Association of short time to blood culture positivity with disease severity in - a population-based cohort study

Supplementary appendix

Contents

1. Rationale

Definition and incidence of bloodstream infections

Bloodstream infections (BSIs) are defined as infections with growth of a relevant pathogen in blood cultures. When a BSI is suspected, patient blood is added to a blood culture medium (in a bottle), which is subsequently incubated at 37°C in a blood culture cabinet.[1] The time from incubation to reaching a detection threshold is time to positivity (TTP).

Bloodstream infections are medical emergencies, with a short term mortality of 10-20%, causing > 150000 deaths in Europe per year.[2,3] In addition, several studies have shown an increasing incidence of BSIs, with the largest increase in the oldest individuals.[4–7] Population projections unanimously predicts that this group will increase substantially, potentially leading to a further increase in BSI incidence. This emphasises the need to improve management of bloodstream infections. Challenges in BSI management include predicting which patients with suspected BSI that will deteriorate, requiring continuous monitoring, and who will not.

Conflicting results in previous studies

Several previous studies have examined the association between TTP and outcomes, including endocarditis and mortality.[8–21] These studies have found conflicting results, with some studies finding a strong association between TTP and outcome, while others have found no association at all.[12,15] This has led to a discussion on the clinical value of TTP, and as of today, the only recommendation for clinical use of TTP is for line-associated bloodstream infections.[22,23]

There has been no clear explanation for the conflicting results in previous studies. Some factors associated with the validity of TTP have been described, including blood volume and time from venepuncture to incubation.[24] In a recent large population-based study, the authors state that small study samples may have contributed.[15] Indeed, a recent systematic review / meta-analysis of 24 studies included 4738 patients, indicating that the average study sample is rather small.[25] Upon studying previous literature, we noted that all of the previous studies have used two different modelling approaches for the association. Many previous studies have categorised TTP (which is an inherently continuous variable) with data-driven categorisations, based on either the distribution (median, lowest tertile, lowest quartile) or on ROC / discrimination / Youden index.[8,13–15,17,18]. These have generally found an association. Others have used a continuous, linear approach and found no association.[9,12] Bacterial growth is an inherently exponential phenomenon, where *Escherichia coli* could double every 20 minutes in ideal conditions. Thus, during optima conditions, using a fixed detection threshold, a TTP difference of one hour would represent an eight-fold difference in the original bacterial load and a two-hour difference a 64-fold. Therefore, we hypothesised that if the bacterial load in blood is a marker of disease severity, the association of TTP and disease severity is likely to be exponential. However, no previous study has modelled the association in this way.

Lack of high-resolution disease severity markers

Previous studies have used singular outcomes, typically mortality, ICU admission or endocarditis.[8,13–15,17,18,20,25] Although clinically relevant, these outcomes are dichotomous and have low resolution. There may also be confounding factors associated with both short TTP and the outcome (e.g. immunosuppression, high age, having received antibiotics prior to culture etc.). Therefore, we strived to 1) add several other parameters of disease severity, including inflammatory biomarkers and vital signs at the time of culturing and the following days and 2) adjust for more potential confounders.

2. Methods

Setting

Geography and healthcare organisation in Skåne

The Skåne region in southern Sweden has a population of approximately 1.4 million people (1 421 781in 2023). Skåne is a peninsula surrounded by water on three sides with the majority of the population living in the southwestern part. The population is served by ten acute care hospitals, with the Skåne university hospital, with sites in Malmö and Lund, providing tertiary care. The geography and hospital placement entails that almost all citizens will seek acute care at a Skåne hospital and vice versa: almost all patients in Skåne hospitals will be residents. This makes Skåne ideal for population-based studies using hospital-based case-finding.[7]

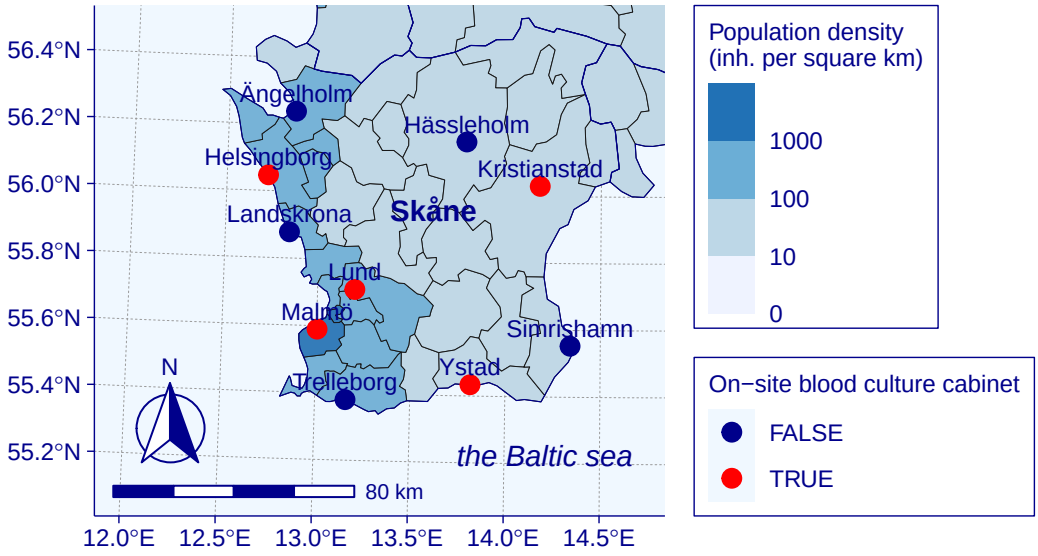

Figure 1: Geography, population density, hospital locations and placement of blood culturing cabinets within the Skåne region in southern Sweden

Clinical microbiology and blood culture systems in Skåne

Upon the suspicion of a BSI, blood cultures are collected, typically by obtaining four bottles (two BD Bactec Plus aerobic medium and two BD Bactec Lytic anaerobic medium) from the same venepuncture. However, in pediatric patients, singular bottles may be obtained. The bottles are then transported to

blood culturing cabinets (BACTEC FX, BectonDickinson, Franklin Lakes, United States) for incubation. In Skåne, the five largest hospitals (in Malmö, Lund, Helsingborg, Kristianstad and Ystad) have on-site blood culturing cabinets, while the others do not. Blood cultures taken in hospitals without cabinets are transported to the nearest hospital with a cabinet for incubation. We hypothesised that this would lead to measurement error in terms of TTP, as bacteria may start to duplicate in the bottles in room temperature before incubation.[26] After a bottle has indicated the growth of a pathogen in a cabinet, it is transported to the laboratory of clinical microbiology in Lund for further downstream procedures including microscopy, species identification and testing for antimicrobial susceptibility. There is only one microbiology lab in the region and all samples are sent to this lab.

Healthcare databases in Skåne

Microbiology data is stored using two different information systems, wwLab which was used up until March 2021 and LIMS (Laboratory Information Management System), which has been used since. All ten hospitals in the Skåne region use the same system for electronical medical records, Melior (Siemens Healthcare Services, Upplands Väsby, Sweden). In this system, laboratory values, vital signs, medications, diagnoses etc. are recorded. Data from primary care is recorded in another system, with diagnoses, labs, medications etc. For medications, a specific database also contains data on pick-up at pharmacies. There is also a separate database on diagnoses. Data from these different systems are hosted by a regional service, which is updated daily. Data extraction from the regional databases as well as matching is provided by a specialised team after obtaining approvals from the Swedish Ethical Review Authority and a regional board approving all database extractions.

Data management

General aspects

- We used R for data management and statistical analyses: https://www.r-project.org, with the R studio https://posit.co/download/rstudio-desktop/
- For reproducibility, we used Quarto markdown for reports and manuscript (https://quarto.org/docs/authoring/markdown-basics.html).
- For transparency, all markdown code and R functions are available at https://www.github.com/gtorisson. This code covers all steps from the raw data to the rendering of this supplementary and the manuscript, including all statistical analyses, results, and figures. Due to privacy concerns, raw data can not be uploaded, which would have increased transparency further.
- Many functions were written in-house and are heavily dependent on local data structure and not generic.
- We used .parquet format for data storage throughout, see https://arrow.apache.org/docs/r/index.html

- We used `tidyverse` and the principles of tidy data with `janitor::clean_names()` naming conventions throughout (small_snake_case_only)

Data retrieval

Case-finding was done in the microbiology database by staff at the Department of Clinical Microbiology in Lund. This database was queried for all blood culture bottles taken from 2021 through 2023. The process was overseen by the authors (TS and KO). This data was then linked to other regional healthcare database(s) in Skåne Region. Data retrieval and linkage was done by a dedicated data retrieval service (Kliniska Studier Sverige - Forum Söder), in close co-operation with the study researchers (OL and GT). Cases from the microbiology database were then matched by personal identification number and sample date to data from the other systems. After this, data without direct identifiers was retrieved, with no key.

Data preprocessing

In all, the dataset consisted of 31 files. Some of the files were large and required data reduction. Some variables were duplicates or more of a technical character. We manually checked all variables for redundancy. We removed redundant variables and converted all files to .parquet files, to facilitate further handling.

Data management of microbiology data

There were four microbiology files, three from the LIMS system (one for each year) and one from wwLab, which was used up until 2021-03-14, after which LIMS was used. First, LIMS files from 2021, 2022, and 2023 were combined. There are several differences between the wwLab and LIMS systems to account for, most notably, the wwLab systems display two blood culture bottles (one set) per row and LIMS one. In addition, polymicrobial findings may render several rows in the datasets. The four files were read, cleaned, harmonised and then merged into a new dataset. Time to positivity was changed to NA if the culture was negative.

Categorisation of pathogens The raw variable that contains microbiological findings included some swedish names, misspellings or old names, and was also differently represented in wwLab and LIMS (Fully CAPITALISED in ww Lab). Therefore, all findings were renamed into a "species" variable which is consistent and according to current taxonomy. Species were then also collapsed (e.g. *Klebsiella pneumoniae* and *Klebsiella oxytoca* collapsed into *Klebsiella* species). A list of all species were saved as an external excel file. This list was reviewed by all authors, including two specialists in clinical Microbiology (TS and KO) and we determined which species were considered potential contaminants (e.g., coagulase-negative staphylococci). In addition, we categorised all species into two categorical levels, first being gram-negative, gram-positive, anaerobic or fungal. Of course, anaerobic bacteria

are also negative or positive on gram stains. The rationale here was to supply clinically meaningful categories that might warrant different treatments. For the next level, we added somewhat higher resolution, the full classification, named "pathogen_classification_2021_2023.xlsx" is found in a separate supplementary file.

Definition of contaminants, polymicrobial findings and deduplication Using this classification, all positive findings with potential contaminants were flagged (species considered potential contaminants). Then we defined contaminants as those cultures where a potential contaminant was found in only one blood culture set on the index date, i.e., the date of the first culture of an individual during the study period. All other positive findings were labelled as relevant.

Subsequently, we determined the episodes for each culture. All cultures in the 30 days after index date (the prespecified deduplication period) were considered to belong to the same episode and were deduplicated. Any cultures after 30 days were considered a new episode, with new index date etc.

If an individual had several relevant findings on the index date, the result was flagged as polymicrobial. If results showed one relevant pathogen (e.g., *Escherichia coli*) and one contaminant (e.g., coagulase-negative staphylococci), this was considered a monomicrobial relevant finding (of *Escherichia coli* in this example). If two different contaminants were found, this was considered a contamination.

Then, we deduplicated episodes into only one result for each individual and episode. If a relevant finding and a contaminant was coincident, the relevant finding was prioritised, as in clinical routine. Polymicrobial findings were included once and categorised as "polymicrobial". If everything else was alike, we prioritised findings with non-missing TTP and used the shortest TTP if several positive bottles were found. After deduplication, all BSI episodes could have one result only, belonging to one of the following categories:

- Non-anaerobic gram-positive bacteria
  - *Staphylococcus aureus*
  - *Staphylococcus* species (those considered relevant)
  - *Streptococcus pneumoniae*
  - Beta-hemolytic streptococci
  - Alpha-hemolytic streptococci
  - *Enterococcus* species
  - Other gram-positive
- Non-anaerobic gram-negative bacteria
  - *Escherichia coli*
  - *Klebsiella* species
  - Other enterobacterales
  - Other gram-negative
- Anaerobic bacteria
- Fungal (basically only *Candida* species)

- Contaminations
- Polymicrobial findings

Data management of other data

Hospitalisation data Hospitalisation data was cleaned and added to the episode data. A hospitalisation was considered linked to a suspected BSI episode if the index date was in the time period of three days before hospital admission to hospital discharge. If there were several hospitalisations in the three days preceding index date, these were all considered linked to the same episode, using the first admission date and the last discharge date. For all hospitalisations, we added the admission and discharge dates, and calculated length-of-stay (LOS). In addition, we added the variable "recent hospitalisation", defined as an inpatient stay in the preceding 90 days, excluding the index hospitalisation. We also defined the variable "nosocomial" - whether the index culture had been obtained > 48 hours after admission.

Dialysis data We added data on dialysis as this group is highly prone to BSIs. Dialysis data was cleaned, but did not seem complete, as there were virtually no patients with dialysis dates more than once a month. We considered the documentation to be incomplete. Therefore, we prioritised the first date of dialysis. If this date was before or on the index date of each culturing episode, the patient was considered to have had chronic dialysis before the current episode. However, this may have misclassified a few patients that have been on and off dialysis. e.g. those having undergone transplantation.

Medications data All medications for two months before or after the index date were retrieved. Data was cleaned to include anatomical therapeutical classification (ATC) group, drug name and date of collection / administration. From this data we added the variable "antibiotics before" - defined as the collection / administration of a drug within ATC group J01 - "antibacterials for systemic use" during the 1-14 days preceding the index date. We also added "immunosuppression before", defined as collection / administration of a drug within ATC groups L01 or L04 for two months preceding the index date. Both these variables were considered potential confounders, i.e., associated with both TTP and patient outcome, which is why they were included.

Diagnosis data Diagnosis data was cleaned and combined into one file, containing diagnosis date, diagnosis code (ICD-10), diagnosis text, and hospitalisation id. We searched for diagnoses of chronic diseases during the index hospitalisation and the 365 days preceding culturing date for each episode. These were used to estimate the Charlson comorbidity index, according to a previous validation for Swedish healthcare registers.[27] This was used as a combined measure of comorbidity but the Charlson index lacks several important risk factors for BSI, why we also categorised comorbidities further, the full documentation including specific ICD-10 codes is found in the `add_comorbidities()` function at https://www.github.com/gtorisson

Laboratory values Raw lab data included lab values and time stamps. We filtered this lab data and included the prespecified parameters with CRP and Procalcitonin being related to inflammation and Lactate, Creatinine, Bilirubin and Platelets to organ failure, with the latter three included in the sequential organ failure assessment (SOFA) score.[28] We excluded all samples taken in other locales than blood (e.g., lactate from cerebrospinal fluids). When a value had a floor or ceiling result, e.g. a CRP of "<5 mg/L" we removed the "<" sign and the value was considered to be 5 mg/L.

Using laboratory values to approximate culturing time. The index date of blood cultures was available in all cases. However, the exact time of culturing was not always provided. For some of our analyses, we needed a baseline time which should be as exact as possible. Therefore, if the culturing time had not been provided, we noted whether other blood samples had been obtained on the index date and presumed that blood cultures had been taken at the same time, with the same venepuncture. If no other blood samples had been obtained, we noted whether the hospitalisation had started on the index date and presumed that blood cultures had been obtained at the same time (at least not before) as the hospitalisation started.

Determining baseline lab values When the culturing time had been established, the difference in time from culturing was estimated for each lab result. We defined baseline lab values as those obtained within ± 24 hours from the time of blood culturing. If several were available, the one closest in time to culturing time was prioritised as the baseline value.

Vital signs Data on vital signs is recorded using the NEWS2 scoring system in Skåne (NEWS = National Early Warning Score), including heart rate, blood pressure, respiratory rate, oygen saturation, temperature, mental alteration, and an aggregate NEWS2 score. This data contained vital sign values as well as time stamps. There were several issues with this data:

- Missing data: the first vital signs when arriving at hospital are typically noted on paper during triage and not included in the database. Therefore, the important first vital signs are most likely missing in many cases. In addition, the ICUs document vital signs continously on sheets and do not register NEWS2. This could have introduced selection bias, where the group with missing NEWS2 values may not be representative.
- Data quality: There were quite a few implausible values, most likely due to entry errors (negative temperature etc.). Values outside plausible limits (seen in the `clean_news_data()` function) were filtered and considered NA. There was a misspecification in obstretric NEWS for blood pressure, which we corrected. Different scales (RLS and ACVPU) were used for mental alteration, why this was dichotomised.

Patient outcomes We recorded two patient outcomes, mortality and ICU admission. Vital status was determined on March 12 2024 and ICU admission was noted along with date and we only included ICU admissions starting at or after the index date (blood cultures obtained in patients already in the ICU were not considered to have an ICU admission outcome). These were used to determine in-hospital mortality, 30-day mortality, 30-day ICU admissions. However, the primary patient outcome was "ICU admission or death within 30 days", which we think is the best representation of disease severity. For the primary analysis, we considered one non-missing value (of deceased at 30 days or icu admission within 30 days) enough for this variable, but as a sensitivity analysis we required both to be non-missing.

Statistical analysis

Epidemiological analysis

We have previously described BSI epidemiology in the region in detail.[7] However, we aimed to provide a summary here of culturing frequency, positivity rate, the effect of 30-day deduplication and an incidence estimate, to facilitate comparisons with other settings.

General thoughts on categorised vs continuous TTP

One of our main hypotheses was that TTP had a non-linear association with outcome. Therefore, we generally aimed to use continuous methods. The downside of this is that such methods often require graphical interpretation rather than well known measures of association and hypothesis testing such as Odds ratios, p values etc. Therefore, we aimed to provide both methods whenever possible and appropriate. However, we consider continuous modelling superior from a mechanisitic and a statistical perspective.

TTP vs baseline characteristics

Using the TTP cutoff in a large recent study (10 hours) we compared baseline characteristics for three groups: "Negative", "ttp $\leq$ 10 hours", "ttp > 10 hours". As our material is quite large, even clinically meaningless differences may reach statistical significance why no hypothesis testing was done. All baseline characteristics were chosen due to possibly confounding potential and were later included in the adjusted analyses.

TTP vs disease severity at baseline

For baseline disease severity markers (laboratory values and vital signs), we constructed scatterplots with a regression fit using a GAM (generalized additive model) smoother including 95% confidence intervals. We considered a continuous vs continuous analysis the most appropriate here. However, we

also provided a table of categorised TTP vs disease severity as well, including hypothesis tests (Mann-Whitney if continuous, Chi-square if categorical) for easier interpretation.

TTP vs disease severity in the first 72 hours.

For this analysis, we plotted a scatterplot with hours from blood culture on the x axis and the value of disease severity markers on the y axis. We included all available values, thus some patients may not contribute at all (if the disease severity marker was not obtained) while others will have many repeated measures. The aim was to try to determine the change over time in the first three days. We used the three global TTP strata and again used the GAM model. As an alternative to the GAM model, we also provide mean values for every 4-hour period during the first 72 hours for easier interpretation.

Exploring a non-linear association between TTP and 30-day mortality

As stated before, we aimed to explore a global, non-linear association between TTP (continuous) and 30-day mortality (dichotomous). First, we plotted a scatterplot with a locally estimated scatterplot smoother (LOESS), using a span of 0.75 and a 95% confidence intervals. For comparison, we also used a generalized additive model (GAM) with a penalized cubic regression spline, as well as a logistic regression using a five-knot restricted cubic spline. The reason for using several methods was 1) to determine how congruent they were and 2) to enable comparisons of model fit, using the logistic regression with a spline fit. To compare with modelling in previous studies, we also estimated the association using a logistic regression with TTP as a continuous variable and a categorised variable using the global TTP cutoff of 10 hours. These models, linear, categorised and spline were compared using a Likelihood ratio test, to evaluate which one had the best model fit.

TTP vs outcome

We provide crude rates for the outcomes based on the TTP cutoff at 10 hours. As a sensitivity analysis, we also changed this cutoff to 9, 8, and 7 hours, respectively. In addition, we provide Odds ratios from logistic regression using the TTP cutoff at 10 hours, with 95% confidence interval to quantify risk differences. For the outcome mortality or ICU we performed a sensitivity analysis without those cases with missing ICU status (see exploratory data analysis below). The logistic regression was also adjusted for all baseline characteristics to adjust for confounders, e.g. receiving antibiotics before culture. To plot this, we also provide three-dimensional cumulative hazard plots where the proportion with outcome is plotted on the z axis, TTP on the x axis and follow-up time on the y axis.

TTP vs disese severity, stratified by species

Previous studies have shown that TTP varies with species, in line with the fact that pathogens have different growth rates (e.g. fast for streptococci, slow for Candida). We described the median and interquartile range for our pathogen categories. In addition, we determined the association with disease severity across these pathogen categories, using separate TTP cutoffs for each pathogen category (the first quartile of TTP vs Q2-Q4), as done in the study by Laupland et al.[15]

Sensitivity analysis 1: hospitals without on-site cabinets

We performed a separate analysis for sites without on-site cabinets, using the non-linear plot of TTP vs 30-day mortality. The rationale to examine the effect of longer transportation times on this association

Sensitivity analysis 2: pediatric bottles

We performed a separate analysis in pediatric bottles. These may be filled with a smaller initial blood volume, and often only one bottle is used.

3. Exploratory data analysis

In exploratory data analysis, we screened all variables for missing values, implausible values and determined the distribution of categorical and continuous variables.

Epidemiology

In total, there were 511458 blood culture bottles obtained in Skåne from 2021 to 2023, representing 260012 blood cultures sets, equivalent to an annual culturing rate of 6134 sets per 100.000 inhabitants. After 30-day deduplication from the index culture, 100473 unique suspected BSI episodes remained. These represented 12915 separate BSI episodes after removal of contaminants, and an annual incidence rate of 305 per 100.000 inhabitants, very similar to previously published data from the region.[7] Of the 100473 culturing episodes, 89546 (89%) were obtained in hospitals with on-site cabinets and constituted the final study sample.

Microbiology data

Estimating the time of sampling Sample date was available for all 89546 episodes and was evenly distributed over time. However, sample time was not always provided and was therefore estimated using the time of other blood samples or the hospitalisation start, as described earlier.

Source of culture time data	count	% of total
Culture time provided	82181	92%
From venepuncture time	5709	6%
From hospitalisation start	1066	1%
None of the above	590	1%

Table 1. Source of information regarding timing of blood cultures

Findings In the deduplicated dataset, microbiological findings were available in all 89546 episodes, with 14508 (16.2%) being positive. Of the positive findings, 2986 were considered contaminants, and 11522 relevant findings. Of the relevant findings, 1407 were polymicrobial, i.e. had two or more relevant findings.

Pathogen	count	% of relevant findings
Escherichia coli	3051	26.5%
Staphylococcus aureus	1506	13.1%
Polymicrobial	1407	12.2%
Staphylococcus species	785	6.8%
Klebsiella species	758	6.6%
Beta-hemolytic streptococci	682	5.9%
Other enterobacterales	616	5.3%
Alpha-hemolytic streptococci	597	5.2%
Anaerobic bacteria	543	4.7%
Other gram_negative	423	3.7%
Streptococcus pneumoniae	393	3.4%
Other gram_positive	365	3.2%
Enterococcus species	333	2.9%
Fungal	63	0.5%

Table 2. Distribution of relevant pathogens in BSI episodes in Skåne 2021-2023

Time to positivity Time to positivity was available in 11282/11522 (97.9%) of the BSI episodes and the distribution was left skewed, as seen in figure 2.

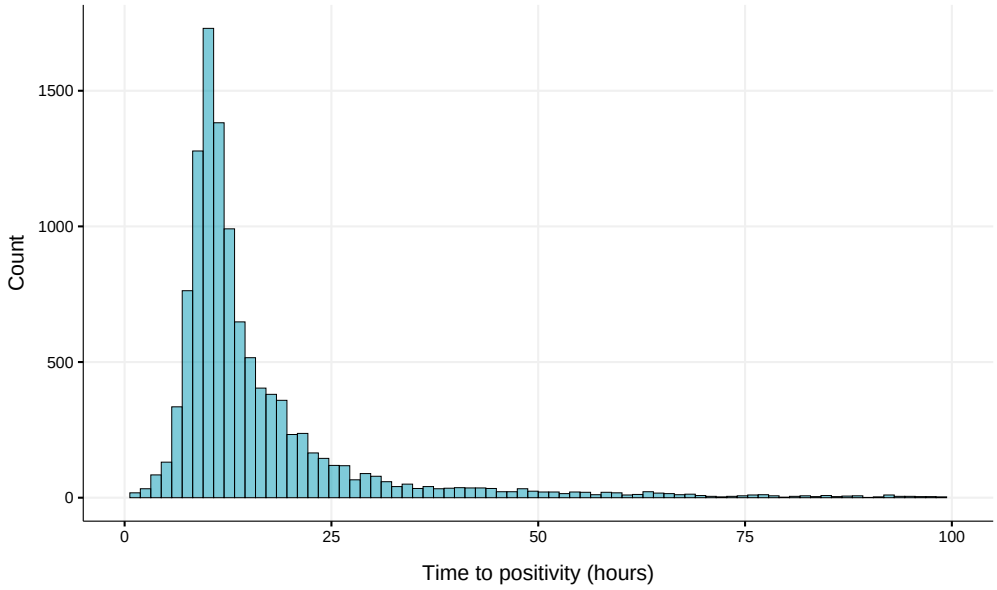

Figure 2: Distribution of time to positivity in BSI episodes

TTP in sites with vs without on-site cabinets

Previous studies have showed that time to incubation has a inverted effect on TTP, defined as time under incubation, as the bacteria in the bottle start to multiply in room temperature before being incubated.[26] Therefore, we compared hospitals with on-site cabinets with those without on-site cabinets (with longer transportation in room temperature). Upon comparison, it was clear that TTP in the hospitals with on-site culturing cabinets differed from those without. In hospitals with cabinets, transfer time was much shorter and more consistent. In these hospitals, TTP was also longer and more consistent. When dichotomised, the proportion with short TTP was much higher and variable in hospitals without on-site cabinets. Thus, we considered it inappropriate to include data from hospitals without on-site cabinets.

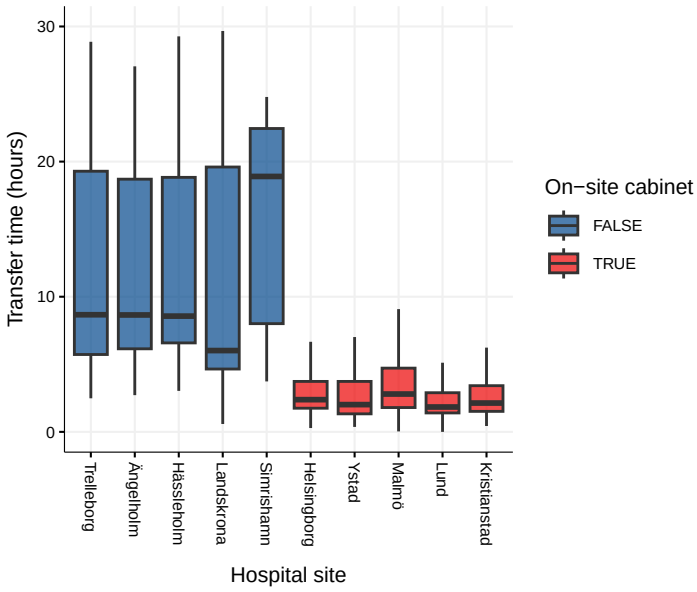

Figure 3: Transfer time in hours from venepuncture to start of incubation by hospital site and on-site cabinet status

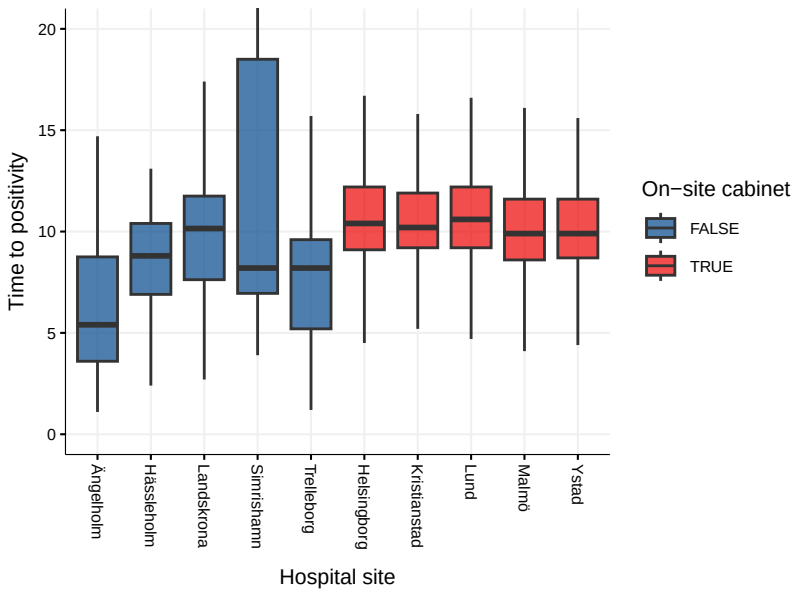

Figure 4: Time to positivity by hospital site and on-site cabinet status. The plot includes *Escherichia coli* only (to avoid bias due to differences in prevalence of species)

Hospital	Has cabinet	Median TTP (hrs)	% with TTP ≤ 10 hours
Lund	TRUE	14.1	21%
Kristianstad	TRUE	14.0	23%
Ystad	TRUE	13.9	26%
Malmö	TRUE	13.6	27%
Helsingborg	TRUE	13.1	25%
Trelleborg	FALSE	12.9	39%
Landskrona	FALSE	11.7	38%
Simrishamn	FALSE	11.2	40%
Hässleholm	FALSE	10.9	42%
Ängelholm	FALSE	9.3	53%

Table 3. Time to positivity in hospitals by cabinet status

Other baseline data

Demographic data Age had no missing data and ranged from 0 to 104 years, with a median of 70 years. As expected, the distribution showed a spike of newborn and older persons. Sex was also complete with 47993 (54%) being males. Site data was available for all 89546 episodes.

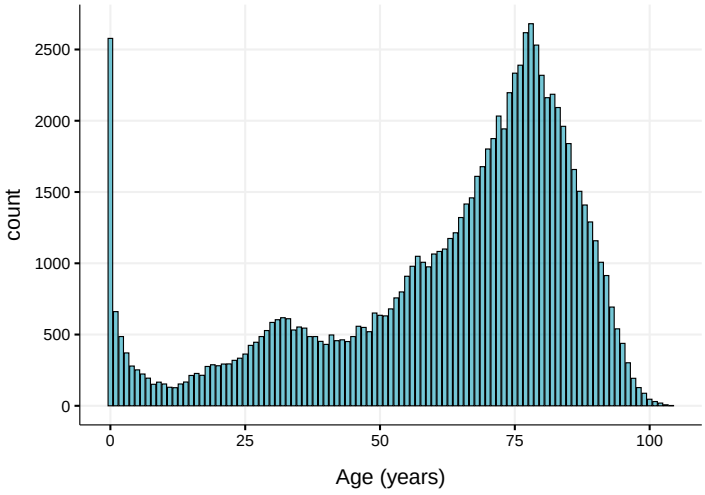

Figure 5: Age distribution of suspected BSI episodes in Skåne Region 2021-2023, N = 89546

Hospitalisation data The suspected BSI episode could be linked to an in-hospital stay in 85714 (95.7%) episodes, with a median length-of-stay of 5.7 days. In the other suspected BSI episodes, the blood cultures had been obtained in an outpatient setting, without a related hospital admission (e.g., a hospital-at-home program or in palliative home care). In total, 12295 (13.7%) episodes were labelled nosocomial, i.e. the culture had been obtained > 48 hours from admission, and the patient had been recently hospitalised in 23333 (26.1%) episodes.

Comorbidities We used ICD-10 codes from the linked hospitalisation and for the year preceding index date to determine comorbidities. Missing diagnoses were labelled absent and thus this data was complete. The most common comorbidities were hypertension and chronic cardiac disease.

Comorbidity	count	% of episodes
Hypertension	34754	39%
Cardiac disease	30899	35%
Musculoskeletal disease	26533	30%
Psychiatric disorder	22415	25%
Genitourinary disease	21159	24%
Malignancy	20933	23%
Neurologic disease	20047	22%
Diabetes	19124	21%
Skin disease	18285	20%
Pulmonary disease	17227	19%
Anemia	12029	13%
Peripheral vascular disease	10220	11%
Immunodeficiency	4211	5%
Hepatic disease	2847	3%
Dialysis	1285	1%

Table 3. The most common comorbidities at suspected BSI episodes

We also estimated Charlson comorbidity index from diagnostic codes; the median Charlson index was 1 points, ranging from 0 to 17, with 32081 (36.1%) patients having 0 points.

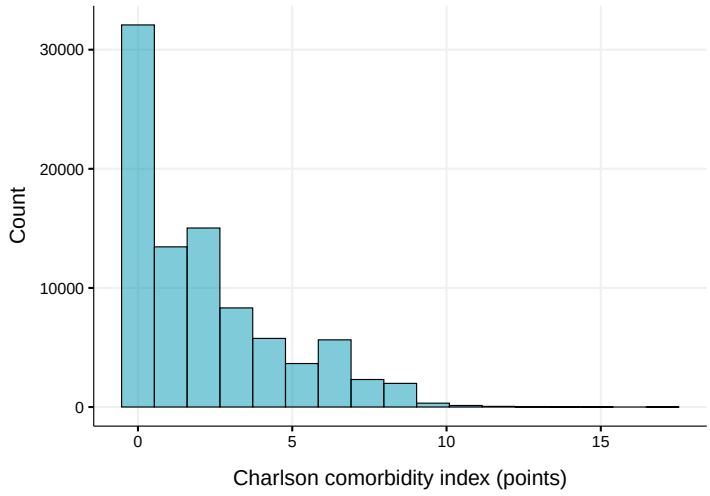

Figure 6: Distribution of Charlson comorbidity index score

Disease severity markers

At baseline We included C-reactive protein, lactate and procalcitonin as inflammatory biomarkers. In addition, bilirubin, creatinine, and platelets where included as markers of organ dysfunction and since they are included in the SOFA score. Among laboratory values, CRP and creatinine were most commonly taken at baseline (within ± 24 hours of culturing time), see table. Approximately two thirds of the patients had registered vital signs within ± 24 hours from blood cultures in the database. This may seem low but could be dependent on the issues described regarding raw data on vital signs above.

	Count	% of episodes
Crp	79164	88%
Creatinine	73424	82%
Lactate	54965	61%
Bilirubin	34958	39%
Platelets	47681	53%
Procalcitonin	10357	12%
Heart rate	56997	64%
Mental alteration	56780	63%
Oxygen saturation	56969	64%
Systolic blood pressure	56967	64%
Respiratory rate	56983	64%
Temperature	56982	64%
News total score	56731	63%

Table 4. Baseline disease severity markers ± 24 hours from sampling

The distribution of baseline disease severity values is shown in figure 7. The timing of baseline disease severity markers is shown in figure 8. Generally, the majority of baseline lab values had been obtained at the time of culturing or slightly before, except for procalcitonin that to a larger extent was taken after the culturing. For vital signs, there was a larger timing spread, with values generally being registered after the blood cultures. The reason for this may be that initial vital signs would be missing from the database as they are noted on sheets, as stated above.

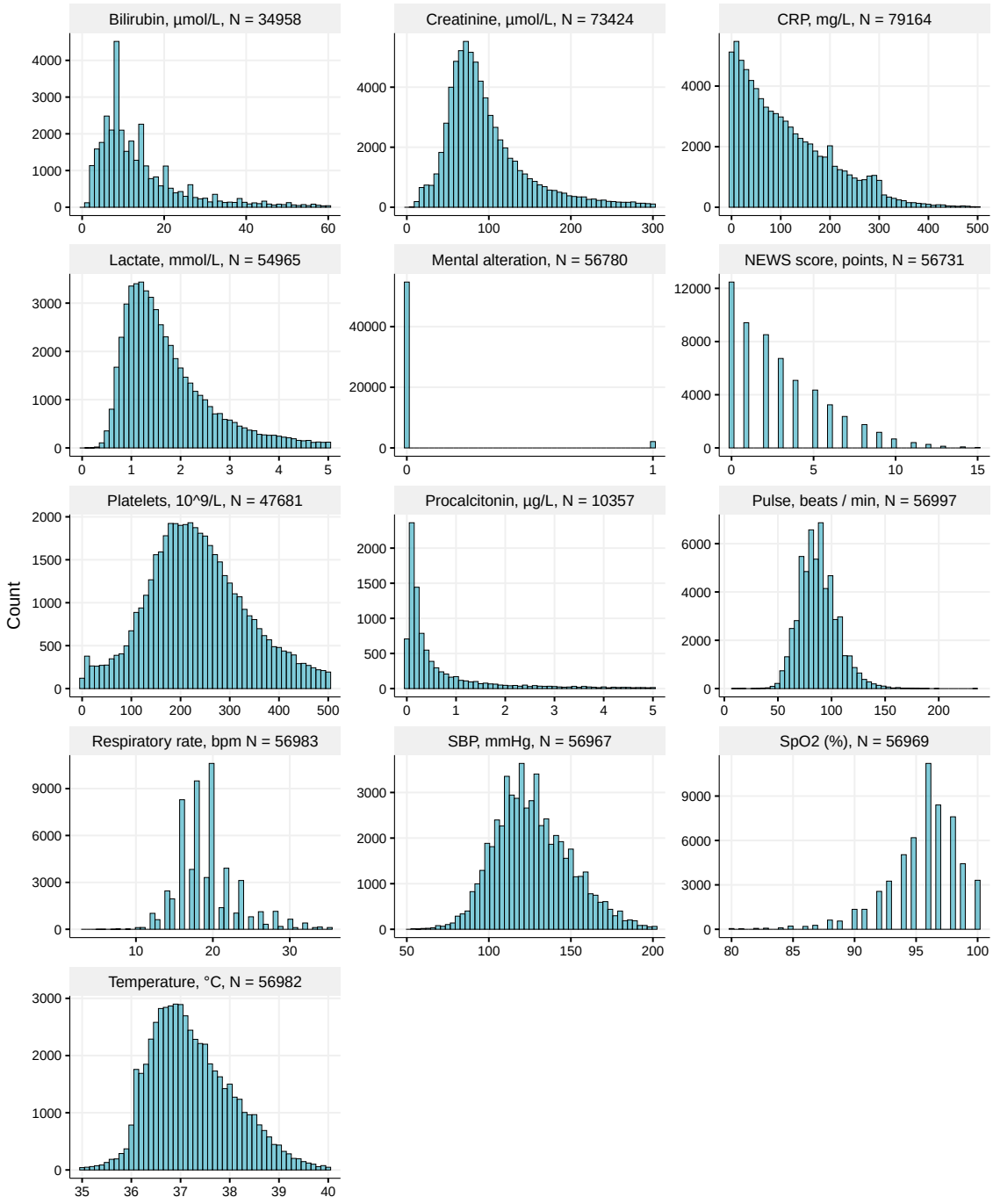

Figure 7: Distribution of disease severity markers in all suspected BSI episodes, at baseline (± 24 hours from culturing). CRP = C-reactive protein, NEWS = National Early Warning System scale, bpm = breaths per minute, SBP = Systolic blood pressure, SpO2 = peripheral oxygen saturation. Note: different scales on both axes

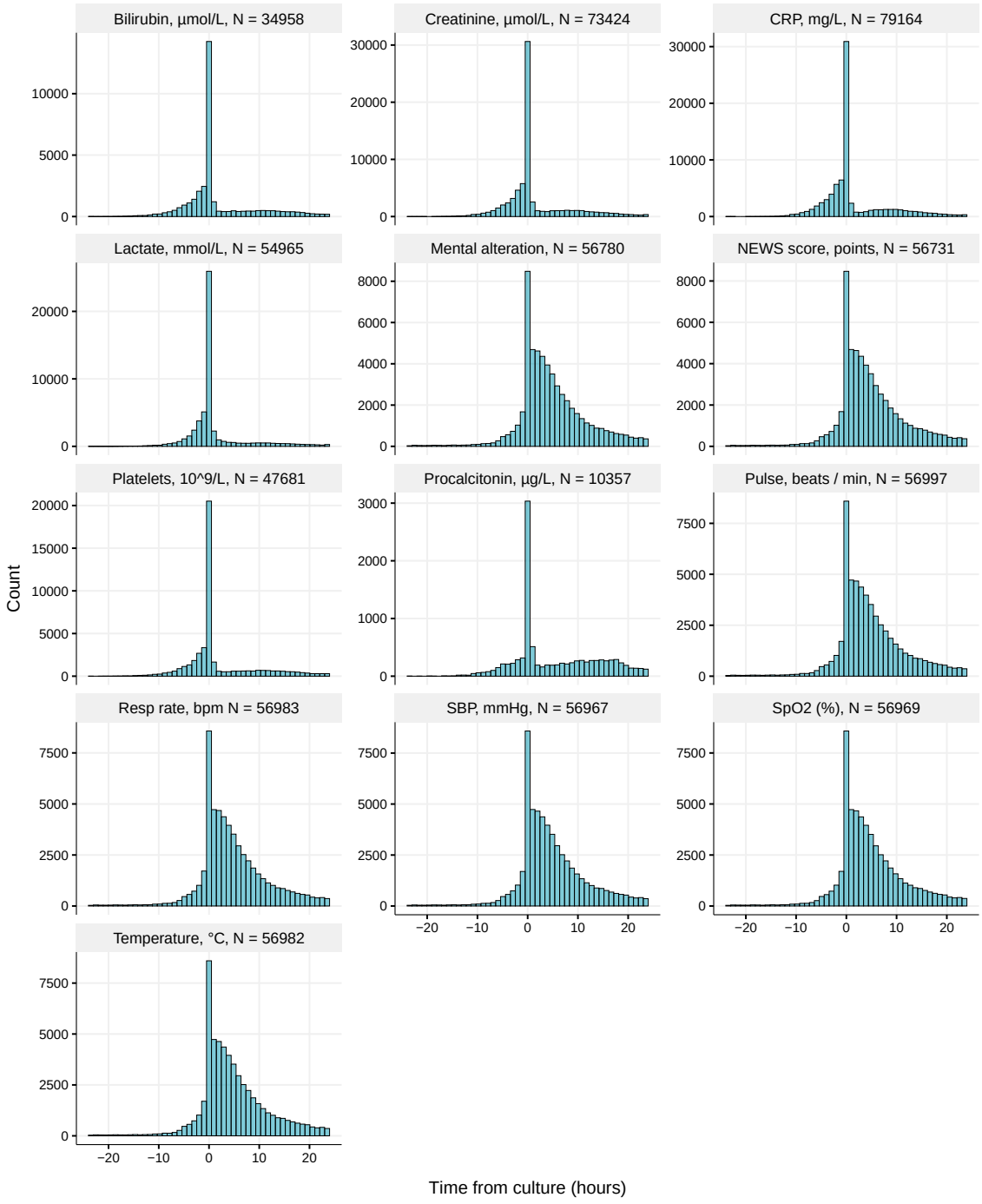

Figure 8: Time between baseline disease severity markers and blood culturing in all suspected BSI episodes, CRP = C-reactive protein, NEWS = National Early Warning System scale, bpm = breaths per minute, SBP = Systolic blood pressure, SpO2 = peripheral oxygen saturation.

Disease severity markers up to 72 hours When the time period was extended to 72 hours after culturing, almost all patient had at least one CRP value taken. Lactate was the lab value most often taken repeatedly. Vital signs were recorded more frequently (often 4 times per day) as compared to the laboratory values.

Marker	Results	Episodes	Percent	Average samples / episode
Bilirubin	77864	39804	44 %	2.0
CRP	227697	85164	95 %	2.7
Creatinine	264309	79339	89 %	3.3
Lactate	230465	57667	64 %	4.0
Platelets	97768	55010	61 %	1.8
Procalcitonin	20625	14351	16 %	1.4
Heart rate	582975	62783	70 %	9.3
Mental alteration	577659	62594	70 %	9.2
NEWS total score	577934	62542	70 %	9.2
Oxygen saturation	582692	62748	70 %	9.3
Respiratory rate	583095	62764	70 %	9.3
Systolic blood pressure	589758	62758	70 %	9.4
Temperature	583020	62777	70 %	9.3

Table 5. Disease severity markers up to 72 hours after blood cultures

Outcomes

Mortality Data on 30-day mortality was available in 88357 / 89546 (98.7%) suspected BSI episodes. The reason for missingness here is unknown - patients were not available in regional registers at the point of follow-up. This could have been due patients having moved outside the region and thus being inaccessible for follow-up. We considered these to be missing at random and used complete case analysis. The overall 30-day mortality in patients with suspected BSI was 8470 / 88357 (9.6%). Among the patients with confirmed BSI and available mortality data, the mortality rate was 1527 / 11392 (13.4%).

ICU Data on ICU admission within 30 days was available in 80840 / 89546 (90.3%) suspected BSI episodes. The reason for missingness here was an misspecification in the data retrieval phase, resulting in missing ICU outcome in nosocomial cases. In total, 3165 / 80840 (3.9%) episodes resulted in ICU admission within 30 days. Among patients with a confirmed BSI, the ICU admission rate was 631 / 10434 (6.0%).

Mortality or ICU admission When we used the first definition (stating that one non-missing outcome was enough), data on 30-day mortality or ICU admission was available in 89205 / 89546 (99.6%) episodes. In total, 11070 / 89205 (12.4%) episodes resulted in death or ICU admission within 30 days. Among patients with a confirmed BSI, the ICU admission rate was 1996 / 11490 (17.4%).

When we used the second definition (stating that both outcomes had to be non-missing for a valid result), data on 30-day mortality or ICU admission was available in 80225 / 89546 (89.6%) episodes. In total,

9595 / 80225 (12.0%) episodes resulted in death or ICU admission within 30 days. Among patients with a confirmed BSI, the ICU admission rate was 1713 / 10361 (16.5%).

4. Results

TTP vs disease severity at baseline

Time-to-positivity from 0 to 36 hours was plotted against the different disease severity markers at baseline in the main manuscript, figure 1. We considered a continuous vs continuous non-linear approach the most appropiate here, as it neither reduces nor categorises data. However, the model may be unfamiliar (the generalized additive model) and the graphical display with different y axes may be difficult to interpret. To show the findings in another way, we present a table here, where TTP is categorised, in which all severity markers were worse in patients with TTP < 10 hours. For some parameters the differences were clinically insignifianct, albeit statistically significant.

Disease severity marker	**<= 10 hours**, N = 3,397	**> 10 hours**, N = 7,885	**p-value**
CRP, mg/L	121.5 [45.0, 222.0]	114.0 [49.0, 204.0]	0.088
Creatinine, µmol/L	113.0 [82.0, 168.0]	100.0 [74.0, 150.0]	<0.001
Lactate, mmol/L	2.3 [1.6, 3.7]	1.8 [1.3, 2.8]	<0.001
Bilirubin, µmol/L	17.0 [10.0, 31.5]	13.0 [8.0, 23.0]	<0.001
Platelets, 10^9/	179.0 [126.0, 240.0]	214.0 [155.0, 293.0]	<0.001
Procalcitonin, µg/L	19.0 [2.4, 56.0]	1.3 [0.3, 13.0]	<0.001
Heart rate / min	90.0 [79.0, 104.0]	88.0 [77.0, 101.0]	<0.001
Mental alteration	172 (7.3%)	262 (4.8%)	<0.001
SpO2, %	96.0 [94.0, 97.0]	96.0 [94.0, 98.0]	0.005
SBP, mmHg	115.0 [101.0, 134.0]	120.0 [106.0, 138.0]	<0.001
Respiratory rate / min	20.0 [18.0, 23.0]	19.0 [17.0, 22.0]	<0.001
Temperature, °C	37.4 [36.8, 38.1]	37.2 [36.6, 37.9]	<0.001
NEWS, points	3.0 [1.0, 6.0]	3.0 [1.0, 5.0]	<0.001

TTP vs dynamics of disease severity markers

We plotted TTP vs severity markers in the first 72 hours, as seen in figure 2 in the main article. As the Generalised additive model may seem difficult to interpret, we provide the 4-hour means for the values for comparison.

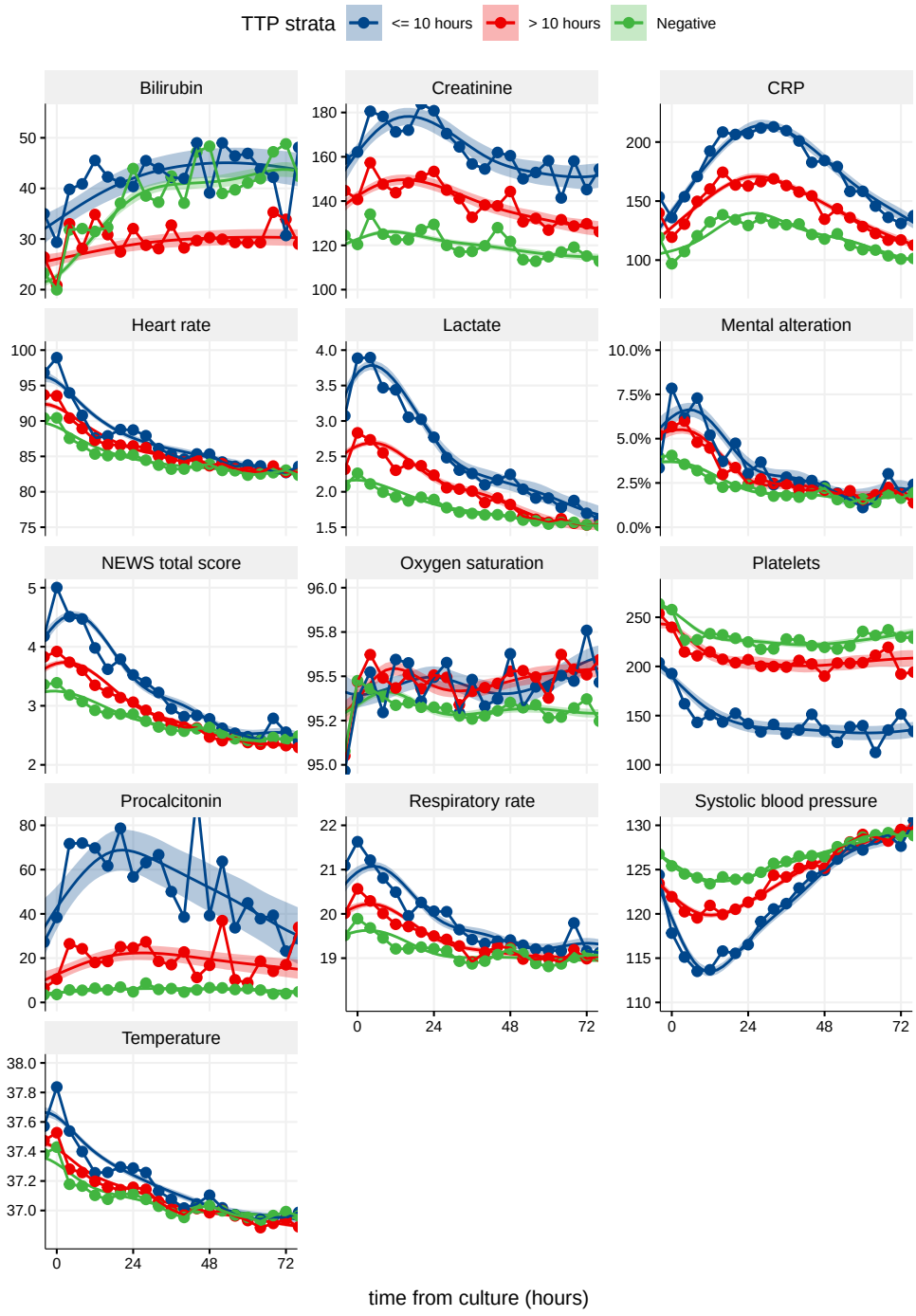

Figure 9: Dynamics of disease severity markers during the first 72 hours by TTP strata, displayed using Generalised additive models (line = point estimate, shade = 95% confidence interval) as well as the mean for each four-hour period (dots and lines)

Exploring the association between TTP and 30-day mortality

Logistic regression using linear TTP

The logistic regression model using TTP as a continuous linear predictor measured in hours found no clear overall relationship with TTP and mortality, Odds ratio (95%CI): 0.999 (0.995-1.003), p value = 0.56. From what we understand, this seems to be the method used by Hamilton et al. who provide odds ratios per incremental TTP hour and found no clear overall relationship between mortality and TTP.[12]. The lack of association can also be seen in the plot of the predicted values from this model.

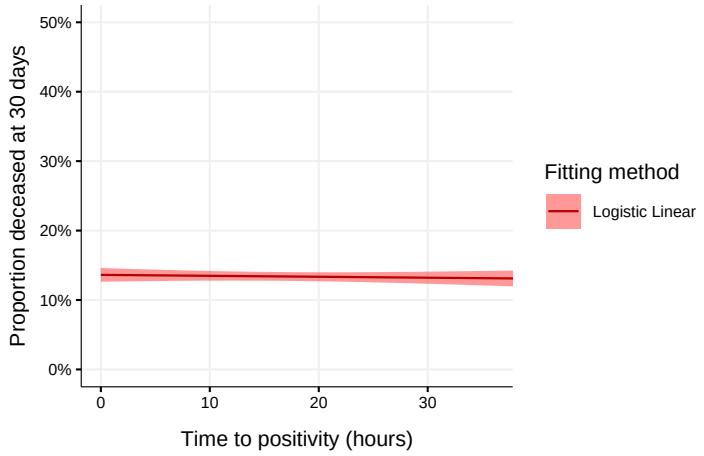

Figure 10: **Linear logistic regression model** Line = regression fit, shaded area = 95% confidence interval.

Logistic regression using categorised TTP

For the categorised model, using a global cutoff at TTP $\leq$ 10 hours, 514/3361 (15.3%) patients within the group with shorter TTP died in 30 days, as compared to 980/7791 (12.6%) in the group with longer TTP, resulting in an Odds ratio of 1.25 (1.12-1.41), p value = 0.0001 for shorter vs longer TTP. This is the approach used by Laupland et al.[15] According to their abstract, the case-fatality rate in the group with a TTP shorter than 10 hours was 2606 / 17879 (14.6%), compared to an aggregate rate of (2834 + 2378 + 2752) / (24272 + 20359 + 22431) = 7964 / 67062 (11.9%) in the group with a longer TTP. This is equivalent to a crude odds ratio (95% CI) of 1.27 (1.21 - 1.33), which is very close to our estimate. It is also noteworthy that the quartiles and overall mortality rates were also similar, indicating that results are robust across these settings.

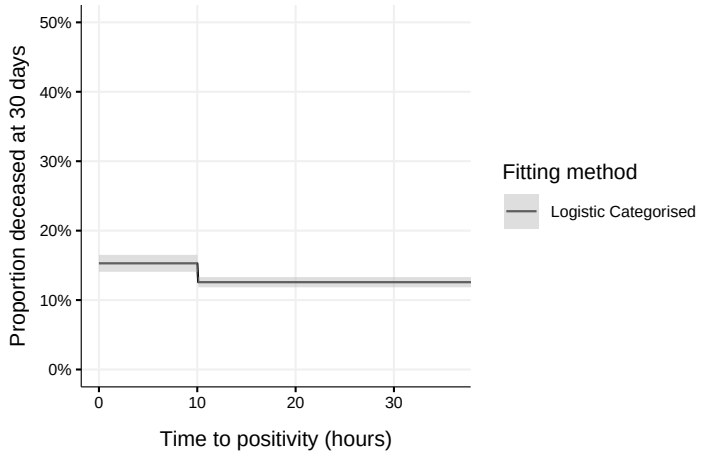

Figure 11: **Categorised logistic regression model** The model used a TTP cutoff at 10 hours. Line = regression fit, shaded area = 95% confidence interval.

However, when the cutoff was changed to 9, 8, and 7 hours, the 30-day mortality rate changed to 355 / 2122 (16.7%), 223 / 1167 (19.1%), 145 / 646 (22.4%), illustrating that the risk increase with shorter TTP is likely to be exponential.

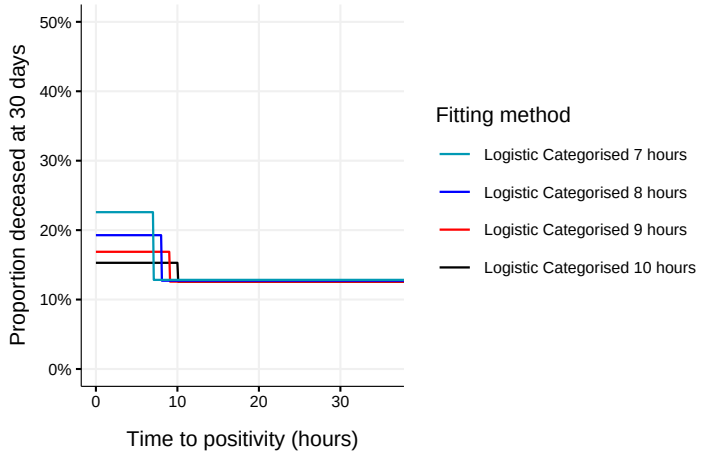

Figure 12: **Categorised logistic regression model** Using TTP cutoffs at 7, 8, 9, and 10 hours. Line = regression fit, shaded area = 95% confidence interval.

Non-linear models

The three non-linear models (the logistic regression using a five-knot restricted cubic spline, the generalised additive model (GAM), and the locally estimated regression smoother (LOESS) model) all confirmed an exponential association and the model fits were very similar.

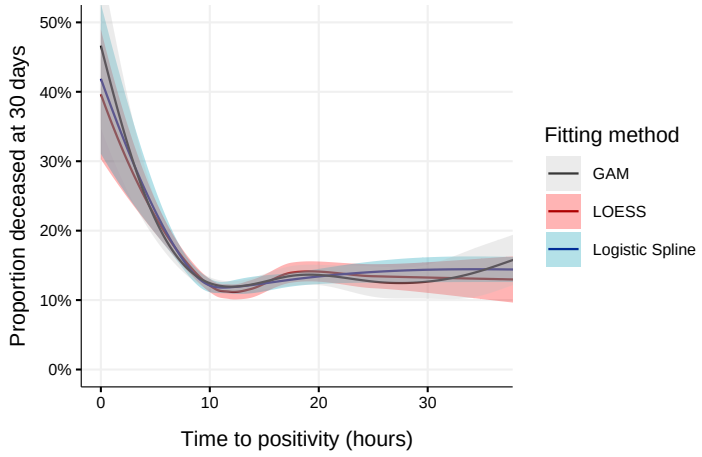

Figure 13: **Non-linear models.** The assocation between time to positivity and 30-day mortality using non-linear models. Lines represent regression models, shaded areas 95% confidence intervals.

Comparing models When comparing these models for model fit using the Likelihood ratio test it was evident that the categorised model with a 10 hour cut-off had better fit than the linear model, Likelihood ratio test, chi-square: 13.7, p value = 0.0002. However, the non-linear model performed much better than both the categorised fit, Likelihood ratio test, chi-square: 37.5, p value < 0.0001, and the linear fit, Likelihood ratio test, chi-square: 51.8, p value < 0.0001.

Conclusion We conclude that the association between TTP and mortality is non-linear and should be modelled as such. This has biological plausibility. By modelling the association in different ways, we could replicate the results of previous studies and thus explain their previously conflicting results.

Timing of 30-day mortality

Using the categorised TTP, a traditional Cumulative hazard plot is seen in figure 14.

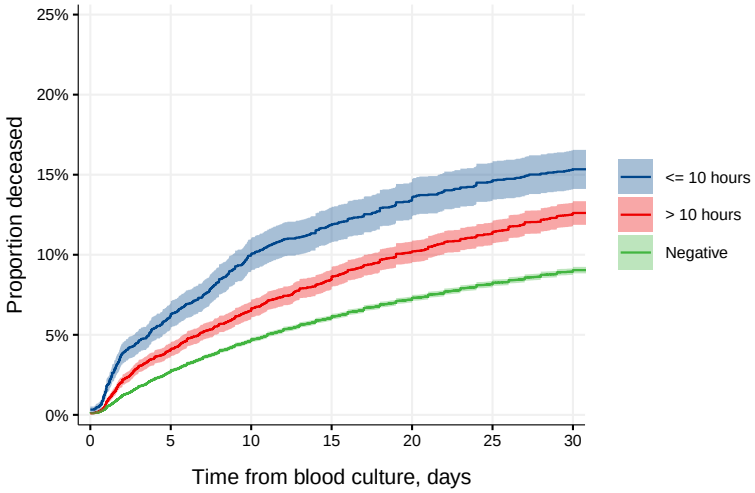

Figure 14: Cumulative hazard plot of 30-day all-cause mortality by TTP strata

However, as discussed above, using a single arbitrary cutoff doesn´t necessarily make sense for an exponential association. To illustrate this we also present a perspective plot with cumulative mortality on the z axis, with TTP and follow-up time on the x and y axes, respectively.

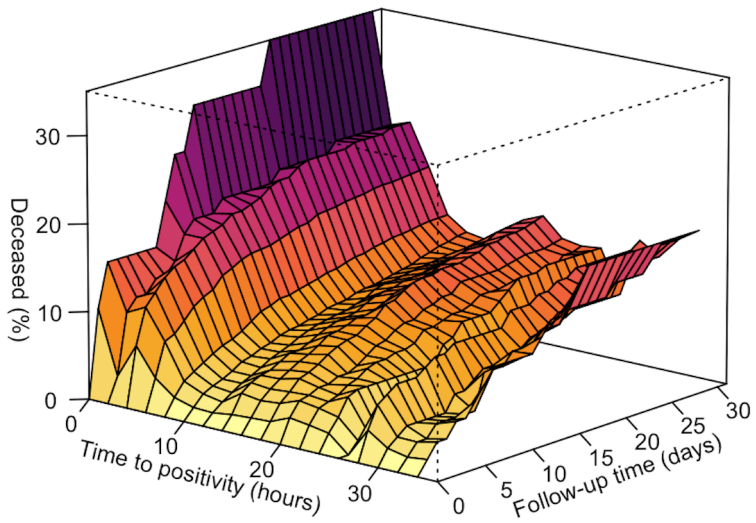

Figure 15: Cumulative hazard plot of TTP vs mortality over time

TTP vs ICU admission

As for the ICU outcome, a TTP ≤ 10 hours was associated with higher 30-day ICU admission rate, with 269/3091 (8.7%), as compared to 348/7160 (4.9%) in those with TTP > 10 hours. This was equivalent to an Odds ratio of 1.87 (1.58 - 2.20) for short vs long TTP. And again, when replicating the non-linear plot of TTP vs mortality, it was clear that the association with ICU admissions had a similar functional form.

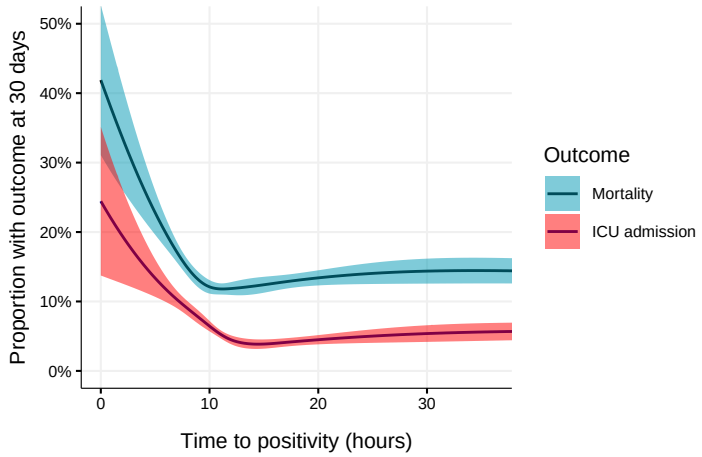

Figure 16: Non-linear modelling of the association between TTP and ICU admission and mortality at 30 days. Line = regression fit, shaded area = 95% confidence interval.

Timing of 30-day ICU admission

When categorised TTP was used, the cumulative hazard plot showed that the majority of ICU admissions occurred during the first days.

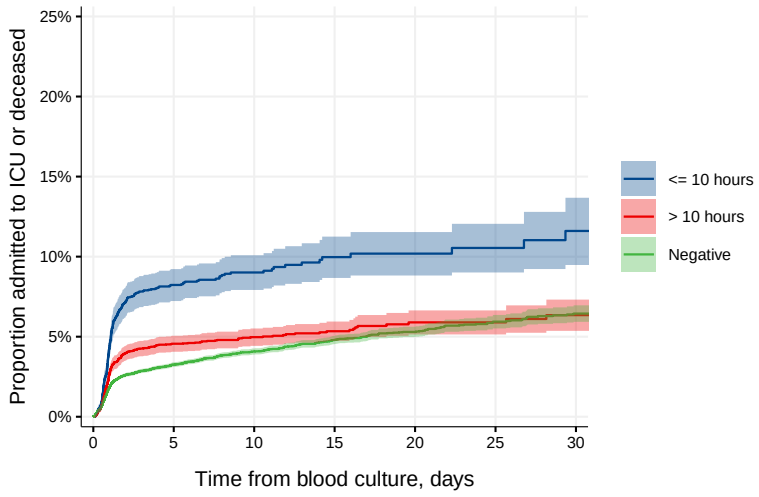

Figure 17: Cumulative hazard plot of 30-day ICU admission by TTP strata

This was also seen in the three-dimensional cumulative hazard plot.

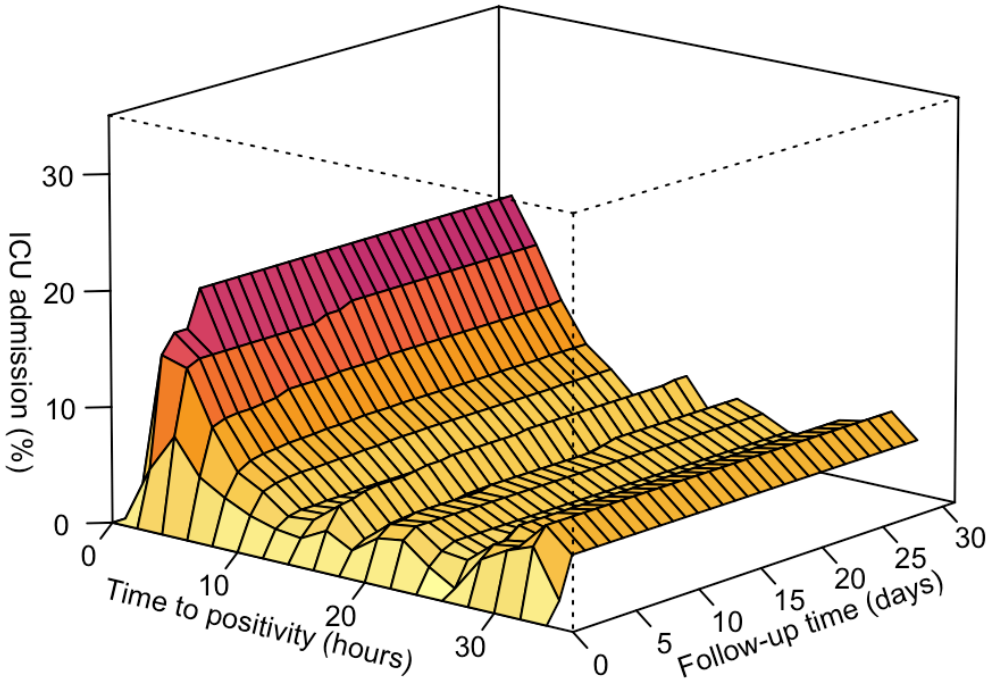

Figure 18: Cumulative hazard plot of TTP vs ICU admission over time

TTP vs ICU admission or mortality As for the combined outcome of ICU admission or mortality in 30 days, a TTP ≤ 10 hours was associated with higher risk, with 709 / 3397 (20.9%), as compared to 1245 / 7885 (15.8%). in those with TTP > 10 hours. This was equivalent to a Odds ratio of 1.41 (1.27 - 1.56) for shorter vs longer TTP. We performed a sensitivity analysis where both outcomes had to be non-missing and the results did not change substantially, with a OR of 1.42 (1.27 - 1.59). For this outcome, we also adjusted for all baseline characteristics (all variables in table 1 in the manuscript) and the association between TTP and outcome was similar, with a OR (95% CI) of 1.40 (1.26 - 1.56).

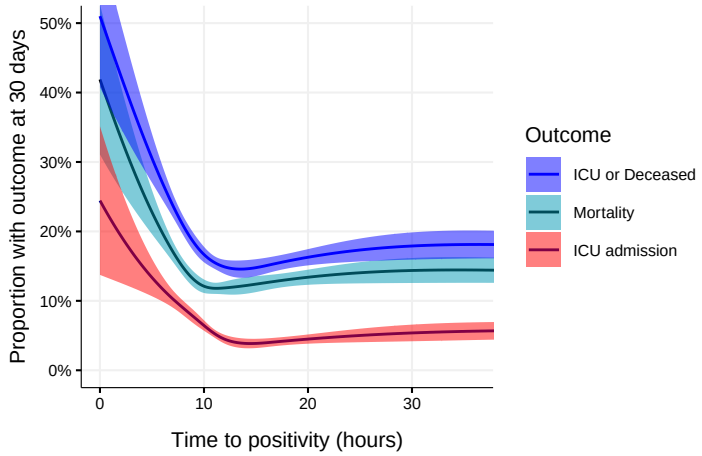

Figure 19: Non-linear modelling of the association between TTP and ICU admission, mortality, or a combination outcome at 30 days. Line = regression fit, shaded area = 95% confidence interval.

Timing of ICU admission or mortality Again, the majority of events occurred in the first days, as seen in the stratified cumulative hazard plot below. This is also seen in the three-dimensional plot, figure 4, in the main manuscript.

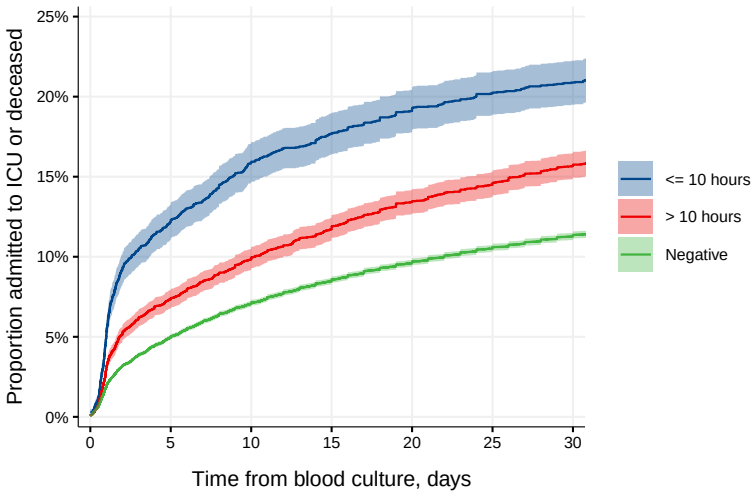

Figure 20: Cumulative hazard plot of ICU admission or mortality within 30 days of culturing, by TTP strata.

TTP vs outcome, stratified by species

We estimated the median and interquartile range of TTP for each pathogen category, see table 7. Then we compared categorical TTP (Q1 vs Q2-Q4) against ICU admission or mortality in 30 days after culturing, estimating the Odds ratios in each species strata, as seen in figure 5 in the main manuscript.

Pathogen category	count	TTP, hours, median (IQR)
Beta hemolytic streptococci	682	9.2 (8.0 - 10.6)
Escherichia coli	3051	10.3 (8.9 - 11.9)
Streptococcus pneumoniae	393	10.6 (8.9 - 11.7)
Klebsiella species	758	10.9 (9.4 - 13.1)
Polymicrobial finding	1407	11.7 (9.4 - 16.6)
Other Enterobacterales	616	11.9 (10.0 - 13.9)
Enterococcus species	333	12.0 (10.3 - 14.4)
Staphylococcus aureus	1506	13.0 (10.1 - 16.4)
Alpha hemolytic streptococci	597	16.4 (12.0 - 22.2)
Staphylococcus species	785	18.4 (16.1 - 21.2)
Other non-anaerobic gram-negative	423	18.5 (14.5 - 32.5)
Contaminants	2986	22.8 (19.0 - 32.7)
Other non-anaerobic gram-positive	365	31.1 (19.6 - 46.1)
Anaerobic bacteria	543	31.1 (23.6 - 52.2)
Fungal infection	63	34.3 (23.7 - 48.6)

Table 7. Time to positivity for different pathogen categories.

Sensitivity analysis 1: Testing the association in sites without cabinets To further explore the validity of findings from sites without cabinets on-site, we plotted the same non-linear plot for these samples only and it was obvious that the difference in transfer time had completely invalidated the results. Thus, prolonged transfer time has to be accounted for in future analyses.

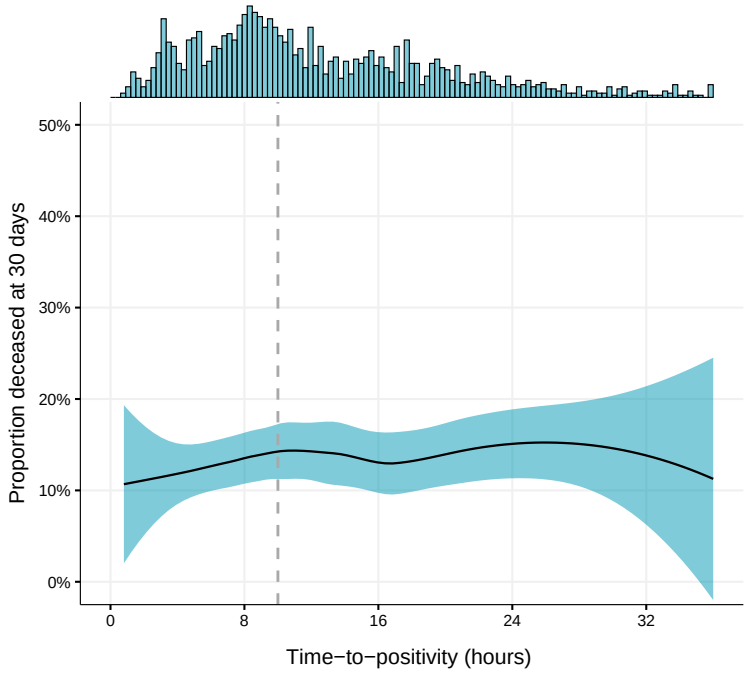

Figure 21: In hospitals withouh on-site cabinets, there was no association between time-to-positivity within 0 to 36 hours and 30-day mortality, using a non-linear LOESS (locally estimated scatterplot smoother), with 95% confidence interval. Dashed line = suggested TTP cutoff at 10 hours. Marginal histogram shows distribution of TTP.

Sensitivity analysis 2: Testing the association in pediatric bottles. The association between TTP and 30-day mortality was similar in pediatric patients.

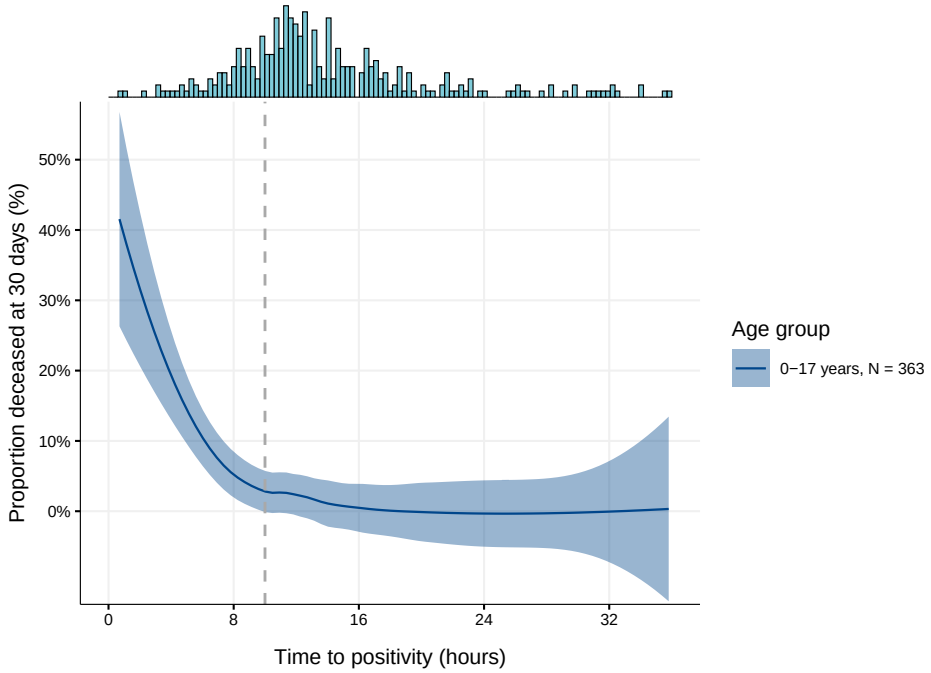

Figure 22: Association between TTP and 30-day all-cause mortality in pediatric patients in hospitals with on-site cabinets. Line = LOESS (locally estimated scatterplot smoother) fit, with 95% confidence interval (shaded area). Dashed line = suggested TTP cutoff at 10 hours. Marginal histogram shows distribution of TTP.

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