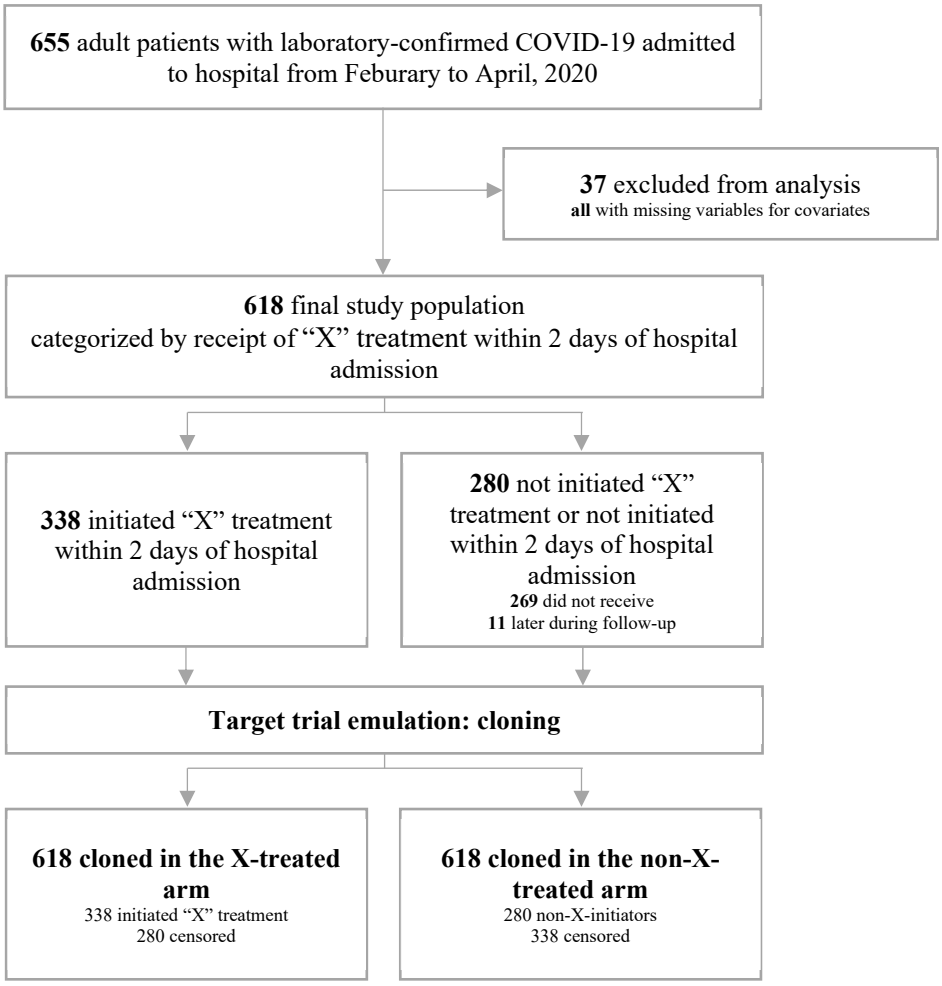
*Life* **Journal**

**Supplementary Material**

Martinuka O, von Cube M, Hazard D, Marateb HR, Mansourian M, Sami R, Hajian MR, Wolkewitz M. Target trial emulation using hospital-based observational data: Demonstration and application in COVID-19.

**Content**

**Supplementary Figure S1.** Study cohort and emulated target trial flowchart




**Abbreviation:** COVID-19, Coronavirus disease 2019.

**Supplementary Table S1.** STROBE checklist - Statistical Methods for Observational Data on Evaluating Treatment: Demonstration of target trial emulation and application in COVID-19

| | Item No. | Recommendation | Page No. |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 1 |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 1 |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 2 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 2 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 2 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 3-4 |
| Participants | 6 | Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up | 3-4 |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 3,6 |
| Data sources/ measurement | 8 | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | NA |
| Bias | 9 | Describe any efforts to address potential sources of bias | 3-7 |
| Study size | 10 | Explain how the study size was arrived at | NA |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | NA |
| **Statistical methods** | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 4-8 |
| | | (*b*) Describe any methods used to examine subgroups and interactions | NA |
| | | (*c*) Explain how missing data were addressed | NA |
| | | (*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed | NA |
| | | (*e*) Describe any sensitivity analyses | 8 |

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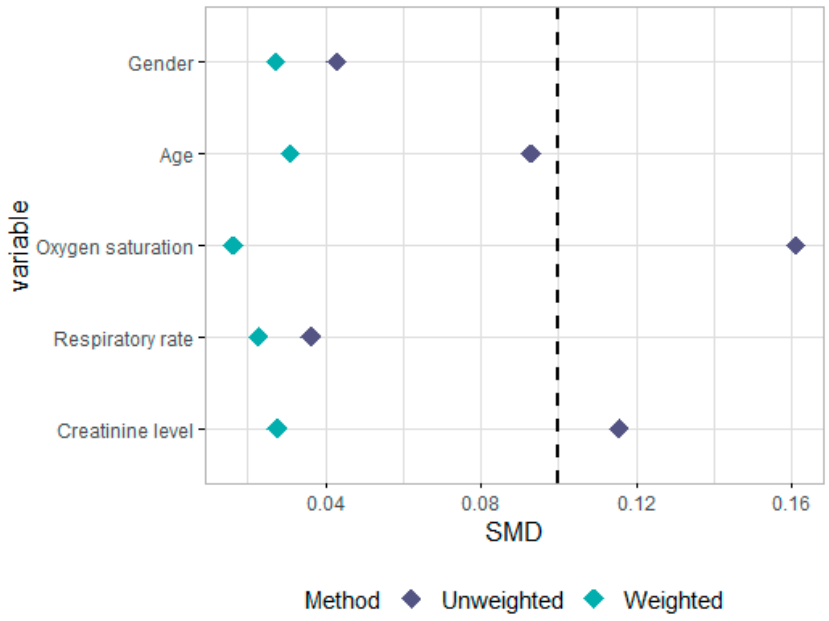
| | | | |
|---|---|---|---|
| **Results** | | | |
| Participants | 13 | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | Figure S1 |
| | | (b) Give reasons for non-participation at each stage | Figure S1 |
| | | (c) Consider use of a flow diagram | Figure S1 |
| Descriptive data | 14 | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | Table S2 |
| | | (b) Indicate number of participants with missing data for each variable of interest | NA |
| | | (c) Summarise follow-up time (eg, average and total amount) | 9 |
| Outcome data | 15 | Report numbers of outcome events or summary measures over time | 8-9 |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | Table S3 |
| | | (*b*) Report category boundaries when continuous variables were categorized | Table S2 |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | 9, Table S3, Figure 3, S3 |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 10, Table S4-S5 |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 10-11 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 11 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 10-11 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | NA |
| **Other information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 12 |

**Supplementary Table S2.** Baseline characteristics of patients hospitalized with COVID-19

| Characteristic | n |
|---|---|
| **Overall sample** | 618 |
| **Gender** | |
| Male | 384 (62.1 %) |
| Female | 234 (37.9 %) |
| **Age, years** | |
| Mean (SD) | 57.2 |
| Median [Min, Max] | 58.0 [20.0, 92.0] |
| **O2 saturation [%]** | |
| Mean (SD) | 89 (7.2) |
| Median [Min, Max] | 91 [35, 100] |
| **Respiratory rate [breaths per minute]** | |
| Mean (SD) | 21.9 (5.2) |
| Median [Min, Max] | 20 [12, 45] |
| **Creatinine [mg/dL]** | |
| Mean (SD) | 1.20 (1.02) |
| Median [Min, Max] | 0.96 [0.41, 9.89] |

**Abbreviation:** SD, standard deviation.

**Supplementary Figure S2.** Balance in demographic and clinical characteristics for the emulated cohort based on standardized mean differences in the unweighted and weighted clone samples




Notes: SMD < 0.1: adequate balance, 0.1-0.25: irrelevant imbalance, >0.25 serious imbalance[1].
Abbreviation: SMD, standardized mean difference.

The standardized differences were examined to ensure that the weights eliminated any imbalance between treatment arms. [1] A threshold below 10% of the standardized mean differences is considered a meaningful balance. [2]

**References**

1. Willems, S.; Schat, A.; van Noorden, M.S.; Fiocco, M. Correcting for dependent censoring in routine outcome monitoring data by applying the inverse probability censoring weighted estimator. Stat. Methods Med. Res. 2018, 27, 323–335, doi:10.1177/0962280216628900.
2. Stuart, E.A.; Lee, B.K.; Leacy, F.P. Prognostic score-based balance measures can be a useful diagnostic for propensity score methods in comparative effectiveness research. J. Clin. Epidemiol. 2013, 66, S84-S90.e1, doi:10.1016/j.jclinepi.2013.01.013.

**Supplementary Table S3.** Summary of the corresponding measures of interest taking the parametric approach and estimated weighted results at the end of follow-up (60 days)

| Corresponding measure | Mathematical formulation | Results [95% CI] |
|---|---|---|
| **Constant hazards** | | |
| Death hazard w/o treatment | $\lambda_{02}$ | 0.010 |
| Discharge hazard w/o treatment | $\lambda_{03}$ | 0.120 |
| Hazard w/o treatment | $\lambda_0 = \lambda_{02} + \lambda_{03}$ | 0.130 |
| Death hazard with treatment | $\lambda_{12}$ | 0.008 |
| Discharge hazard with treatment | $\lambda_{13}$ | 0.124 |
| Hazard with treatment | $\lambda_1 = \lambda_{12} + \lambda_{13}$ | 0.131 |
| **Mortality** | | |
| Mortality risk w/o treatment at day 60 | $MR_0 = \frac{\lambda_{02}}{\lambda_{02} + \lambda_{03}}$ | 0.074 [0.031-0.085] |
| Mortality risk with treatment at day 60 | $MR_1 = \frac{\lambda_{12}}{\lambda_{12} + \lambda_{13}}$ | 0.058 [0.037-0.076] |
| Mortality risk ratio at day 60 | $\frac{\lambda_{12}/\lambda_{02}}{(\lambda_{13} + \lambda_{12})/(\lambda_{02} + \lambda_{03})}$ | 0.785 [0.535-1.316] |
| Difference in mortality at day 60 | $MR_1 - MR_0$ | -0.016 [-0.033, 0.030] |
| **Occurrence of outcome and treatment effect estimands** | | |
| Hazard ratio of death (treatment *vs.* w/o treatment) | $HR_2 = \frac{\lambda_{12}}{\lambda_{02}}$ | 0.793 [0.480-1.247] |
| Hazard ratio of discharge (treatment *vs.* w/o treatment) | $HR_3 = \frac{\lambda_{13}}{\lambda_{03}}$ | 1.027 [0.806-1.045] |
| Differences in hazard ratios of death (treatment *vs.* w/o treatment) at day 60 | $\lambda_{12} - \lambda_{02}$ | -0.002 [-0.005, 0.003] |
| Differences in hazard ratios of discharge (treatment *vs.* w/o treatment) at day 60 | $\lambda_{13} - \lambda_{03}$ | 0.003 [-0.023, 0.005] |
| Cumulative risk of death w/o treatment at time t | $CIF_{02} = \frac{\lambda_{02}}{\lambda_0} \times (1 - \exp(-\lambda_0 t))$ | 0.074 [0.031-0.085] |
| Cumulative risk of discharge w/o treatment at time t | $CIF_{03} = \frac{\lambda_{03}}{\lambda_0} \times (1 - \exp(-\lambda_0 t))$ | 0.924 [0.913-0.968] |
| Cumulative risk of death with treatment at time t | $CIF_{12} = \frac{\lambda_{12}}{\lambda_1} \times (1 - \exp(-\lambda_1 t))$ | 0.058 [0.037-0.076] |
| Cumulative risk of discharge with treatment at time t | $CIF_{12} = \frac{\lambda_{13}}{\lambda_1} \times (1 - \exp(-\lambda_1 t))$ | 0.940 [0.921-0.961] |
| Risk difference functions for death at day 60 | $RD_2 = CIF_{12} - CIF_{02}$ | -0.016 [-0.033, 0.030] |
| Risk difference functions for discharge at day 60 | $RD_3 = CIF_{13} - CIF_{03}$ | 0.016 [-0.031, 0.032] |
| Risk ratios for death at day 60 | $RR_2 = \frac{CIF_{12}}{CIF_{02}}$ | 0.785 [0.053, 1.309] |

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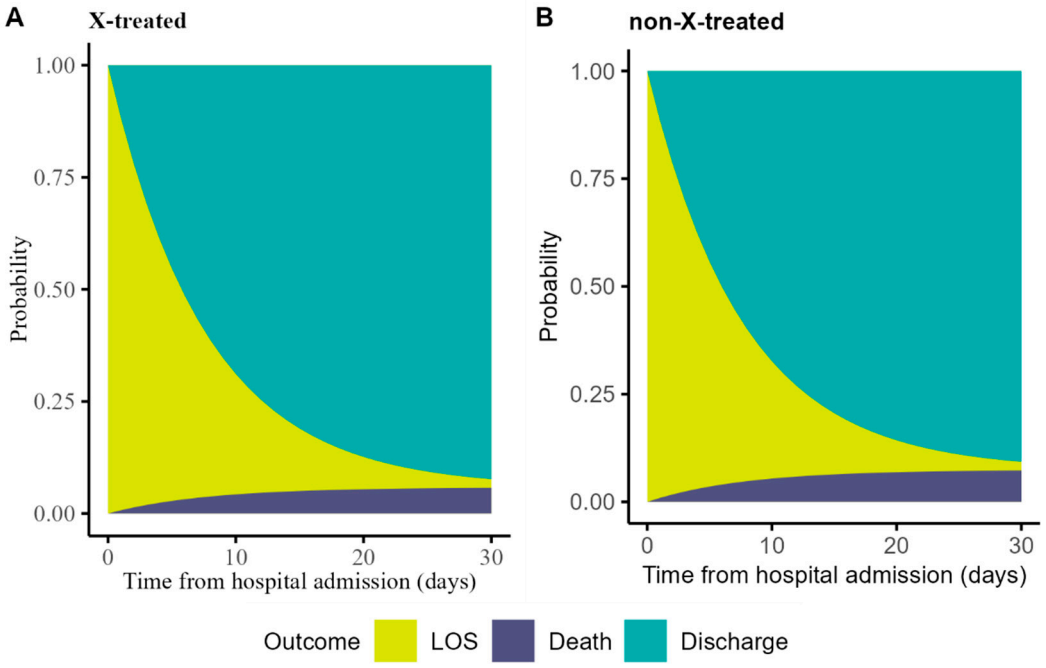
| Corresponding measure | Mathematical formulation | Results |
|---|---|---|
| Risk ratios for discharge at day 60 | $RR_3 = \frac{CIF_{13}}{CIF_{03}}$ | 1.017<br>[0.965, 1.035] |
| **Length of stay** | | |
| Length of stay w/o treatment | $LOS_0 = \frac{1}{\lambda_{02} + \lambda_{03}}$ | 7.69 days<br>[7.00, 8.39] |
| Length of stay with treatment | $LOS_1 = \frac{1}{\lambda_{12} + \lambda_{13}}$ | 7.61 days<br>[7.21, 8.03] |
| Difference in length of stay | $LOS_1 - LOS_0$ | -0.08 |

Notes: 0 = from non-X-treated state, 1 = from X-treated state.
Abbreviations: CIF, cumulative incidence function; HR, hazard ratio; LOS, length of stay; MR, mortality risk, RD, risk difference; RR, risk ratio; w/o, without.

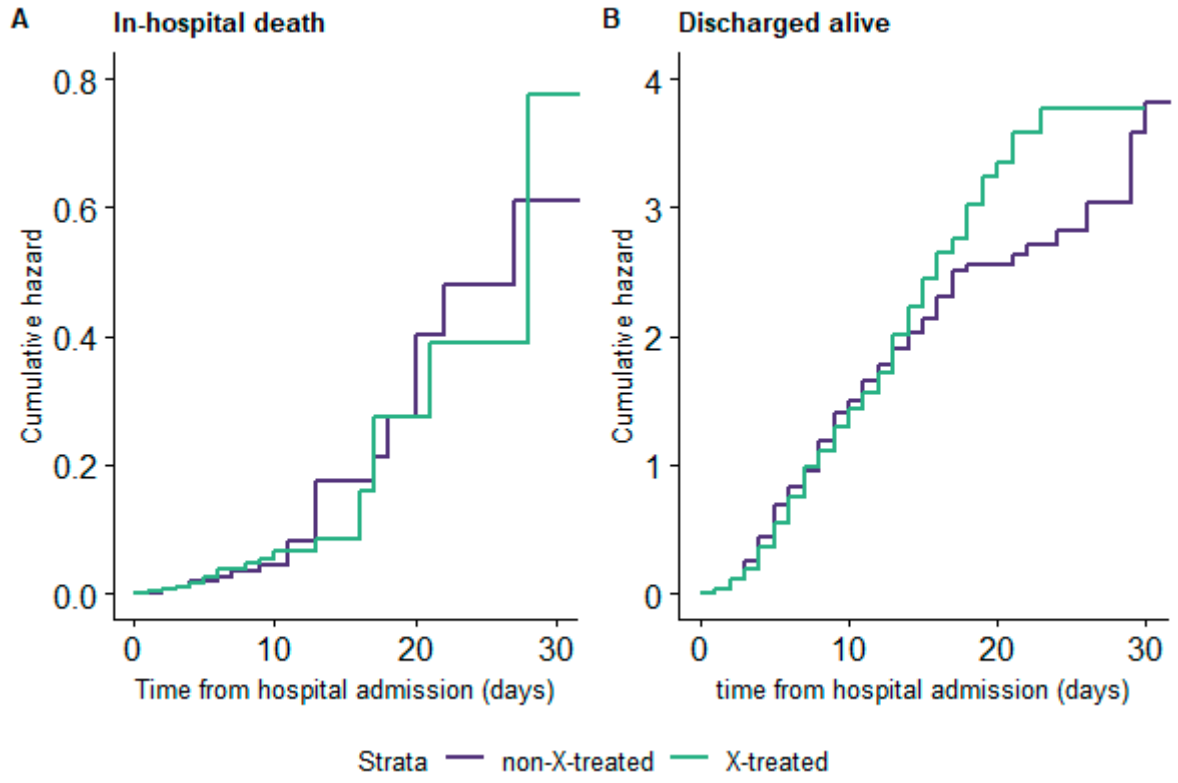
**Supplementary Figure S3.** Effect on hospital length of stay in X-treated (A) and non-X-treated (B) patients



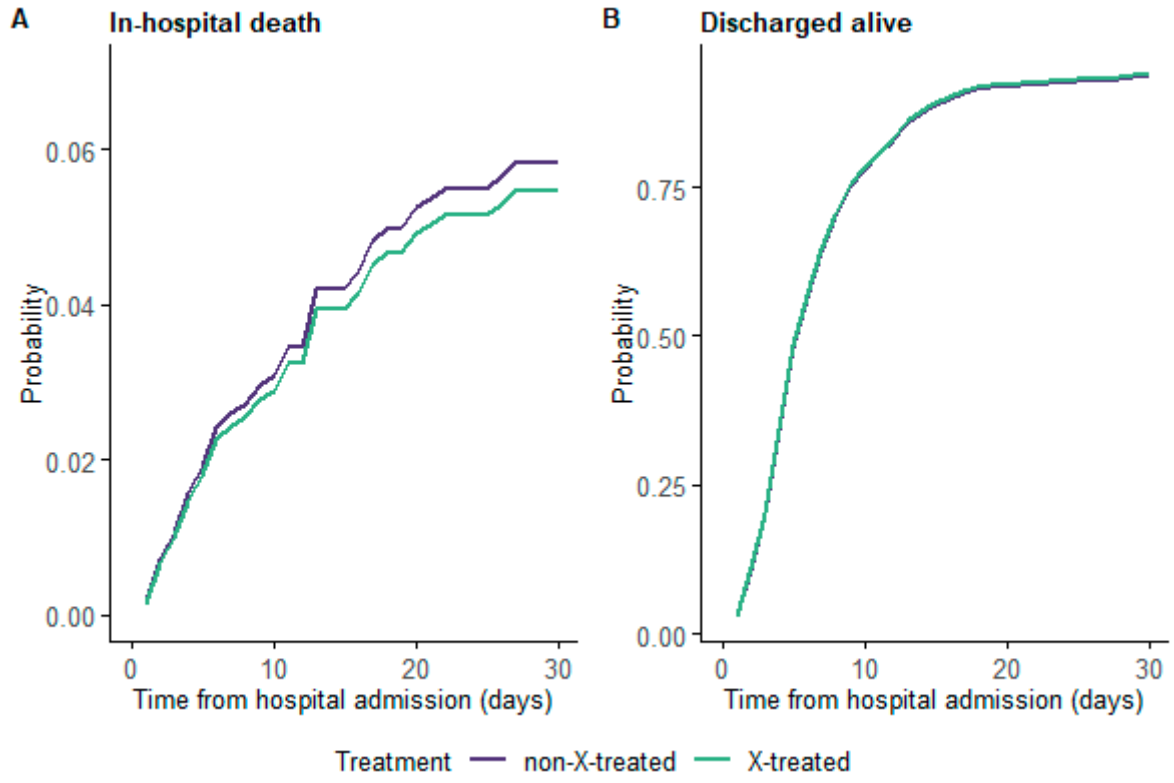

Abbreviation: LOS, length of stay.

**Results from the additional analyses**

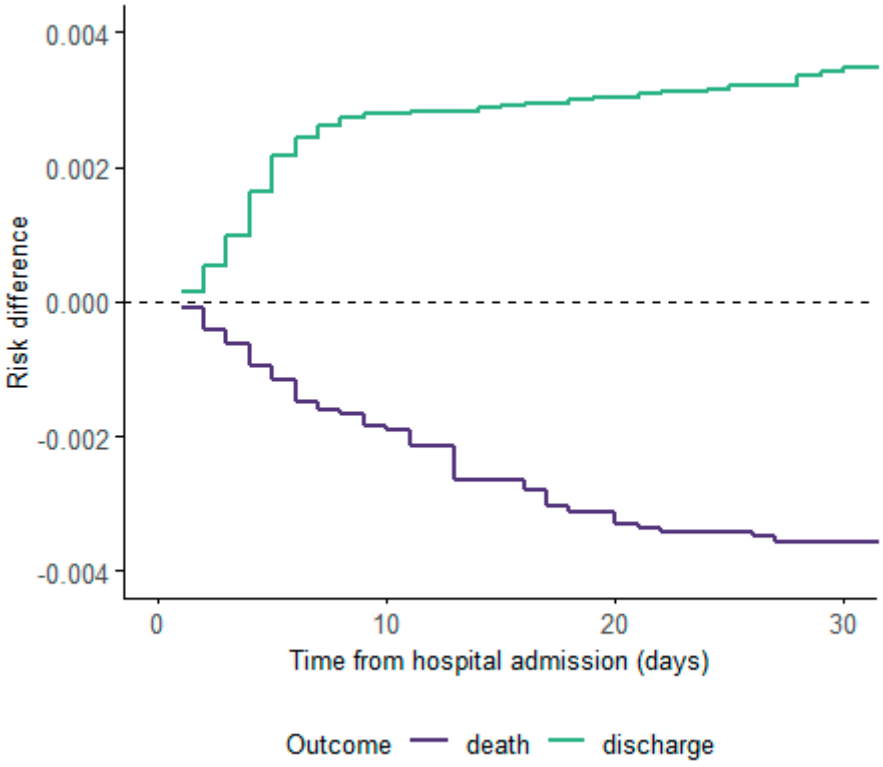
**Supplementary Figure S4.** Weighted cause-specific cumulative hazards estimated from the Nelson-Aalen estimator for in-hospital death (A) and discharged alive (B) outcomes



**Supplementary Figure S5.** Weighted cause-specific cumulative incidences estimated using the Aalen-Johansen estimator for in-hospital death (A) and discharged alive (B)



**Supplementary Figure S6.** Risk differences for in-hospital death and discharged alive outcomes over the follow-up period using the Aalen-Johansen approach



**Supplementary Table S4.** Summary results of the sensitivity analysis with one-day grace period at the end of follow-up (60 days)

| Corresponding measure | Mathematical formulation | Results [95% CI] |
|---|---|---|
| **Constant hazards** | | |
| Death hazard w/o treatment | $\lambda_{02}$ | 0.010 |
| Discharge hazard w/o treatment | $\lambda_{03}$ | 0.121 |
| Hazard w/o treatment | $\lambda_0 = \lambda_{02} + \lambda_{03}$ | 0.130 |
| Death hazard with treatment | $\lambda_{12}$ | 0.007 |
| Discharge hazard with treatment | $\lambda_{13}$ | 0.124 |
| Hazard with treatment | $\lambda_1 = \lambda_{12} + \lambda_{13}$ | 0.131 |
| **Mortality** | | |
| Mortality risk w/o treatment at day 60 | $MR_0 = \frac{\lambda_{02}}{\lambda_{02} + \lambda_{03}}$ | 0.074 [0.031-0.085] |
| Mortality risk with treatment at day 60 | $MR_1 = \frac{\lambda_{12}}{\lambda_{12} + \lambda_{13}}$ | 0.055 [0.029-0.068] |
| Mortality risk ratio at day 60 | $\frac{\lambda_{12}/\lambda_{02}}{(\lambda_{13} + \lambda_{12})/(\lambda_{02} + \lambda_{03})}$ | 0.750 [0.411-1.197] |
| Difference in mortality at day 60 | $MR_1 - MR_0$ | -0.018 [-0.041-0.023] |
| **Occurrence of outcome and treatment effect estimands** | | |
| Hazard ratio of death (treatment *vs.* w/o treatment) | $HR_2 = \frac{\lambda_{12}}{\lambda_{02}}$ | 0.754 [0.352-1.125] |
| Hazard ratio of discharge (treatment *vs.* w/o treatment) | $HR_3 = \frac{\lambda_{13}}{\lambda_{03}}$ | 1.025 [0.801-1.042] |
| Differences in hazard ratios of death (treatment *vs.* w/o treatment) at day 60 | $\lambda_{12} - \lambda_{02}$ | -0.002 [-0.006, 0.002] |
| Differences in hazard ratios of discharge (treatment *vs.* w/o treatment) at day 60 | $\lambda_{13} - \lambda_{03}$ | 0.003 [-0.024, 0.005] |
| Cumulative risk of death w/o treatment at time t | $CIF_{02} = \frac{\lambda_{02}}{\lambda_0} \times (1 - \exp(-\lambda_0 t))$ | 0.073 [0.031-0.085] |
| Cumulative risk of discharge w/o treatment at time t | $CIF_{03} = \frac{\lambda_{03}}{\lambda_0} \times (1 - \exp(-\lambda_0 t))$ | 0.925 [0.913-0.968] |
| Cumulative risk of death with treatment at time t | $CIF_{12} = \frac{\lambda_{12}}{\lambda_1} \times (1 - \exp(-\lambda_1 t))$ | 0.055 [0.029-0.068] |
| Cumulative risk of discharge with treatment at time t | $CIF_{12} = \frac{\lambda_{13}}{\lambda_1} \times (1 - \exp(-\lambda_1 t))$ | 0.943 [0.929-0.968] |
| Risk difference functions for death at day 60 | $RD_2 = CIF_{12} - CIF_{02}$ | -0.018 [-0.041, 0.023] |
| Risk difference functions for discharge at day 60 | $RD_3 = CIF_{13} - CIF_{03}$ | 0.018 [-0.024, 0.040] |
| Risk ratios for death at day 60 | $RR_2 = \frac{CIF_{12}}{CIF_{02}}$ | 0.750 [0.041, 1.196] |

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| Corresponding measure | Mathematical formulation | Results |
|---|---|---|
| Risk ratios for discharge at day 60 | $RR_3 = \frac{CIF_{13}}{CIF_{03}}$ | 1.020 [0.973, 1.044] |
| **Length of stay** | | |
| Length of stay w/o treatment | $LOS_0 = \frac{1}{\lambda_{02} + \lambda_{03}}$ | 7.66 days [6.89, 8.31] |
| Length of stay with treatment | $LOS_1 = \frac{1}{\lambda_{12} + \lambda_{13}}$ | 7.63 days [7.84, 8.71] |
| Difference in length of stay | $LOS_1 - LOS_0$ | -0.03 |

Notes: 0 = from non-X-treated state, 1 = from X-treated state.
Abbreviations: CIF, cumulative incidence function; HR, hazard ratio; LOS, length of stay; MR, mortality risk, RD, risk difference; RR, risk ratio; w/o, without.

Mean of the weights 1.608 for the treated and 1.837 for the control group.

**Supplementary Table S5.** Summary results of the sensitivity analysis with a grace period (of three days) at the end of follow-up (60 days)

| Corresponding measure | Mathematical formulation | Results [95% CI] |
|---|---|---|
| **Constant hazards** | | |
| Death hazard w/o treatment | $\lambda_{02}$ | 0.010 |
| Discharge hazard w/o treatment | $\lambda_{03}$ | 0.121 |
| Hazard w/o treatment | $\lambda_0 = \lambda_{02} + \lambda_{03}$ | 0.131 |
| Death hazard with treatment | $\lambda_{12}$ | 0.007 |
| Discharge hazard with treatment | $\lambda_{13}$ | 0.124 |
| Hazard with treatment | $\lambda_1 = \lambda_{12} + \lambda_{13}$ | 0.132 |
| **Mortality** | | |
| Mortality risk w/o treatment at day 60 | $MR_0 = \frac{\lambda_{02}}{\lambda_{02} + \lambda_{03}}$ | 0.076 [0.033-0.088] |
| Mortality risk with treatment at day 60 | $MR_1 = \frac{\lambda_{12}}{\lambda_{12} + \lambda_{13}}$ | 0.055 [0.034-0.070] |
| Mortality risk ratio at day 60 | $\frac{\lambda_{12}/\lambda_{02}}{(\lambda_{13} + \lambda_{12})/(\lambda_{02} + \lambda_{03})}$ | 0.728 [0.469-1.176] |
| Difference in mortality at day 60 | $MR_1 - MR_0$ | -0.021 [-0.034, 0.022] |
| **Occurrence of outcome and treatment effect estimands** | | |
| Hazard ratio of death (treatment *vs.* w/o treatment) | $HR_2 = \frac{\lambda_{12}}{\lambda_{02}}$ | 0.730 [0.415-1.102] |
| Hazard ratio of discharge (treatment *vs.* w/o treatment) | $HR_3 = \frac{\lambda_{13}}{\lambda_{03}}$ | 1.026 [0.806-1.040] |
| Differences in hazard ratios of death (treatment *vs.* w/o treatment) at day 60 | $\lambda_{12} - \lambda_{02}$ | -0.003 [-0.006, 0.002] |
| Differences in hazard ratios of discharge (treatment *vs.* w/o treatment) at day 60 | $\lambda_{13} - \lambda_{03}$ | 0.003 [-0.023, 0.005] |
| Cumulative risk of death w/o treatment at time t | $CIF_{02} = \frac{\lambda_{02}}{\lambda_0} \times (1 - \exp(-\lambda_0 t))$ | 0.076 [0.033-0.088] |
| Cumulative risk of discharge w/o treatment at time t | $CIF_{03} = \frac{\lambda_{03}}{\lambda_0} \times (1 - \exp(-\lambda_0 t))$ | 0.923 [0.910-0.966] |
| Cumulative risk of death with treatment at time t | $CIF_{12} = \frac{\lambda_{12}}{\lambda_1} \times (1 - \exp(-\lambda_1 t))$ | 0.055 [0.034-0.070] |
| Cumulative risk of discharge with treatment at time t | $CIF_{12} = \frac{\lambda_{13}}{\lambda_1} \times (1 - \exp(-\lambda_1 t))$ | 0.943 [0.927-0.964] |
| Risk difference functions for death at day 60 | $RD_2 = CIF_{12} - CIF_{02}$ | -0.021 [-0.039, 0.022] |
| Risk difference functions for discharge at day 60 | $RD_3 = CIF_{13} - CIF_{03}$ | 0.021 [-0.024, 0.038] |
| Risk ratios for death at day 60 | $RR_2 = \frac{CIF_{12}}{CIF_{02}}$ | 0.728 [0.047, 1.175] |

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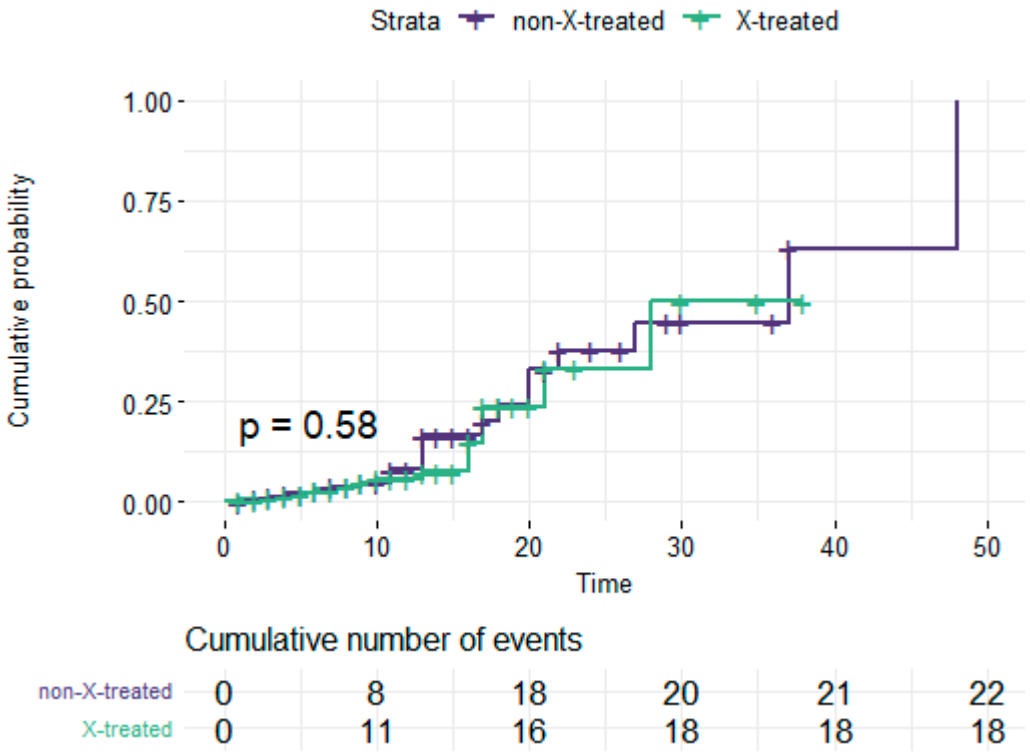
| Corresponding measure | Mathematical formulation | Results |
|---|---|---|
| Risk ratios for discharge at day 60 | $RR_3 = \frac{CIF_{13}}{CIF_{03}}$ | 1.022 [0.973, 1.042] |
| **Length of stay** | | |
| Length of stay w/o treatment | $LOS_0 = \frac{1}{\lambda_{02} + \lambda_{03}}$ | 7.62 days [6.87, 8.30] |
| Length of stay with treatment | $LOS_1 = \frac{1}{\lambda_{12} + \lambda_{13}}$ | 7.60 days [7.84, 8.66] |
| Difference in length of stay | $LOS_1 - LOS_0$ | -0.02 |

Notes: 0 = from non-X-treated state, 1 = from X-treated state.
Abbreviations: CIF, cumulative incidence function; HR, hazard ratio; LOS, length of stay; MR, mortality risk, RD, risk difference; RR, risk ratio; w/o, without.

Mean of the weights 1.303 for the treated and 1.863 for the control group.

**Supplementary Figure S7.** Kaplan-Meier estimates of the cumulative probability (%) of in-hospital death




*Using an immortal-time dataset, all treated patients were classified as treated from time zero

**The Kaplan-Meier analysis**

We used the standard Kaplan-Meier survival analysis (that is, one minus survival function) to calculate the cumulative probability of in-hospital death over time. Competing risk bias was artificially introduced using the Kaplan-Meier method that treats all discharged events as right-censored.