

Statistical Analysis Plan for Final Analysis

**Optimization of sepsis therapy based on patient-specific digital precision diagnostics
using next generation sequencing
(DigiSep-Trial)**

Trial registration no.:

NCT04571801

Signatures

The original SAP was written in German language and was signed by all parties in December 2023. By signing this translated English version of the SAP, we confirm the accuracy of the translation.

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1 Objective of the trial

This randomized-controlled, multicentre intervention study entitled “Optimization of sepsis therapy based on patient-specific digital precision diagnostics using next generation sequencing” (DigiSep-Trial) is intended to optimize sepsis treatment in a patient-centered and digitally supported manner through timely and reliable identification of the pathogen using next generation sequencing (NGS)-based precision diagnostics (in addition to routine microbiological diagnostics) and anti-infective therapy management based on this. In addition, treating physicians have the option of using a web-based infectiology (interdisciplinary) expert consultation if required, in which NGS findings and the resulting treatment decisions can be discussed. This allows complex findings to be discussed with independent experts and treatment decisions to be further optimized if necessary. Ideally, patients can be treated more quickly, safely, efficiently and cost-effectively.

2 Study design

The study presented here is a prospective, multicenter, randomized, controlled intervention study (RCT).

3 Analysis sets

DigiSep includes patients in Intensive Care Units (ICU) & Intermediate Care (IMC) wards of maximum care providers (MVs) who are suffering from sepsis/septic shock (Sepsis-3). In the project presented here, wards requiring monitoring are deliberately combined with ICU and IMC wards in order to achieve comparability of the collected outcome data regardless of the underlying level of care.

The corresponding inclusion and exclusion criteria for participation in the study are defined as follows:

Inclusion criteria:

- Sepsis/septic shock (according to Sepsis-3), onset <24h
- Written consent by the study participant or its legal representative
- Age ≥18 years
- Statutory health insurance, as funding is provided by the innovation fund of the Federal Joint Committee (Gemeinsamer Bundesausschuss (G-BA))

Exclusion criteria:

- Age <18 years
- Refusal to participate in the study

- Transfer from ICU/IMC <72h after study inclusion likely
- Palliative treatment goal
- Existing treatment goal limitation
- Death of the patient is already foreseeable at the time of study inclusion
- Private health insurance, as funding is provided by the innovation fund of the Federal Joint Committee (Gemeinsamer Bundesausschuss (G-BA))

Patients who have already been included cannot be included again.

The main analysis population, hereinafter referred to as the Full Analysis Set (FAS), includes all patients and the analysis is performed according to the intention-to-treat principle, i.e. patients are analyzed in the group (intervention group (IG), control group (CG)) in which they were randomized to, regardless of which treatment they received. Patients who did not fulfill inclusion criteria or fulfilled an exclusion criterion, but were nevertheless included in the study, are not included in the analysis.

4 Definitions of endpoints to be analyzed

4.1 Primary endpoint

DOOR/RADAR score, based on Overall Clinical Outcome (OCO) according to the DOOR (Desirability of Outcome Ranking) principle with adjustment according to RADAR (Response Adjusted for Duration of Antibiotic Risk). The following classification is used:

1. Group: Survival* + ICU/IMC LOS (length of stay) <12 days without ARF (acute renal failure)**
2. Group: Survival* + ICU/IMC LOS < 12 days with ARF**
3. Group: Survival * + ICU/IMC LOS ≥ 12 days without ARF**
4. Group: Survival * + ICU/IMC LOS ≥ 12 days with ARF**
5. Group: Death*

* assessed after 28 days or until transfer to a normal ward. Transfer to a normal ward is counted as survival. If no complete data on survival or transfer to a normal ward is available (censoring), these missing values are dealt with in accordance with Section 7.1.

** with the need for renal replacement therapy.

The duration of the anti-infective therapy is then included and the DOOR/RADAR score is calculated as follows:

DOOR/RADAR-Score:

$$\begin{cases} = \text{OCO} + \left[\frac{\text{number of days with anti-infective treatment}}{\text{max. number of days on anti-infective treatment} + 1 \text{ day}} \right], & \text{if } \text{OCO} \leq 4 \\ = 5 & , \text{if } \text{OCO} = 5 \end{cases}$$

4.2 Secondary Endpoints

The analysis of the secondary endpoints will be performed at two time points: the first, short-term evaluation accompanies the evaluation of the primary endpoint 28 days after study inclusion. The second, long-term evaluation of the other secondary endpoints takes place 180 days after study inclusion and thus at the end of the study for each patient. For each secondary endpoint, it is specified whether it is evaluated at the first and/or second time point.

4.2.1 Long-term mortality

The endpoint “long-term mortality” is defined as the time between randomization and death or the end of the study. Patients who are “lost-to-follow-up” are censored. Long-term mortality is evaluated at the first and second time point.

4.2.2 Duration of ventilation

The endpoint “duration of ventilation” is defined as the cumulative duration of ventilation over the entire course of the study, relative to the duration of survival. Patients not requiring ventilation are scored as 0. If the survival time is censored, the ventilation time is to be evaluated as a missing value. The subgroup of surviving patients is recorded in absolute days. The duration of ventilation is evaluated at the first and second time point.

4.2.3 Time to shock resolution

The endpoint “time to shock resolution” is defined as the time between the onset of septic shock and the end of septic shock, measured by the cumulative duration of catecholamine dependency, relative to survival time. Patients without catecholamine requirement are scored as 0. If the survival time is censored, the “time to shock resolution” is to be evaluated as a missing value. The subgroup of surviving patients is recorded in absolute days. The time to shock resolution is evaluated at the first and second time point.

4.2.4 Ongoing need for renal replacement therapy during follow-up

The endpoint “ongoing need for renal replacement therapy during follow-up” is defined as the cumulative time of renal replacement therapy dependency over the entire course of the study, relative to survival time. Patients without renal replacement therapy dependency are rated as 0. If the survival time is censored, the “ongoing renal replacement therapy requirement during

follow-up” is to be evaluated as a missing value. The subgroup of surviving patients is recorded in absolute days. The ongoing renal replacement therapy requirement during follow-up is evaluated at the first and second time point.

4.2.5 Total length of hospital stay

The endpoint “total length of hospital stay” is defined as the time between randomization and discharge from hospital. The total length of hospital stay is recorded in relative terms, as the proportion of days since randomization that the patient has stayed in hospital. If the patient dies in hospital, the proportion is counted as 100%. On the other hand, for the subgroup of surviving patients, the total length of hospital stay is recorded in absolute days. The total length of hospital stay is evaluated at the first and second time point.

4.2.6 Cumulative use of anti-infectives

The endpoint “cumulative use of anti-infectives” is defined as the number of days in which the patient received an anti-infective treatment. The number of days is recorded separately for each antiinfective drug and summed up. This figure is then evaluated relative to the survival time. In addition, the cumulative use of anti-infectives is recorded in absolute days for the subgroup of surviving patients. The cumulative use of anti-infectives is only evaluated at the first time point.

4.2.7 Time of initiation of a targeted anti-infective therapy

The exact time of initiation of a targeted anti-infective therapy was not recorded. As a result, this endpoint cannot be evaluated.

4.2.8 Retrospective evaluation of the expert panel

The evaluation of the 3-member expert panel takes place via a questionnaire consisting of 6 items.

For each item, the rates of “complete agreement” of the 3 raters, “majority agreement” of the 3 raters and the rate of “no agreement” are also reported. The rating is as follows:

+++;---	Complete agreement
++-; +-; ++missing; --missing; -missing, missing; + missing, missing	Majority agreement
+ - missing; missing, missing, missing	No agreement

4.2.9 Quality of Life

This is part of the health economic evaluation carried out by the Department of Health Economics and Health Care Management at Bielefeld University.

5 Data handling

Patients who withdrew their informed consent before the database was closed and did not agree to the further use of the data were excluded from the study.

The Glasgow Coma Scale (GCS) can often not be collected uniformly in intensive care patients (current GCS at the time of data collection with analgesic medication, if applicable, vs. GCS at the last possible time without analgesic medication). As a result, a modified SOFA score is calculated instead of the “classical” SOFA score (see 11.2), which excludes the GCS.

6 Statistical methods

6.1 Descriptive methods

Continuous variables and scores are described with mean, standard deviation, median, Q1, Q3, minimum and maximum, categorical variables are described with absolute and relative frequencies per treatment group and overall. The number of missing values is given, respectively; relative frequencies refer to the number of patients without missing values. Continuous variables are compared between the two treatment groups using t-tests, ordinal and categorical variables using chi-square test.

6.2 General medical history and baseline data

The following data are collected at V1.

Demographic Data:

- Age
- Sex (male/female)
- BMI (calculated from height and weight)
- Type of admission (surgical emergency, non-surgical emergency, elective)
- Location before admission to ICU (emergency room, shock room, normal ward (internal), other ICU/IMC (internal), other hospital, other location)

Comorbidity:

- Charlson Comorbidity Index (CCI)

Immune status:

- History of neutropenia (yes, no, unknown)
- Condition after allogenic stem cell transplantation (yes, no, unknown)
- Prolonged use of corticosteroids (yes, no, unknown)
- Treatment with other T-cell immunosuppressants (yes, no, unknown)
- Congenital severe immunodeficiency syndromes (yes, no, unknown)

The variables are reported using descriptive methods.

6.3 Sepsis criteria

The following data are collected at V1 and V2:

Sepsis criteria

- Presence of an infection (yes, no)
- Degree of certainty (clinically suspected, microbiologically proven)
- Origin of the infection (outpatient, nosocomial)
- Localization known (yes (incl. specific information), no)
- Organ dysfunction (yes, no)
- Lactate (mmol/l, mg/dl (incl. maximum value within 24 hours))
- Need to stabilize the circulation with vasopressors to achieve a mean arterial pressure of 65 mmHg and an increase in lactate concentration to over 2 mmol/l (18 mg/dl) despite adequate fluid intake (yes, no)
- Time span of presumed onset of sepsis and randomization (in hours)

The variables are reported at both time points using descriptive methods.

6.4 Neuroscores

The following data are collected from V1 to V5 (if the study is terminated prematurely: up to V4):

- RASS (ordinal: -5, -4, ..., 4)
- CAM-ICU
 - CAM-ICU measureable (yes, no)
 - Feature 1: Acute onset or fluctuating course (present, absent)
 - Feature 2: Inattention (present, absent)
 - Feature 3: Disorganized thinking (present, absent)
 - Feature 4: Altered level of consciousness (present, absent)
 - Overall CAM-ICU (present, absent)
- GCS
 - GCS measureable (yes, no)
 - Time point of GCS (current value, before the start of sedation)
 - Ocular response (spontaneously, response to voice, response to pain, no)
 - Oral response ([oriented, clear], confused, inappropriate words, incomprehensible sounds, no sounds)
 - Motor response (obeys commands, moves to localize pain, Flexion / Withdrawal from painful stimuli, abnormal flexion, abnormal extension, makes no movements)
 - GCS-Score (ordinal: 3,4, ..., 15)

The variables are reported at each time point using descriptive methods.

6.5 Study check

The following data are collected at V1 in the IG if “Blood samples collected for NGS” is answered with “Yes”. Absolute and relative frequencies of the variable “Blood samples collected for NGS” are reported.

- Change in the anti-infective treatment regimen due to the NGS findings from V1/day 0 (yes, no)
 - Changes made (expansion of the anti-infective bacterial spectrum, narrowing of the anti-infective bacterial spectrum, complete discontinuation of anti-infectives, other (free text))

The following data are collected at V2 in the IG if “Blood samples for NGS are collected” is answered with “Yes”:

- Change in the anti-infective treatment regimen due to the NGS findings from V2/day 3 (yes, no)
 - Changes made (expansion of the anti-infective bacterial spectrum, narrowing of the anti-infective bacterial spectrum, complete discontinuation of anti-infectives, other (free text))

The following data are collected from V1 to V4:

- Have blood samples been collected for an extended immunomonitoring and for the transcriptome analysis? (yes, no)

The variables are reported at each time point using descriptive methods.

6.6 Further blood cultures

The following data are collected from V2 to V4:

- Have further blood cultures (2 sets each: 2x aerobic & 2x anaerobic) been collected since the last scheduled visit? (yes (date), no)
- Have unscheduled blood samples been collected for an extended immunomonitoring and for the transcriptome analysis since the last regular sample collection? (yes, no)

The following data from V2 to V4 are only collected in the IG:

- Have additional NGS samples been collected since the last scheduled visit? (yes, no)
 - Change in anti-infective treatment regimen due to NGS findings? (yes, no)
 - Changes made (expansion of the anti-infective bacterial spectrum, narrowing of the anti-infective bacterial spectrum, complete discontinuation of anti-infectives, other (free text))

The variables are reported at each time point using descriptive methods.

6.7 Physiology, catecholamines, fluid balances, routine laboratory

The following data are collected from V2 to V5. If the measurements are done in different units, they are converted, if necessary.

Physiologie

- Heart rate (yes, no),
- Heart rate (minimum and maximum in 1/min),
- Systolic blood pressure (yes, no)
- Systolic blood pressure (minimum and maximum in mmHg),
- Mean arterial blood pressure (yes, no)
- Mean arterial blood pressure (minimum and maximum in mmHg),
- Body temperature (yes, no)
- Body temperature (minimum and maximum in °C)
- Respiratory rate (yes, no)
- Respiratory rate (minimum and maximum in 1/min)
- Bicarbonate (yes, no)
- Bicarbonate (minimum and maximum in mmol/l),
- pH (yes, no)
- pH (minimum and maximum in pH value)
- Horowitz quotient (yes, no);
- Horowitz quotient (measured value in (kPa, mmHg)),
- PaO₂ (yes, no)
- PaO₂ (measured value in (kPa, mmHg))
- FiO₂ (yes, no)
- FiO₂ (measured value in %)
- PaCO₂ (yes, no)
- PaCO₂ (measured value in (kPa, mmHg)),
- Type of ventilation (none, non-invasive, invasive)
- Urine output (yes, no)
- Amount of urine output (ml)
- Duration of urine output (h)
- Renal replacement therapy (yes, no)
- Renal replacement therapy: Dialysis method (CVVH (continuous venovenous hemofiltration), CVVHD (continuous venovenous hemodialysis), CVVHDF (continuous venovenous hemodiafiltration), IHD (intermittent hemodialysis), SLED (sustained low efficiency dialysis), other (more detailed description (free text)))
- Type of anticoagulation (none, unfractionated heparin, citrate anticoagulation, other (free text))
- ECMO (yes, no)
- ECMO (type: venoarterial, venovenous)

Catecholamines

- Use of vasopressors and inotropics (yes, no)
- Use of adrenaline (yes, no)

- Use of adrenaline (maximum dosage in ($\mu\text{g/kg/min}$, $\mu\text{g/min}$, mg/h))
- Use of noradrenaline (yes, no)
- Use of noradrenaline (maximum dosage in ($\mu\text{g/kg/min}$, $\mu\text{g/min}$, mg/h))
- Use of dopamine (yes, no)
- Use of dopamine (maximum dosage in ($\mu\text{g/kg/min}$, $\mu\text{g/min}$, mg/h))
- Use of dobutamine (yes, no)
- Use of dobutamine (maximum dosage in ($\mu\text{g/kg/min}$, $\mu\text{g/min}$, mg/h))
- Use of vasopressin (yes, no)
- Use of vasopressin (maximum dosage in ($\mu\text{g/kg/min}$, $\mu\text{g/min}$, mg/h , IU/h))
- Use of hydrocortisone (yes, no)
- Use of hydrocortisone (maximum dosage (mg/day))
- Use of dexamethasone (yes, no)
- Use of dexamethasone (maximum dosage (mg/day))

Fluid Balance

Volume therapy

- Has a volume therapy been performed? (yes, no)
- Crystalloid solutions (yes, no)
- Crystalloid solutions (total amount (ml/24h)),
- Human albumin 20% (yes, no)
- Human albumin 20% (total amount (ml/24h))
- Human albumin 4-5% (yes, no)
- Human albumin 4-5% (total amount (ml/24h))
- Red packed blood cells (yes, no)
- Red packed blood cells (total quantity (ml/24h))
- Fresh frozen plasmas (yes, no)
- Fresh frozen plasmas (total quantity (ml/24h)))
- Platelet concentrates (yes, no)
- Platelet concentrates (total quantity (ml/24h))
- Gelatine preparations (yes (total quantity (ml/24h)), no)
- Gelatine preparations (yes (total quantity (ml/24h)), no)
- HES 6% [200/0.5] (yes, no)
- HES 6% [200/0.5] (total amount (ml/24h))
- HES 10% [200/0.5] (yes, no)
- HES 10% [200/0.5] (total quantity (ml/24h))
- HES 6% [130/0.4] (yes, no)
- HES 6% [130/0.4] (total quantity (ml/24h))
- HES [70/0.5] (yes, no)
- HES [70/0.5] (total quantity (ml/24h))
- Dextrans (yes, no)
- Dextrans (total amount (ml/24h))
- Other colloidal solutions (yes, no)
- Other colloidal solutions (total amount (ml/24h))

Fluid losses

- Urine output (yes, no)
- Urine output (total amount (ml/24h))
- Dialysis (yes, no)
- Dialysis (total amount of ultrafiltration (ml/24h))
- Drains (yes, no)

- Drains (total amount (ml/24h))
- Blood loss (yes, no)
- Blood loss (total amount (ml/24h))
- Stool/stoma (yes, no)
- Stool/stoma (total amount (ml/24h))
- Reflux/vomiting (yes, no)
- Reflux/vomiting (total amount (ml/24h))

Routine laboratory parameters

- Sodium (multiple, single, none)
- Sodium (minimum & maximum for repeated, value for single in (mmol/l, mg/dl))
- Potassium (multiple, single, none)
- Potassium (minimum & maximum for repeated, value for single in (mmol/l, mg/dl))
- Urea (multiple, single, none)
- Urea (minimum & maximum for repeated, value for single in (mmol/l, μ mol/l, mg/dl))
- Creatinine (multiple, single, none)
- Creatinine (minimum & maximum for repeated tests, value for single test in (mg/dl, μ mol/l))
- GFR ([Yes, according to Cockcroft-Gault], [Yes, according to MDRD], [not collected]),
- GFR (multiple, single, none)
- GFR (minimum & maximum for multiple, value for single in ml/min)
- Total bilirubin (multiple, single, none)
- Total bilirubin (minimum & maximum for multiple, value for single in (mg/dl, μ mol/l))
- Lactate (multiple, single, none)
- Lactate (minimum & maximum for repeated tests, value for single test in (mg/dl, μ mol/l))
- Albumin (multiple, single, none)
- Albumin (minimum & maximum for multiple tests, value for single test in (g/l, g/dl))
- CRP (multiple, single, none)
- CRP (minimum & maximum for multiple tests, value for single test in (mg/l, mg/dl, μ mol/l))
- PCT (multiple, single, none)
- PCT (minimum & maximum for multiple tests, value for single test in (μ g/l, ng/ml))
- Total protein (multiple, single, none)
- Total protein (minimum & maximum for repeated, value for single) in (g/l, g/dl)
- ALAT (GPT) (multiple, single, none)
- ALAT (GPT) (minimum & maximum for repeated, value for single) in (μ mol/l, U/l)
- ASAT (GOT) (multiple, single, none)
- ASAT (GOT) (minimum & maximum for repeated, value for single) in (μ mol/l, U/l)
- Alkaline phosphatase (AP) (multiple, single, none)
- Alkaline phosphatase (AP) (minimum & maximum for repeated, value for single) in (μ mol/l, U/l)
- Hematocrit (multiple, single, none)
- Hematocrit (minimum & maximum for repeated, value for single) in %
- Leukocytes (multiple, single, none)
- Leukocytes (minimum & maximum for multiple, value for single) in (gpt/l, $\times 10^3/\mu$ l, /nl)
- Platelets (multiple, single, none)
- Platelets (minimum & maximum for multiple, value for single) in (gpt/l, $\times 10^3/\mu$ l, /nl)
- Quick's value (multiple, single, none)
- Quick's value (minimum & maximum for repeated, value for single) in %

- aPTT (multiple, single, none)
- aPTT (minimum & maximum for multiple, value for single) in sec
- INR (multiple, single, none)
- INR (minimum & maximum for multiple, value for single)

The variables are reported at each time point using descriptive methods. Comparisons for continuous variables across all time points are performed using a repeated measures ANOVA and presented graphically using boxplots.

6.8 Source control, anti-infectives, microbiology, virology

The following data inquiries are „repeatable instruments“

Source control

- Conducted? (yes, no)
- Time (day since study inclusion)
- Type of source control (surg. source control, removal of intravascular catheter, interventional source control)
- Detailed description (free text)

Anti-infectives

- Choice of substance (antibiotics, antivirals, antimycotics)
- Type & further description (selection and free text if applicable)
- Start of administration (-3d, -2d, -1d, 0d (= sepsis onset), +1d, +2d, +3d, +4d, +5d, +6d, +7d)
- Continuous administration of anti-infectives? (yes, no)
- End of administration (-3d, -2d, -1d, 0d (= sepsis onset), +1d, +2d, +3d, +4d, +5d, +6d, +7d, +>7d)
- Strategy of the administration of anti-infectives (consistently empirical, consistently targeted, initially started empirically but then continued in a targeted manner)

Microbiology:

- Sample material (blood culture, respiratory secretion, fresh drainage material, old drainage material, surgical swab, wound swab, punctate, catheter tip, fresh urine, old urine, cerebrospinal fluid, tissue, hygiene swab, stool)
- Study-related collection of blood cultures (2x aerobic & 2x anaerobic) at the time of sepsis onset (yes, no)
- Study-related collection of blood cultures (2x aerobic & 2x anaerobic) at 72h after sepsis onset (yes, no)
- Sample type (aerobic, anaerobic)
- Site of collection (arterial, venous)
- Access (peripheral, central)
- Site of collection (surgical drainage, interventional drainage)
- Type (Easyflow, Robinson, suction irrigation drainage, T-drainage, other)
- Type (abscess or retention drainage, PTCD, other)
- Type of material (CVC, artery, EVD, PTCD, other)
- Site of collection (rectal, nasal)

- Is the time of sample collection given (yes, no)?
- Day of collection (-3d, -2d, -1d, 0d (= sepsis onset), +1d, +2d, +3d, +4d, +5d, +6d)
- Positive findings (yes, no)
- Classification (AER, 3-MRGN, 4-MRGN)
- Classification (MRSA)
- Type of pathogen (*Enterococcus faecium*, *Enterococcus faecalis*)
- Type of pathogen (*Escherichia* spp., *Pseudomonas* spp., *Klebsiella* spp., *Citrobacter* spp., *Enterobacter* spp., *Serratia* spp., *Proteus* spp., *Acinetobacter* spp., others)
- Cocci (yes, no):
If yes, indicate type of pathogen, detection and therapy (see CRF)
- Enterobacteriaceae (yes, no):
If yes, then state type of pathogen, detection, therapy, ESBL phenotype, ceftriaxone and cefotaxime resistance and MIC with value (cf. CRF)
- Non-fermenter (yes, no):
If yes, indicate type of pathogen, detection and therapy (cf. CRF)
- Fungi (yes, no):
If yes, then specify type of pathogen, detection and therapy (cf. CRF)
- Relevant pathogens (yes, no) (comes from stool sample):
If yes, then state type of pathogen, detection and therapy (cf. CRF)

Virology:

- Sample material (blood, respiratory secretions, swab, cerebrospinal fluid, stool)
- Type of diagnostics (CMV-PCR, VZV-PCR, HSV1-PCR, pp65-Ag, Asp.-Ag, SARS-CoV2-PCR, SARS-CoV2-AG, RSV-PCR, Para-Influenza-PCR, Influenza-PCR, other)
- Is the time of sample collection given (yes, no)?
- Day of collection (-3d, -2d, -1d, 0d (= sepsis onset), +1d, +2d, +3d, +4d, +5d, +6d)
- Findings positive (yes, no)
- Norovirus (yes, no)
- Rotavirus (yes, no)
- Astrovirus (yes, no)
- Other (yes, no)
- Depending on the type of diagnosis in [number of copies, no unit or 1/500000 cells].

The variables are reported at each time point using descriptive methods. Furthermore, for microbiology and virology, the total number of samples per day and the number of samples per patient are reported using descriptive methods.

7 Analysis of primary endpoint

7.1 Primary analysis

The primary hypothesis of the study is that there is a difference between the IG and the CG, based on the FAS, with regard to the DOOR/RADAR score (for definition see 4.1).

The null hypothesis

$$H_0: P(DOOR/RADAR_{IG} > DOOR/RADAR_{KG}) = P(DOOR/RADAR_{IG} < DOOR/RADAR_{KG})$$

is tested against the alternative hypothesis

$$H_1: P(DOOR/RADAR_{IG} > DOOR/RADAR_{KG}) \neq P(DOOR/RADAR_{IG} < DOOR/RADAR_{KG})$$

using a Mann-Whitney-U Test with a two-sided significance level of $\alpha = 0.05$. In addition to the (asymptotic) p-value, normal approximative 95% confidence intervals are given.

Handling of missing data

For missing values in the variables that form the components of the DOOR/RADAR score, multiple imputation is performed for the respective individual variables. The method used is “predictive mean matching”, which includes the variables age, SOFA score at onset of sepsis and survival status. The number of donors for the predictive mean matching is $d = 5$.

Multiple imputation generates $m = 10$ imputed data sets. Since an appropriate number of burn-in iterations cannot be specified with certainty without knowledge of the missing pattern, 50 burn-in iterations are used as a starting point and adjusted if necessary. The seed used for multiple imputation is 230842.

The analyses regarding the primary endpoint are performed separately for each created data set and combined according to Rubin's Rule (Rubin 1987).

7.2 Sensitivity analyses

The multiple imputation is supported by various sensitivity analyses:

- Firstly, “best case” and “worst case” analyses are performed to provide a framework for the assessment of the imputation. In addition, a “complete case” analysis is performed in which all patients with missing values for the DOOR/RADAR score are excluded.
- Furthermore, the number of donors for the predictive mean matching is varied (to $d = 3$ and $d = 10$) to ensure the robustness of the results. An additional variation of the number of imputed data sets m (to $m = 5$ and $m = 20$) also supports this.

The primary analysis is supplemented by a logistic regression. Here, the actually ordinal DOOR/RADAR score is transformed into a binary variable by distinguishing between a DOOR/RADAR score of <2 and a DOOR/RADAR score of ≥2. In addition to group membership (IG, KG), age, gender, SOFA score and the Charlson comorbidity index are included in the model as exogenous variables. Odds ratios including 95% confidence intervals and corresponding p-values are given for all covariates.

A modified DOOR/RADAR score is calculated for the first 7 days to take into account the administration of various anti-infectives. This refers to the anti-infective days. An anti-infective day is defined as a day with one anti-infective administered, i.e. a patient who has been given anti-infective A for 4 days and anti-infective B for 3 days, for example, is scored as having 7 anti-infective days.

modified DOOR/RADAR – Score:

$$\left\{ \begin{array}{l} = \text{OCO} + \left[\frac{\text{Anti-infective day}}{\text{Number of anti-infectives administered} * \text{Max. number of days with anti-infectives} + 1 \text{ day}} \right], \\ \quad \text{if } \text{OCO} \leq 4 \\ = 5, \text{ if } \text{OCO} = 5 \end{array} \right.$$

An analysis analogous to the primary analysis (see 7.1) is then conducted.

7.3 Subgroup analyses

The primary analysis of the DOOR/RADAR score is also performed for various subgroups. Categories can be combined if the subgroups are too small for valid analyses.

For missing values, the imputed data sets from the multiple imputation of the primary analysis are used and combined according to Rubin's Rule (Rubin 1987).

The subgroups are:

- Time of collection NGS:
 - Patients without time-delayed collection
 - Patients with delayed collection
- Disease severity in the course:
 - Septic shock: V1- No and V2 - No
 - Septic shock: V1- Yes and V2 - No
 - Septic shock: V1- No and V2 - Yes
 - Septic shock: V1- Yes and V2 - Yes
- Focus (Confirmed or Suspected):
 - Pneumonia, other upper or lower respiratory tract, thoracic
 - Gastrointestinal, biliary tract, intra-abdominal / peritonitis
 - Bacteremia, catheter infection
 - Bone / soft tissue, surg. Wound infection

- CNS
 - Cardiovascular
 - Urogenital
 - Other
- Presence of infection
 - Degree of certainty
 - Clinically suspected
 - Microbiologically proven
 - Origin of the infection
 - Outpatient
 - Nosocomial
- Immunosuppression
 - Immunosuppression present
 - Immunosuppression not present
- Charlson Comorbidity Index at baseline
 - Charlson Comorbidity Index > 4
 - Charlson Comorbidity Index ≤ 4
 - Charlson Comorbidity Index less than/equal to median
 - Charlson Comorbidity Index greater than median
 - Charlson Comorbidity Index greater than 3rd quartile
- Modified SOFA-Score at baseline:
 - Less than/equal to median
 - Greater than median
 - Greater than 3rd quartile
- Blood culture
 - Positive (patients with at least one positive microbiology result)
 - Negative
- Source control
 - Yes
 - No
 - Type of source control
 - surgical source control
 - Removal of intravascular catheters
 - Interventional source control
- Therapy change: If a therapy change was performed or proposed for a patient based on NGS diagnostics, regardless of the timing, this patient is to be assigned to the “Change” subgroup

- Change implemented in IG vs. proposed change in CG (retrospectively by expert panel)
- Proposed change in IG (retrospectively by expert panel) vs. proposed change in CG (retrospectively by expert panel)
- No change implemented in IG vs. no proposed change in CG
- No proposed change in IG (retrospectively by expert panel) vs. no proposed change in CG

8 Analysis of secondary endpoints

The analysis of all secondary endpoints is based on the FAS.

8.1 Long-term mortality

Long-term mortality is analyzed at day 28 and day 180 after study inclusion.

Descriptively, the number of events up to the respective time point is given for IG and KG as well as the entire patient collective. Subsequently, Kaplan-Meier plots are created, which differentiate between the IG and KG. In addition, a descriptive log-rank test is calculated, the p-value of which is also given. The 28-day, 90-day and 180-day survival are described using the Kaplan-Meier estimator with the associated 95% confidence interval per group and overall.

8.2 Duration of mechanical ventilation

The duration of mechanical ventilation is analyzed at day 28 and day 180 after study inclusion.

The derived variables (see Chapter 4.2) are analyzed using descriptive methods. The difference between the two study groups is reported via the mean value with the associated 95% confidence interval.

8.3 Duration until shock resolution

The time to shock resolution is analyzed at day 28 and day 180 after study inclusion.

The derived variables (see Chapter 4.2) are analyzed using descriptive methods. The difference between the two study groups is reported using the mean value with the associated 95% confidence interval.

8.4 Ongoing need for renal replacement therapy during follow-up

The ongoing need for renal replacement therapy during follow-up is analyzed at day 28 and day 180 after study inclusion.

The derived variables (see Chapter 4.2) are analyzed using descriptive methods. The difference between the two study groups is reported via the mean value with the associated 95% confidence interval.

8.5 Total length of stay in hospital

The total length of stay in hospital is analyzed at day 28 and day 180 after study inclusion.

The derived variables (see Chapter 4.2) are analyzed using descriptive methods. The difference between the study two groups is reported using the mean value with the associated 95% confidence interval.

8.6 Cumulative use of anti-infectives

The cumulative anti-infective use is analyzed at day 28 after study inclusion.

The derived variable (see Chapter 4.2) is analyzed using descriptive methods. The difference between the two study groups is reported via the mean value with the associated 95% confidence interval.

8.7 Retrospective evaluation of the expert panel

Per item, the rate of “complete agreement”, “majority agreement” and “no agreement” is reported using descriptive methods. All percentages are reported in total and also separately by treatment group with exact confidence intervals (Pearson-Clopper).

Each item of the questionnaire is then analyzed with descriptive methods for the cases with agreement (complete or majority).

In addition, the comparison between recommended and actual treatment change is presented in the IG using a four-field table and evaluated with a chi-square test.

9 Analysis of safety

The following safety data are collected:

- Has an AE occurred (yes, no)?
- Event (text)
- Start of the event (date)
- Intensity (mild, moderate, severe)
- SAE (yes, no)
- Outcome (recovered, improvement, not recovered, recovered with consequences, death, unknown)
 - If “Outcome = Recovered” or “Outcome = Recovered with consequences”:
Termination of the event (date)

The duration of the event is calculated and reported from the recorded start of the event and the end. The variables are reported using descriptive methods. The number of patients with AEs and SAEs and the number of AEs and SAEs per patient are also analyzed using descriptive methods. In addition, a listing of all AEs that occurred is created.

10 Software

All analyses are performed in a validated R environment based on R version $\geq 4.2.0$. The following external packages are used for the specified purpose:

- For imputation: MICE
- For plots in general: ggplot2
- For survival plots: survminer
- For survival analysis: survival
- For data preparation: tidyverse

11 Appendix

11.1 Visit matrix

Data Collection Instrument	Screening	Expert council Days 0 - 14	V1 Day 0	V2 Day 3	V3 Day 7	V4 Day 14	V5 Day 28	V6 Day 90	V7 Day 180	Log Pages
Consent	●									
Inclusion/ exclusion criteria	●									
Expert council		●								
Randomization			●							
Demography			●							
Comorbidity			●							
Immune status			●							
Sepsis criteria			●	●						
Outcome							●	●	●	
Neuroscores			●	●	●	●	●*			
Study check			●	●	●	●				
Additional blood cultures				●	●	●				
Physiology			●	●	●	●	●*			
Catecholamine			●	●	●	●	●*			
Balance			●	●	●	●	●*			
Routine laboratory			●	●	●	●	●*			
Quality of Life			●					●*	●*	
Source control					●*					
Antiinfectives					●*					
Microbiology					●*					
Virology					●*					
Signature									●	
Adverse Events										●*
Protocol violation										●*

* repeatable instruments

*can only be filled out if study was not terminated prematurely

11.2 Modified SOFA-Score

modified SOFA-Score		0	1	2	3	4
Cardio-vascular system	MAP [mmHg] / vasopressors	≥ 70	< 70	dopamine < 5* or dobutamine	dopamine 5-15* or epinephrine ≤ 0.1* or norepinephrine ≤ 0.1*	dopamine >15* or epinephrine > 0.1* or norepinephrine > 0.1*
Respiratory system	paO2/FiO2	≥ 400	< 400	< 300	< 200	< 100
Liver	Bilirubin [mg/dl]	< 1,2	1,2 - 1,9	2,0 - 5,9	6,0 - 11,9	> 12
Coagulation	Platelets x10³/μl	≥ 150	< 150	< 100	< 50	< 20
Renal function	Creatinine [mg/dl]	< 1,2	1,2 - 1,9	2,0 - 3,4	3,5 - 4,9	> 5
	Urine [ml/d]				< 500	< 200

0-4 points are awarded for each organ system, the range of the modified SOFA score is therefore 0-20. If several measurements are available for a period, the respective maximum is used to calculate the modified SOFA score.

11.3 Expert panel questionnaire

Are the NGS findings plausible?	Day 0 (Sepsis Onset)	Yes	No
Are the NGS findings plausible?	Day 3	Yes	No
Based on the NGS findings, would you consider continuing the empirical anti-infective therapy unchanged?	Based on the NGS findings, would you consider continuing the empirical anti-infective therapy unchanged?	Yes	No
In what way would you consider changing the empirical anti-infective treatment strategy?	a. Would you narrow down the spectrum of anti-infective activity on the basis of the NGS findings?	Yes	No
In what way would you consider changing the empirical anti-infective treatment strategy?	b. Would you expand the anti-infective spectrum of activity on the basis of the NGS findings?	Yes	No
In what way would you consider changing the empirical anti-infective treatment strategy?	c. Would negative NGS findings prompt you to shorten or even discontinue the entire anti-infective therapy?	Yes	No

12 Literature

Rubin, D.B., 1987. Multiple Imputation for Nonresponse in Surveys, 1st ed, Wiley Series in Probability and Statistics. Wiley. <https://doi.org/10.1002/9780470316696>