) of patients at day 30, and 3% of patients at day 60 had a cumulative predicted risk of CRE infection ≤10%. The model showed acceptable 60-day discrimination and prediction accuracy for CRE infection when assessed against the derivation AUC 74.6 (95% 70.9-78.4), Brier index 16.3 (95%CI 6.7-25.9) and bootstrapped validation dataset AUC 73.9 (95%CI 67.7-79.1), Brier index 16.6 (95%CI 14.6-19.1) (Figure 5). Discrimination and calibration were also evaluated by centre and were consistent with the overall performance, except for higher variability and lower AUCs in centres with lower number of recruited patients (see Supplementary Figure 2). The score was designed to be used in the immediate peri-transplant period, ideally from the day of transplantation up to 2-3 weeks after transplantation. During this period, the patient should be frequently evaluated for carriage status, multisite colonization, prolonged MV, AKI, and/or re-intervention. According with the presence of such variables the cumulative risk of CRE infection within 30 and 60 days after LT can be predicted (see examples in Figure 6 and mobile application at https://idbologna.shinyapps.io/CREPostOLTPredictionModel/, instructions for using app are further provided as supplementary material).

Finally, we explored the potential clinical utility of our model using a decision-curve analysis (DCA) to examine the "net benefit" of applying the prediction model across a range of CRE infection threshold probabilities [23]. A theoretical risk-model guided strategy (i.e. empiric administration of CRE-active antibiotics) is compared against two default strategies- "treat all" and "treat none." A model is only

298 useful at a given disease threshold if it has a higher net benefit than treat all or treat none. Our
299 analysis suggested that the model-directed interventions for CRE post LT would show net benefit
300 over default strategies when the overall CRE infection threshold probability exceeded 10% (Fig. 7).

301

302 **DISCUSSION**

303 Assessing a large multinational cohort of LT recipients colonized with CRE (n=840) we have found
304 that one third developed CRE infection after LT. This rate was similar among pre- and post-LT CRE
305 carriers. Most infection episodes occurred within 2 months after LT. The time to infection was earlier
306 for pre-LT CRE carriers, and the clinical severity was higher in this group. All-cause 6-month mortality
307 among carriers was as high as 30%, with similar rates between pre- and post-LT CRE carriers.
308 However, it reached 58% among patients who developed CRE infection.

309 We confirmed that the CRE carriage status is strongly associated with the risk of CRE infection after
310 LT, either if detected before or after LT [6, 7]. However, we also confirmed our previous hypothesis
311 that, given a probability of CRE infection higher than 10%, a strategy of treating all CRE carriers could
312 be inferior to a targeted strategy in terms of efficacy, as well as safety, considering potential adverse
313 events of anti-CRE drugs and the risk for further selection pressure. A longitudinal risk stratified
314 approach, could be useful for several reasons. In general, LT is not an elective surgery, thus it could
315 be difficult to implement a strategy before transplantation other than in a minority of patients
316 known to be colonized at the time of inclusion or while on waiting list. Another opportunity for
317 intervention could be surgical prophylaxis. However, data about colonization is frequently available
318 only during the immediate post-operative period. Finally, in most patients CRE colonization becomes
319 evident in the first weeks after LT, typically with a complicated post-operative course, which also
320 increases the risk of infection.

Along with pre- and post-LT colonization status, multisite colonization after LT was a predictor of infection. The relationship between multisite colonization with infection development has been already observed in other settings [3]. It is indicative of high colonization burden leading to an increased risk of CRE infection [24]. Indeed, its inclusion in our prognostic model improved model performance. Nevertheless, multisite colonization was not systematically screened in this study. Therefore, we cannot rule out that colonization from different sites may be weighted differently in the model if this risk factor had been systematically screened, as patients with a more complicated course may have been cultured more frequently.

The other complications included in our score are widely described as factors involved in the increase risk of infection after LT [25]. AKI has been also associated with microbiome dysbiosis that could favour prolonged CRE carriage and infection [26]. As for MV, it is worth mentioning that we considered such variable, as a qualitative data, from LT to CRE infection, death or end of follow-up. A more detailed information about timing and duration of MV may improve the model performance.

Our study has several limitations. First, the retrospective design could have limited identification of all CRE carriers. However, the presence of a local prospective registry of CRE carriers and/or good collaboration with local microbiology laboratories reduced this bias. Heterogeneity in local assays and criteria for identification and determination of CRE could exist. However, obtained data are in line with international literature. The majority of involved centres were high endemic CRE hospitals, with KPC as main mechanism of CRE. Thus, our findings could not apply to transplant centres with low prevalence of CRE, or with KPC alternative main mechanisms among CRE. Finally, we analysed the CRE infection risk only in colonized patients, thus the performance of our prediction tool should be confirmed in unselected LT patients.

To conclude, one third of CRE carriers develop infection after LT with a dramatic impact on patient survival. Effective preventive or pre-emptive strategies are needed. Our clinical prediction tool could enable better targeting of early interventions for CRE infection after transplant as opposed to

346 universal prophylaxis or treatment in high-prevalence centres. Further studies are needed to further
347 validate the model and establish which strategy would be more effective, in terms of adverse events
348 and collateral resistance impact and, ultimately patient survival after transplantation.

349

350

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CONFLICT OF INTEREST

Authors have no conflict of interest related to the present study.

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439 **Table 1.** Characteristics of study population according to the timing of CRE carriage detection

	Total N=840 (%)	**pre-OLT carrier N=203 (%)**	**post-OLT carrier N=637 (%)**	**P**
Demographic data				
Age (years) [median (IQR)]	55 (46-62)	53 (42-60)	56 (47-62)	0.005
Sex, male	549 (65.4)	132 (65)	417 (65.5)	0.9
Comorbidities				
Myocardial infarction	23 (2.7)	5 (2.5)	18 (2.8)	0.827
Congestive heart failure	29 (3.5)	7 (3.4)	22 (3.5)	0.9
Peripheral vascular disease	33 (3.9)	6 (3)	27 (4.2)	0.4
Cerebrovascular disease	22 (2.6)	8 (3.9)	14 (2.2)	0.18
COPD	46 (5.5)	6 (3)	40 (6.3)	0.07
Connective tissue disease	9 (1.1)	3 (1.5)	6 (0.9)	0.38
Peptic ulcer disease	62 (7.4)	20 (9.9)	42 (6.6)	0.09
Diabetes without organ damage	147 (17.5)	38 (18.7)	109 (17.1)	0.3
Moderate/severe renal disease	82 (9.8)	28 (13.8)	54 (8.5)	0.03
Diabetes with organ damage	76 (9)	15 (7.4)	61 (9.6)	0.2
Any tumor within 5 years	271 (32.3)	45 (22.2)	226 (35.5)	0.001
Moderate/severe liver disease	820 (97.6)	199 (98)	621 (97.5)	0.79
Charlson index [median (IQR)]	5 (4-7)	5 (3-6)	5 (4-7)	0.004
Underlying liver disease				
Viral hepatitis	376 (44.8)	74 (36.5)	302 (47.4)	0.006
Alcohol	207 (24.6)	55 (27.1)	152 (23.9)	0.2
Metabolic disease	69 (8.2)	12 (5.9)	57 (8.9)	0.1
Autoimmune disease	41 (4.9)	12 (5.9)	29 (4.6)	0.45
Fulminant hepatitis	52 (6.2)	7 (3.4)	45 (7.1)	0.04
Hepatocellular carcinoma	247 (29.4)	37 (18.2)	210 (33)	<0.001
Prior transplant	39 (4.6)	20 (9.9)	19 (3)	<0.001
MELD at waiting list inclusion (median, IQR)	19 (14-25)	21 (16-28)	19 (14-24)	<0.001
MELD at OLT (median, IQR)	23 (16-30)	27 (21-32)	22 (15-29)	<0.001
Variables relative to the last 90 days before OLT				
Hepato-renal syndrome	235 (28)	88 (43.3)	147 (23.1)	<0.001
Ascites grade III/refractory	385 (45.8)	108 (53.2)	277 (43.5)	0.01
GI bleeding	138 (16.4)	43 (21.2)	95 (14.9)	0.025
Encephalopathy	343 (40.8)	96 (47.3)	247 (38.8)	0.02
ICU admission	161 (19.2)	70 (34.5)	91 (14.3)	<0.001
ACLF	98 (11.7)	44 (21.7)	54 (8.5)	<0.001
Any infection	326 (38.8)	137 (67.5)	189 (29.7)	<0.001
Intraoperative variables				
Combined transplant	45 (5.4)	12 (5.9)	33 (5.2)	0.7
Donor age	51 (36-65)	50 (35-65)	51 (36-65)	0.4
Cold ischemia time (hours) (median, IQR)	7 (6-8)	7 (6-8)	7 (6-8)	0.62
Biliary anastomosis				
Duct to duct without Kehr	382 (45.5)	84 (41.4)	298 (46.8)	0.19
Duct to duct with Kehr	347 (41.3)	76 (37.4)	271 (42.5)	0.20

Choledochojejunostomy	111 (13.2)	43 (21.2)	68 (10.7)	<0.001
Intraoperative bleeding	294 (35)	88 (43.3)	206 (32.3)	0.005
Prolonged surgery (>8 hours)	248 (29.5)	70 (34.5)	178 (27.9)	0.046
Induction regimen				
None	87 (10.4)	24 (11.8)	63 (9.9)	0.51
Bolus of steroids	701 (83.5)	171 (84.2)	530 (83.2)	0.75
Antithymocyte globulins	26 (3.1)	3 (1.5)	23 (3.6)	0.09
Basiliximab	167 (19.9)	37 (18.2)	130 (20.4)	0.55
Rituximab	14 (1.7)	0 (0)	14 (2.2)	0.02
Maintenance regimen				
Steroids	612 (72.9)	162 (79.8)	450 (70.6)	0.011
Cyclosporin	42 (5)	8 (3.9)	34 (5.3)	0.28
Tacrolimus	770 (91.7)	181 (89.2)	589 (92.5)	0.15
Micophenolate mophetil	287 (34.2)	70 (34.5)	217 (34.1)	0.9
Post-operative complications				
Acute renal injury	462 (55)	118 (58.1)	344 (54)	0.33
Renal replacement therapy	299 (35.6)	90 (44.3)	209 (32.8)	0.003
Mechanical ventilation ≥48 hours	266 (31.7)	64 (31.5)	202 (31.7)	0.96
PGNF	128 (15.2)	28 (13.8)	100 (15.7)	0.58
Re-intervention	322 (38.3)	74 (36.5)	248 (38.9)	0.56
Re-transplantation	79 (9.4)	11 (5.4)	68 (10.7)	0.025
Rejection	152 (18.1)	30 (14.8)	122 (19.2)	0.17
CMV treatment	225 (26.8)	46 (22.7)	179 (28.1)	0.15
Infections other than CRE	479 (57)	106 (52.2)	373 (58.6)	0.12
Clostridioides difficile infection	37 (4.4)	8 (3.9)	29 (4.6)	0.44
Candidemia	68 (8.1)	11 (5.4)	57 (8.9)	0.14
CRE carriage				
Time between OLT and carriage detection (days) (median, IQR)	9 (0.25-21)	-6 (-32-0)	13 (7-28)	<0.001
First positive sample				
Rectal swab	719 (85.6)	189 (93.1)	530 (83.2)	0.001
Respiratory sample	45 (5.4)	3 (1.5)	42 (6.6)	0.002
Urine	69 (8.2)	22 (10.8)	47 (7.4)	0.14
Multi-site colonization	113 (13.5)	26 (12.8)	87 (13.7)	0.81
Resistance mechanism				
KPC	600 (71.4)	149 (73.4)	451 (70.8)	0.53
VIM	7 (0.8)	3 (1.5)	4 (0.6)	0.23
OXA-48	17 (2)	6 (3)	11 (1.7)	0.21
Not tested	115 (13.7)	19 (9.4)	96 (15.1)	0.05
Management of CRE colonization				
No strategy adopted	521 (62)	85 (41.9)	436 (68.4)	<0.001
Target perioperative prophylaxis	51 (6.1)	51 (25.1)	0 (0)	<0.001
Decolonization	16 (1.9)	4 (2)	12 (1.9)	0.57
Pre-emptive strategy[1]	21 (2.5)	6 (3)	15 (2.4)	0.4
Outcome				
Length of ICU stay after OLT (days) (median, IQR)	7 (4-15)	7 (4-14)	7 (4-15)	0.38
Length of hospital stay after OLT (days) (median, IQR)	27 (16-50)	23 (14-37)	29 (17-54)	<0.001
All-cause 6-months mortality	263 (31.3)	69 (34)	194 (30.5)	0.39

Time from OLT to death (days) (median, IQR)	70 (29-145)	32 (10.5-77.5)	88.5 (43-162)	<0.001
Hospital re-admissions (median, IQR)	0 (0-1)	0 (0-1)	0 (0-1)	0.07

[1]Start of an anti-CRE treatment if complications occur

Abbreviations: IQR interquartile range, COPD chronic obstructive pulmonary disease, MELD Model End Stage Liver Disease, OLT orthotopic liver transplant, ICU intensive care unit, ACLF acute on chronic liver failure, PGNF primary graft non-function, CMV cytomegalovirus, CRE carbapenem-resistant *Enterobacteriaceae*, KPC *Klebsiella pneumoniae* carbapenemase-producing, SBP spontaneous bacterial peritonitis.

447 **Table 2.** Comparison of patients with and without CRE infection within 180 days after liver transplant

	Patients without CRE infection, n=590 (%)	**Patients with CRE infection, n=250 (%)**	***P***
Demographic data			
Age (years) [median (IQR)]	55 (46-61)	56 (47-62)	0.5
Sex, male	389 (65.9)	160 (64)	0.59
Comorbidities			
Myocardial infarction	17 (2.9)	6 (2.4)	0.7
Congestive heart failure	19 (3.2)	10 (4)	0.57
Peripheral vascular disease	27 (4.6)	6 (2.4)	0.14
Cerebrovascular disease	15 (2.5)	7 (2.8)	0.83
COPD	30 (5.1)	16 (6.4)	0.51
Connective tissue disease	6 (1)	3 (1.2)	0.81
Peptic ulcer disease	44 (7.5)	18 (7.2)	0.89
Diabetes without organ damage	103 (17.5)	44 (17.6)	0.96
Moderate/severe renal disease	45 (7.6)	37 (14.8)	0.001
Diabetes with organ damage	55 (9.3)	21 (8.4)	0.67
Any tumor within 5 years	192 (32.5)	79 (31.6)	0.79
Moderate/severe liver disease	576 (97.6)	244 (97.6)	0.98
Charlson index [median (IQR)]	5 (4-7)	5 (4-7)	0.56
Underlying liver disease			
Viral hepatitis	260 (44.1)	116 (46.4)	0.53
Alcohol	157 (26.6)	50 (20)	0.04
Metabolic disease	48 (8.1)	21 (8.4)	0.89
Autoimmune disease	32 (5.4)	9 (3.6)	0.26
Fulminant hepatitis	36 (6.1)	16 (6.4)	0.87
Hepatocellular carcinoma	174 (29.5)	73 (29.2)	0.93
Prior transplant	24 (4.1)	15 (6)	0.22
MELD at waiting list inclusion (median, IQR)	19 (14-24)	19 (14-26)	0.73
MELD at OLT (median, IQR)	22.5 (16-29)	25 (16-33)	0.02
Events prior 90 days to OLT			
Hepato-renal syndrome	150 (25.4)	85 (34)	0.01
Ascites grade III/refractory	278 (47.1)	107 (42.8)	0.25
Bleeding	89 (15.1)	49 (19.6)	0.11
Encephalopathy	245 (41.5)	98 (39.2)	0.53
ICU admission	110 (18.6)	51 (20.4)	0.55
ACLF	66 (11.2)	32 (12.8)	0.5
Infections	223 (37.8)	103 (41.2)	0.36
Intraoperative variables			
Combined transplant	25 (4.2)	20 (8)	0.03
Donor age	51 (35-65)	51 (36-67)	0.6
Cold ischemia time (hours) (median, IQR)	7 (6-8)	7 (5:50-8)	0.36
Choledochojejunostomy	71 (12)	40 (16)	0.22
Intraoperative bleeding	196 (33.2)	98 (39.2)	0.1
Prolonged surgery	162 (27.5)	86 (34.4)	0.04
Induction regimen			
Bolus of steroids	476 (80.7)	225 (90)	0.001

Antithymocyte globulins	12 (2)	14 (5.6)	0.006
Basiliximab/Daclizumab	113 (19.2)	54 (21.6)	0.42
Rituximab	6 (1)	8 (3.2)	0.02
Maintenance regimen			
Steroids	414 (70.2)	198 (79.2)	0.007
Cyclosporin	31 (5.3)	11 (4.4)	0.6
Tacrolimus	549 (93.1)	221 (88.4)	0.03
Micophenolate mophetil	215 (36.4)	72 (28.8)	0.03
Post-operative complications			
Acute renal injury	286 (48.5)	176 (70.4)	<0.001
Renal replacement therapy	173 (29.3)	126 (50.4)	<0.001
Mechanical ventilation >48 hours	135 (22.9)	131 (52.4)	<0.001
PGNF	74 (12.5)	54 (21.6)	0.001
Re-intervention	200 (33.9)	122 (48.8)	<0.001
Re-transplantation	43 (7.3)	36 (14.4)	0.001
Rejection	101 (17.1)	51 (20.4)	0.26
CMV DNAemia>100,000 copies/mL	156 (26.4)	69 (27.6)	0.73
Infections other than CRE	333 (56.4)	146 (58.4)	0.6
Clostridioides difficile infection	26 (4.4)	11 (4.4)	0.99
Candidemia	36 (6.1)	32 (12.8)	0.001
CRE carriage			
Pre-OLT carriage	136 (23.1)	67 (26.8)	0.25
Post-OLT carriage	454 (76.9)	183 (73.2)	0.25
Rectal swab	517 (87.6)	202 (80.8)	0.01
Respiratory sample	21 (3.6)	24 (9.6)	<0.001
Urine sample	50 (8.5)	19 (7.6)	0.67
Multisite colonization	54 (9.2)	59 (23.6)	<0.001
pre-OLT	17 (2.9)	9 (3.6)	<0.001
post-OLT	37 (6.3)	50 (20)	0.66
Genotype of the colonizing strain			
KPC	418 (70.8)	182 (72.8)	0.61
VIM	4 (0.7)	3 (1.2)	0.45
OXA-48	11 (1.9)	6 (2.4)	0.6
Not tested	76 (12.9)	39 (15.6)	0.3
Management of CRE colonization			
Target perioperative prophylaxis	35 (5.9)	17 (6.8)	0.6
Decolonization	12 (2)	4 (1.6)	0.7
Pre-emptive strategy[1]	11 (1.9)	10 (4)	0.07
Outcome			
Length of ICU stay after OLT (days) (median, IQR)	6 (3-10)	14 (5-29)	<0.001
Length of hospital stay after OLT (days) (median, IQR)	22 (15-40)	43 (24-76)	<0.001
All-cause 6-months mortality	118 (20)	145 (58)	<0.001
Hospital re-admissions (median, IQR)	0 (0-1)	0 (0-1)	<0.001

448 [1]Start of an anti-CRE treatment if complications occur.

449 Abbreviations: IQR interquartile range, COPD chronic obstructive pulmonary disease, MELD Model

450 End Stage Liver Disease, OLT orthotopic liver transplant, ICU intensive care unit, SBP spontaneous

bacterial peritonitis, ACLF acute on chronic liver failure, PGNF primary graft non-function, CMV cytomegalovirus, CRE carbapenem-resistant *Enterobacteriaceae*, KPC *Klebsiella pneumoniae* carbapenemase-producing.

454 **Table 3.** Final multivariable analysis for predicting risk of CRE infection after liver transplantation

Variable	Subhazard ratio (95% CI)	β-coefficient	P value
Reintervention	1.37 (1.04-1.80)	0.30 (0.02-0.57)	0.02
Prolonged mechanical ventilation	2.01 (1.49-2.71)	0.70 (0.40-0.99)	<0.0001
Acute renal injury	1.69 (1.26-2.27)	0.52 (0.23-0.82)	0.001
CRE colonisation 60 days prior to transplant	2.17 (1.33-3.53)	0.78 (0.29-1.26)	0.002
CRE colonization within 60 days after transplant	1.41 (0.91-2.19)	0.35 (-0.09-0.78)	0.12
Multisite colonization within 60 days after transplant	2.87 (1.98-4.20)	1.06 (0.68-1.43)	<0.0001

455

456 Abbreviations: CI confidence interval, CRE carbapenem-resistant Enterobacteriaceae.

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Figure legends:

Figure 1. Study flow chart with detailed data about involved centers.

Figure 2. Cumulative incidence of CRE infection and death post-transplant. The cumulative incidence was derived from the estimated sub-distribution hazard function following regression with a Fine-Gray model.

Figure 3. Model-based nomogram for predicting 30 and 60-day cumulative risk of developing CRE infection. Points are summed for each risk factor.

Figure 4. Distribution of model-predicted (a) 30-day and (b) 60-day risk for CRE infection following LT.

Figure 5. Calibration curves for the (a) derivation dataset and (b) Bootstrap resampled validation dataset. The C-index (AUC) and Brier score are expressed as the point estimates and 95% CI. An AUC > 0.8 is considered to give good discrimnatory accuracy for a clinical prediction model. The Brier score is a measure of the accuracy of probabilistic predictions. The lower the Brier score is for a set of predictions, the better the predictions are calibrated. An ideal model has pairs of observed and predicted probabilites that like on the 45-degree angle line. Dotplots shown the distribution of predicted risk for patients who developed (top) and did not develop (bottom) CRE infection.

Figure 6. 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) LT patient. The blue lines with shading in the nomogram represent the distribution of individual risk factors (0=no, 1=yes) and the distribution of total assigned points on the bottom scale in the original derivation data set . Red points on the upper scale indicate assigned points per risk factor. The red arrowsat the bottom of the nomogram indicate the total calculated points for the patient and corresponding 30-day and 60 day predicted cumulative risk with 95% CI.

Figure 7. Decision-curve analysis of model-directed anti CRE therapy. Net benefit at 60 days represents true cases of CRE infection that would be treated with a CRE-active antibiotic regimen-patients without CRE infection unnecessarily receiving CRE-active therapy. The curves indicate that a model-directed antibiotic strategy should have a higher net benefit than default strategies (all patients receive CRE-active therapy or no patients receive CRE-active therapy) at CRE infection probabilities greater than 10-50 %.