



Necrotizing soft tissue infections - a multicentre, prospective observational study (INFECT): Protocol and statistical analysis plan

Madsen, M. B., Skrede, S., Bruun, T., Arnell, P., Rosén, A., Nekludov, M., ...
Hyldegaard, O.

This article is made publically available in the institutional repository of Wageningen University and Research, under article 25fa of the Dutch Copyright Act, also known as the Amendment Taverne.



Article 25fa states that the author of a short scientific work funded either wholly or partially by Dutch public funds is entitled to make that work publicly available for no consideration following a reasonable period of time after the work was first published, provided that clear reference is made to the source of the first publication of the work.

For questions regarding the public availability of this article, please contact openscience.library@wur.nl.

Please cite this publication as follows:

Madsen, M. B., Skrede, S., Bruun, T., Arnell, P., Rosén, A., Nekludov, M., ...
Hyldegaard, O. (2018). Necrotizing soft tissue infections - a multicentre, prospective observational study (INFECT): Protocol and statistical analysis plan. *Acta Anaesthesiologica Scandinavica*, 62(2), 272-279. <https://doi.org/10.1111/aas.13024>

Necrotizing soft tissue infections – a multicentre, prospective observational study (INFECT): protocol and statistical analysis plan

M. B. Madsen¹ , S. Skrede^{2,3}, T. Bruun², P. Arnell⁴, A. Rosén⁴, M. Nekludov⁵, Y. Karlsson⁶, F. Bergey⁷, E. Saccenti⁸ , V. A. P. Martins dos Santos^{7,8}, A. Perner¹, A. Norrby-Teglund⁹ and O. Hyldegaard¹⁰

¹Department of Intensive Care, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark

²Department of Medicine, Haukeland University Hospital, Bergen, Norway

³Department of Clinical Science, University of Bergen, Bergen, Norway

⁴Department of Anaesthesia and Intensive Care, Sahlgrenska University Hospital, Gothenburg, Sweden

⁵Department of Anaesthesia, Surgical Services and Intensive Care, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

⁶Department of Anaesthesiology and Intensive Care, Blekinge County Hospital, Karlskrona, Sweden

⁷LifeGlimmer GmbH, Berlin, Germany

⁸Laboratory of Systems and Synthetic Biology, Wageningen University & Research, Wageningen, the Netherlands

⁹Centre for Infectious Medicine, Karolinska Institutet, Karolinska University Hospital, Huddinge, Sweden

¹⁰Department of Anaesthesia, Centre of Head and Orthopaedics, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark

Correspondence

M. B. Madsen, Department of Intensive Care
4131, Copenhagen University Hospital,
Rigshospitalet, Blegdamsvej 9, 2100
Copenhagen, Denmark
E-mail: martin.bruun.madsen.01@regionh.dk

Conflicts of interest

The Department of Intensive Care, Rigshospitalet, receives support for research from CSL Behring, Ferring Pharmaceuticals and Fresenius Kabi, but the authors would like to emphasize that the support has not been used as contribution to this study.

Funding

The study is supported by the European Union's Seventh Framework Programme under the grant agreement 305340.

Accepted 8 October 2017; submission 6 October 2017.

Citation

Madsen MB, Skrede S, Bruun T, Arnell P, Rosén A, Nekludov M, Karlsson Y, Bergey F, Saccenti E, Martins dos Santos VAP, Perner A, Norrby-Teglund A, Hyldegaard O. Necrotizing soft tissue infections – a multicentre, prospective observational study (INFECT): protocol and statistical analysis plan. *Acta Anaesthesiologica Scandinavica* 2018

doi: 10.1111/aas.13024

Background: The INFECT project aims to advance our understanding of the pathophysiological mechanisms in necrotizing soft tissue infections (NSTIs). The INFECT observational study is part of the INFECT project with the aim of studying the clinical profile of patients with NSTIs and correlating these to patient-important outcomes. With this protocol and statistical analysis plan we describe the methods used to obtain data and the details of the planned analyses.

Methods: The INFECT study is a multicentre, prospective observational cohort study. Patients with NSTIs are enrolled in five Scandinavian hospitals, which are all referral centres for NSTIs. The primary outcomes are the descriptive variables of the patients. Secondary outcomes include identification of factors associated with 90-day mortality and amputation; associations between affected body part, maximum skin defect and Laboratory Risk Indicator for Necrotizing Fasciitis (LRINEC) score and 90-day mortality; 90-day mortality in patients with and without acute kidney injury (AKI) and LRINEC score of six and above or below six; and association between affected body part at arrival and microbiological findings. Exploratory outcomes include univariate analyses of baseline characteristics associations with 90-day mortality. The statistical analyses will be conducted in accordance with the predefined statistical analysis plan.

Conclusion: Necrotizing soft tissue infections result in severe morbidity and mortality. The INFECT study will be the largest prospective study in patients with NSTIs to date and will provide important data for clinicians, researchers and policy makers on the characteristics and outcomes of these patients.

Necrotizing soft tissue infections (NSTIs) are rare, but devastating diseases with high mortality rates, multiorgan impairment, amputation and impaired quality of life among survivors.^{1,2} NSTIs may affect any layer of the soft tissue and typically have a rapid progression. The aetiology is often polymicrobial, but monomicrobial cases, in particular with beta-haemolytic streptococci, are also common.³ Managing patients with NSTIs requires a multidisciplinary approach including repeated surgery, antibiotics and organ support. The treatments used vary between centres.⁴ Due to low incidence rates, only a few prospective cohort studies have been performed, and knowledge has mainly been derived from retrospective studies.

This observational study is part of the 'Improving Outcome of Necrotizing Fasciitis: Elucidation of Complex Host and Pathogen Signatures that Dictate Severity of Tissue Infection (INFECT)' project – an investigator initiated project to advance the understanding of the pathophysiological mechanisms, diagnosis and prognosis of NSTIs.⁵ The objectives of the INFECT observational study are to describe the clinical profile of patients with NSTI and to correlate these to patient-important outcomes.

Methods

Trial design

INFECT is an international, prospective observational cohort study.

Setting

Patients are enrolled at five hospitals, which are all referral centres for patients with NSTI: Rigshospitalet, Copenhagen University Hospital, Denmark; Karolinska University Hospital, Solna, Sweden; Blekinge Hospital, Karlskrona, Sweden; Sahlgrenska University Hospital, Gothenburg, Sweden, and Haukeland University Hospital, Bergen, Norway.

Population

We screen all adult patients (age ≥ 18 years) admitted to one of the active centres with suspected NSTI and include those with confirmed NSTI as defined by perioperative tissue

characteristics, observed by the surgeon. The diagnosis is based on signs as necrotic or deliquescent soft tissue with widespread undermining of the surrounding tissue.

Study size

A total of 400–500 patients are expected to be included based on yearly rates at the respective sites.⁶

Outcome measures

Primary outcome

The primary objective of the study was to describe the population. The following data have been registered and will be reported:

Baseline characteristics (first 24 h of intensive care unit (ICU) admission)

1. Age (at time of diagnosis)
2. Sex
3. Weight before surgery; if not available then best estimate
4. Body mass index
5. Comorbidities (y/n)
 - a. AIDS (HIV positive with clinical complications as *Pneumocystis jiroveci* pneumonia, Kaposi's sarcoma, lymphoma, tuberculosis or toxoplasma infection)
 - b. Active malignancy
 - c. Metastatic carcinoma (proven metastasis by surgery, C.T. scan or any other method)
 - d. Haematological cancer
 - e. Chronic obstructive pulmonary disease or asthma
 - f. Cardiovascular disease (hypertension, myocardial infarction, angina pectoris, heart failure, stroke)
 - g. Peripheral vascular disease
 - h. Diabetes
 - i. Chronic kidney disease (s-creatinine > 100 before admission for NSTI or any kind of dialysis)
 - j. Chronic liver disease (any history of chronic liver disease)
 - k. Varicella
 - l. Rheumatoid disease
 - m. Chronic wound or other skin disease
 - n. Immunodeficiency (any documented)

- o. No comorbidities
- 6. Preexisting conditions (y/n)
 - a. Previously affected by NSTI
 - b. Surgery before diagnosis of NSTI (any surgery performed within the 4 weeks prior to diagnosis)
 - c. Blunt trauma (a hit by a blunt object or a fall within the 4 weeks prior to diagnosis)
 - d. Penetrating trauma (a hit by a sharp object penetrating the skin within the 4 weeks prior to diagnosis)
 - e. Use of IV drugs (either as a drug addict or use of IV medication outside hospital settings)
 - f. Use of immunosuppressing drugs (medication given for auto-immune or malignant disease)
 - g. Use of steroids
 - h. Active smoking
 - i. Alcohol use above recommended limits (above 14 units of alcohol per week for females, above 21 for males)
- 7. Site of infection (y/n)
 - a. Head, neck and intrathoracic space
 - b. Upper extremity
 - c. Lower extremity
 - d. Ano-genital and abdominal area
- 8. Simplified acute physiology (SAPS) II score
- 9. Sequential organ failure assessment (SOFA) score
- 10. Mechanical ventilation (mechanically ventilated via a tube or continuously via a mask)
- 11. Acute kidney injury (KDIGO stage 3)
- 12. Septic shock (use of vasopressor or inotropic agents and lactate > 2 mmol/l)
- 13. Noradrenaline rate (highest)

Characteristics before diagnosis

 - 1. Clinical characteristics
 - a. Severe pain, in the need of opioids
 - b. Skin bullae
 - c. Skin discolouration
 - d. Skin bruising
 - e. Skin anaesthesia
 - f. Crepitus
 - 2. Blood samples
 - a. White blood cells
 - b. C-reactive protein
 - c. Lactate
 - d. Creatinine
 - e. Glucose
 - f. Haemoglobin
 - g. Sodium
 - 3. Gas on radiology
 - 4. Laboratory Risk Indicator for Necrotizing Fasciitis (LRINEC) score

Treatment characteristics, ICU day 1–7

 - 1. Surgical management
 - a. Maximum skin defect observed during surgery (%)
 - b. Number of operations
 - c. Time from admission to surgery (hours)
 - d. Amputation, any
 - 2. Medical management
 - a. Antibiotics used in the ICU
 - i. Penicillin
 - ii. Dicloxacillin
 - iii. Piperacillin + tazobactam
 - iv. Meropenem
 - v. Cefuroxime
 - vi. Ceftriaxone
 - vii. Cefotaxime
 - viii. Ciprofloxacin
 - ix. Gentamicin
 - x. Moxifloxacin
 - xi. Clindamycin
 - xii. Vancomycin
 - xiii. Linezolid
 - xiv. Metronidazole
 - xv. Other
 - b. Intravenous polyspecific immunoglobulin G (IVIG)
 - c. Number of IVIG doses
 - d. Use of blood products
 - i. Erythrocytes
 - ii. Fresh frozen plasma
 - iii. Platelets
 - 3. Supportive modalities
 - a. Mechanical ventilation during ICU admission
 - b. Renal replacement therapy during ICU admission

- c. Use of vasopressor/inotrope during ICU admission
- d. Use of hyperbaric oxygenation (HBO) at any time
- e. Number of HBO sessions

Microbiological findings

1. Polymicrobial infection
 - a. Polymicrobial infection with presence of beta-haemolytic streptococci
 - i. Polymicrobial infection with presence of group A streptococcus
 - ii. Polymicrobial infection with presence of group B streptococcus
 - iii. Polymicrobial infection with presence of group C/G streptococcus
 - b. Polymicrobial infection with presence of clostridium species
 - c. Polymicrobial infection with presence of *Staphylococcus aureus*
 - d. Other polymicrobial infections
2. Monomicrobial infection
 - a. Monomicrobial infection with presence of beta-haemolytic streptococci
 - i. Monomicrobial infection with presence of group A streptococcus
 - ii. Monomicrobial infection with presence of group B streptococcus
 - iii. Monomicrobial infection with presence of group C/G streptococcus
 - b. Monomicrobial infection with presence of other streptococci
 - c. Monomicrobial infection with presence of clostridium species
 - d. Monomicrobial infection with presence of *Staphylococcus aureus*
 - e. Monomicrobial infection with presence of anaerobic bacteria
 - f. Monomicrobial infection with presence of aerobic gram-negative species

3. Patients with no bacteria detected

Follow-up

1. 30-day mortality (all-cause mortality)
2. 90-day mortality (all-cause mortality)
3. Days in the ICU (index admission)

4. Days on mechanical ventilation (index admission)
5. Days on renal replacement therapy in the 90 days after inclusion (%)
6. Days alive and out of hospital in the 90 days after inclusion (%)

Secondary outcomes

1. Identification of factors associated with 90-day mortality
2. Identification of factors associated with amputation of the extremities within ICU stay
3. 90-day mortality in patients with AKI at baseline vs. non-AKI
4. Association between affected body part at arrival and microbiological findings
5. Association between affected body part at arrival and 90-day mortality
6. Association between maximum skin defect and 90-day mortality
7. Association between LRINEC score and 90-day mortality
8. 90-day mortality in patients with a LRINEC score ≥ 6 vs. < 6

Exploratory outcomes

Association between baseline variables and 90-day mortality

Perspective

Protocols focusing specifically on the detailed microbiological findings, streptococcal infections, HBO treatment, IVIG treatment, patients with diabetes mellitus and long-term follow-up including quality of life are being prepared.

Registered variables

For a full list of registered variables, please see Appendix S1.

Missing data

Primary outcome

Data will be presented as complete cases only except for the following:

SAPS II. If values from blood samples are missing, values from day 2 will be used.

SOFA score. If values from blood samples at day 1 are missing, values from day 2 will be used. If values from blood samples at days 2–7 are missing, last observation carried forward will be used. Due to the nature of eCRF, day 1 can vary from 0 to 24 h. If day 1 is < 12 h, the worst value from either day 1 or day 2 is used. The SOFA score has been modified, as Glasgow coma scale (GCS) is not included.

Kidney Disease, Improving Global Outcomes (KDIGO) staging. KDIGO staging will be modified according to the KDIGO serum creatinine criteria.⁷ Urine output criteria will not be assessed. Preadmission creatinine is not collected; the Modification of Diet in Renal Disease (MDRD) equation will be used to estimate the preadmission creatinine from a eGFR of 75 ml/min per 1.73 m² as recommended by the KDIGO guidelines.⁷

Secondary outcomes

We will perform complete-case analyses. If the frequency of missing data for a given analysis is more than 5%, the analysis will be repeated using multiple imputation based on chained equations (MICE). The variables included in the multiple imputation will be: the variables in the specific analysis, age, SOFA score at day 1, presence of haematological cancer, presence of diabetes, AKI at baseline, shock at baseline, GAS as causative microbiological agent and 90-day mortality. Lastly, we will present unadjusted, non-imputed analysis.

General analytical principles

For all analyses, the level of statistical significance (two sided) is 0.05. Correction for multiple testing will be used when applicable.

Statistical analyses

Trial profile

The flow of patients will be displayed in a Consolidated Standard of Reporting Trials (CONSORT) diagram.

Primary outcome measure

The primary outcome will be presented as number/total number (percentage), median (IQR) or mean (SD).

Secondary outcome measures

Identification of factors associated with 90-day mortality. This will be done by logistic regression to examine the crude and adjusted estimate. Inclusion of covariates will be done in a single step (enter modelling) to give the most conservative estimate. The following covariates will be included:

1. Age^{8–11}
2. AKI at baseline (KDIGO stage 3)^{9,12}
3. Alcohol abuse (above 14 units of alcohol per week for females, above 21 for males)¹³
4. BMI¹²
5. Diabetes mellitus^{12,13}
6. Fluid input (total baseline input, ml/24 h)¹⁴
7. Group A streptococcus as probable causative microorganism^{15,16}
8. Haematological cancer or metastatic cancer¹⁷
9. Lactate (highest baseline lactate)¹⁸
10. Noradrenaline rate (highest baseline rate)^{9,19}
11. Treating centre^{20,21}

Age, fluid input, lactate and noradrenaline rate will be categorized as linear variables, the remaining as categorical variables. BMI will be categorized according to WHO criteria. Results will be presented as relative risk ratios including 95% confidence intervals.

Identification of factors associated with amputation of the extremities within ICU stay. Only patients with NSTIs located on the extremities will be included in the analysis. We will use the Cox proportional hazards model with amputation of the extremities within ICU stay as outcome and SOFA score at day 1 and age as explanatory variables. A variable selection algorithm will be applied using the Bayesian Information Criterion as selection criterion in a stepwise procedure to select variables significantly associated with amputation. In retrospective studies, bacterial aetiology, shock, heart disease, diabetes mellitus, cutaneous gangrene, skin necrosis and high creatinine have been associated with limb

amputation.^{8,16,22} However, the analyses were performed in populations with NSTIs found retrospectively. In order not to be biased by these findings we will include all clinical variables.

Ninety-day mortality in patients with AKI at baseline vs. non-AKI. Will be analysed by logistic regression with 90-day mortality as outcome, AKI as explanatory variable and SOFA score at day 1 and age as covariates. AKI will be defined as modified KDIGO stage 3 (see Missing data section).

Association between affected body part at arrival and microbiological findings. Will be performed by Fisher's exact test to test the association for each bacterial group and subgroup with each body part. *P*-values will be adjusted using the Benjamin-Hochberg procedure controlling the false discovery rate. The definitions of affected body part and microbiological findings are given in the Appendix S1.

Association between affected body part at arrival and 90-day mortality. We will build a logistic regression model with the affected body part as explanatory variable and the SOFA score and age as covariates. The matrix design will be constructed to test the association of a body part against the average association of all body parts instead of testing the association of a body part against a body part taken arbitrary as a reference.

Association between maximum skin defect and 90-day mortality. Will be analysed by logistic regression with 90-day mortality as outcome and maximum skin defect as explanatory variable. If a patient is amputated, the size of the amputated area will be used as maximum skin defect.

Association between LRINEC score and 90-day mortality. Will be analysed by logistic regression with 90-day mortality as outcome and LRINEC score as explanatory variable.

Ninety-day mortality in patients with a LRINEC score ≥ 6 vs. < 6 . Will be analysed by logistic regression with 90-day mortality as outcome, LRINEC

score ≥ 6 as explanatory variable and SOFA score at day 1 and age as covariates.

Explorative outcomes

Associations between baseline variables and 90-day mortality. Identification of variables will be done by univariate analyses using *t*-test for continuous variables and Fisher's exact test for dichotomous variables. Variables with *P*-value < 0.10 are included in a multivariate analysis. All variables in the multivariate analysis will be included in a single step. Results will be presented as relative risks including 95% confidence intervals.

Data collection

Data will be entered into the web-based eCRF by trial personnel. The trial database will be established from the eCRF. In case of technical difficulties, a paper CRF will be used.

Data handling and retention

Data will be handled according to the regulations of the national data protection agencies. All original records (including consent forms, CRFs and relevant correspondences) will be retained at trial sites for 15 years to allow inspection by local authorities. The trial database will be maintained for 15 years.

Ethics

The study will adhere to the Helsinki Declaration and the national laws of the including sites. The study is approved by the ethical committees and data protection agencies in each country of the participating sites. Due to the severity of illness, patients are not able to give informed consent prior to inclusion. Informed consent will be obtained according to national law. Oral information and written information approved by the respective ethical committees will be given to the consenting party. Consent can be withdrawn at any time without any consequences for the consenting party or the patient.

Approvals

The study is registered at ClinicalTrials.gov (NCT01790698) and is approved by the Danish Data Protection Agency (30-0900) and the following ethical committees: the Danish Ethical Committees (1211709), the Swedish Ethical Committee (Dnr 930-12) and the Regional Committee for Ethics in Medical Research (2012/2227) in Western Norway.

Study management and organization

The study is part of INFECT project,⁵ led by project coordinator Anna Norrby-Teglund. The steering committee consists of Ole Hyldegaard, Per Arnell, Ylva Karlsson, Michael Nekludov and Steinar Skrede. Scientific and ethical advisory boards can be found in the Appendix S1. Local research teams are responsible for the inclusion of patients 24/7.

Publication

We will report the study in accordance with the STROBE reporting guidelines for observational studies.²³ The results of the study will be submitted for publication in an international peer-reviewed journal.

Timeline

The first patient was included in February 2013 and last inclusion was in June 2017. After last follow-up, data will be analysed and the paper written and submitted for publication in late 2017.

Discussion

Necrotizing soft tissue infections occur worldwide, but the underlying mechanisms are poorly understood. This study will provide important clinical data and may indicate factors associated with certain outcomes. The strengths of the study include the prospective design, minimizing the risk of inclusion bias. Patients are included in five different centres, making the results more generalizable. To our knowledge, this will be the largest cohort of patients with NSTIs. The study is limited by its

observational design; no causative relations can be made, only associations. Many analyses will be performed, increasing the risk for chance findings.

Conclusion

Necrotizing soft tissue infections result in severe morbidity and mortality. The INFECT study will be the largest prospective study in patients with NSTIs and will provide important data for clinicians, researchers and policy makers on the characteristics and outcomes of these patients.

Acknowledgements

The authors thank the clinical and research personnel at each of the including sites, the microbiological laboratories, patients and their relatives.

Authors' contributions

A. N.: is project coordinator of the INFECT study.

M. B. M.: drafted the first manuscript.

E. S., F. B., V. A. P. M. and A. P.: revised the statistical details of the statistical analysis plan.

O. H., S. S., P. A. and M. N. are national investigators and have contributed to study design and coordinated study conduct.

M. B. M.: is responsible for the eCRF and contributed to patient inclusion and data collection.

All authors contributed to the writing of this manuscript and approved the final version.

References

1. Madsen MB, Hjortrup PB, Hansen MB, Lange T, Norrby-Teglund A, Hyldegaard O, Perner A. Immunoglobulin G for patients with necrotising soft tissue infection (INSTINCT): a randomised, blinded, placebo-controlled trial. *Intensive Care Med* 2017; 43: 1585–93.
2. Hakkarainen TW, Burkette Ikebata N, Bulger E, Evans HL. Moving beyond survival as a measure of success: understanding the patient experience of necrotizing soft-tissue infections. *J Surg Res* 2014; 192: 143–9.

3. Anaya DA, Dellinger EP. Necrotizing soft-tissue infection: diagnosis and management. *Clin Infect Dis* 2007; 44: 705–10.
4. de Prost N, Sbidian E, Chosidow O, Brun-Buisson C, Amathieu R. Management of necrotizing soft tissue infections in the intensive care unit: results of an international survey. *Intensive Care Med* 2015; 41: 1506–8.
5. INFECT – EU-FP7-HEALTH – Systems medicine to understand severe soft tissue infections [Internet]. Available at: <http://www.fp7infect.eu/> (accessed 2 September 2013).
6. Skovsen AP, Bonde J, Andersen JS, Jansen EC, Tvede M. Necrotizing fasciitis. *Ugeskr Laeger* 2010; 172: 440–4.
7. Group KAW. KDIGO clinical practice guideline for acute kidney injury. *Kidney Int Suppl* 2012; 2: 1–138.
8. Dworkin MS, Westercamp MD, Park L, McIntyre A. The epidemiology of necrotizing fasciitis including factors associated with death and amputation. *Epidemiol Infect* 2009; 137: 1609–14.
9. Hua C, Sbidian E, Hemery F, Decousser JW, Bosc R, Amathieu R, Rahmouni A, Wolkenstein P, Valeyrie-Allanore L, Brun-Buisson C, de Prost N, Chosidow O. Prognostic factors in necrotizing soft-tissue infections (NSTI): a cohort study. *J Am Acad Dermatol* 2015; 73: 1006–12. e8.
10. Anaya DA, Bulger EM, Kwon YS, Kao LS, Evans H, Nathens AB. Predicting death in necrotizing soft tissue infections: a clinical score. *Surg Infect (Larchmt)* 2009; 10: 517–22.
11. Elliott DC, Kufera JA, Myers RA. Necrotizing soft tissue infections. Risk factors for mortality and strategies for management. *Ann Surg* 1996; 224: 672–83.
12. Arif N, Yousfi S, Vinnard C. Deaths from necrotizing fasciitis in the United States, 2003–2013. *Epidemiol Infect* 2016; 144: 1338–44.
13. Beck MK, Jensen AB, Nielsen AB, Perner A, Moseley PL, Brunak S. Diagnosis trajectories of prior multi-morbidity predict sepsis mortality. *Sci Rep* 2016; 6: 36624.
14. Hjortrup PB, Haase N, Bundgaard H, Thomsen SL, Winding R, Pettilä V, Aaen A, Lodahl D, Berthelsen RE, Christensen H, Madsen MB, Winkel P, Wetterslev J, Perner A, CLASSIC Trial Group, Scandinavian Critical Care Trials Group. Restricting volumes of resuscitation fluid in adults with septic shock after initial management: the CLASSIC randomised, parallel-group, multicentre feasibility trial. *Intensive Care Med* 2016; 42: 1695–705.
15. Bruun T, Kittang BR, de Hoog BJ, Aardal S, Flaatten HK, Langeland N, Mylvaganam H, Vindenes HA, Skrede S. Necrotizing soft tissue infections caused by *Streptococcus pyogenes* and *Streptococcus dysgalactiae* subsp. *equisimilis* of groups C and G in western Norway. *Clin Microbiol Infect* 2013; 19: E545–50.
16. Anaya DA, McMahon K, Nathens AB, Sullivan SR, Foy H, Bulger E. Predictors of mortality and limb loss in necrotizing soft tissue infections. *Arch Surg* 2005; 140: 151–7; discussion 158.
17. Russell L, Madsen MB, Dahl M, Kampmann P, Perner A. Prediction of bleeding and thrombosis by standard biochemical coagulation variables in haematological intensive care patients. *Acta Anaesthesiol Scand* 2017. In press.
18. Varis E, Pettilä V, Poukkanen M, Jakob SM, Karlsson S, Perner A, Takala J, Wilkman E, FINNAKI Study Group. Evolution of blood lactate and 90-day mortality in septic shock. a post hoc analysis of the FINNAKI study. *Shock* 2017; 47: 574–81.
19. Auchet T, Regnier M-A, Girerd N, Levy B. Outcome of patients with septic shock and high-dose vasopressor therapy. *Ann Intensive Care* 2017; 7: 43.
20. Reinikainen M, Karlsson S, Varpula T, Parviainen I, Ruokonen E, Varpula M, Ala-Kokko T, Pettilä V. Are small hospitals with small intensive care units able to treat patients with severe sepsis? *Intensive Care Med* 2010; 36: 673–9.
21. Vincent J-L, Sakr Y, Sprung CL, Ranieri VM, Reinhart K, Gerlach H, Moreno R, Carlet J, Le Gall J-R, Payen D, Sepsis Occurrence in Acutely Ill Patients Investigators. Sepsis in European intensive care units: results of the SOAP study. *Crit Care Med* 2006; 34: 344–53.
22. Khamnuan P, Chongruksut W, Jearwattananok K, Patumanond J, Tantraworasin A. Necrotizing fasciitis: epidemiology and clinical predictors for amputation. *Int J Gen Med* 2015; 8: 195–202.
23. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet* 2007; 370: 1453–7.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Necrotizing soft tissue infections – a multi-centre, prospective observational study (INFECT): protocol and statistical analysis plan.