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Diabetes and necrotizing soft tissue infections—A prospective observational cohort study: Statistical analysis plan

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Background: Necrotizing soft tissue infections (NSTIs) are rare but carry a high morbidity and mortality. The multicenter INFECT project aims to improve the understanding of the pathogenesis, clinical characteristics, diagnosis, and prognosis of NSTIs. This article describes the study outline and statistical analyses that will be used.

Methods: Within the framework of INFECT project, patients with NSTI at 5 Scandinavian hospitals are enrolled in a prospective observational cohort study. The goal is to evaluate outcome and characteristics for patients with NSTI and diabetes compared to patients with NSTI without diabetes. The primary outcome is mortality at 90 days after inclusion. Secondary outcomes include days alive and out of ICU and hospital, SAPS II, SOFA score, infectious etiology, amputation, affected body area, and renal replacement therapy. Comparison in mortality between patients with diabetes type 1 and 2 as well as between insulin-treated and non-insulin–treated diabetes patients will be made. Clinical data for diabetic patients with NSTI will be reported.

Conclusion: The study will provide important data on patients with NSTI and diabetes.

1 | INTRODUCTION

1.1 | Necrotizing soft tissue infections

Necrotizing soft tissue infections (NSTIs) are uncommon, severe, and potentially lethal conditions.[1,2] Surviving often comes at the prize of

life-lasting morbidity.[3] NSTI typically presents with pain, swelling, and skin discoloration[4]. The infection may involve any layer of soft tissue, causing local ischemia, tissue destruction, and necrosis.[4-6] Bacterial spread proceeds swiftly, frequently along fascial planes. Host immunological response results in systemic toxicity. Shock, organ failure, and eventually death may result.[7-9]

Microbiological findings frequently exhibit a distinguishing pattern with polymicrobial growth in samples from the pelvic region[10,11]

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†INFECT Study Group members are in Appendix 1.

and monomicrobial growth when a limb is affected. In the latter case, group A streptococcus is often found in cultures, but many other bacterial findings have been described as well.[4,12-14]

Mainstays of successful treatment are aggressive resuscitation, intravenous antibiotics, and prompt surgical resection of affected tissue.[4,6,8,10,12] Hyperbaric oxygen treatment (HBOT) is, by some, considered an adjuvant treatment resource.[8,15,16] Due to concurrent circulatory, respiratory, and renal disturbances, most patients are treated at intensive care units (ICU).

Diabetes is associated with an increased risk of infections[4,17,18] and is overrepresented among patients with NSTI, with a reported frequency of 20%-70%.[10,11,15,19-23] In contrast, the World Health Organization (WHO) estimates global diabetes prevalence to be 8.5% in adults.[24] Concomitant diabetes seems to be a negative prognostic factor among patients with NSTI with increased risk of death, amputation, and infectious complications.[22,23,25-27] Hyperglycemia could be a factor associated with outcome in diabetic patients with NSTI.[26]

1.2 | Hypothesis

Our research hypothesis is that there are significant differences in both clinical characteristics, bacterial findings, and outcome among patients with NSTI and diabetes compared to patients with NSTI without diabetes.

1.3 | Study aim

The aim of this study is to report outcome for patients with NSTI and diabetes compared to patients with NSTI without diabetes and to report clinical data for patients with NSTI and diabetes.

2 | METHODS

2.1 | Study design

Prospective observational cohort study.

2.2 | Population

Adult (≥18 years) patients with NSTI.

2.3 | Exposure group

Patients with diabetes type 1 or type 2.

2.4 | Comparison group

Patients without diabetes type 1 or type 2.

2.5 | Outcome

2.5.1 | Primary outcome

1. All-cause mortality 90 days after inclusion.

2.5.2 | Secondary outcomes

1. Days alive and out of ICU at day 30 after inclusion.
2. Days alive and out of hospital at day 90 after inclusion.
3. Amputation of the extremities during ICU stay.
   Site of amputation is defined as either upper arm, lower arm, hand, finger, upper leg, lower leg, foot, toe, or penis.
4. Sequential organ failure assessment score (SOFA score) day 1 in the ICU.
   SOFA score is used to measure severity of disease. It is based on the assessed function of respiratory, nervous, and cardiovascular systems, liver, kidneys, and coagulation.[28,29]
5. SAPS II score at ICU admission.
   SAPS II score is used to measure severity of disease at ICU admission. It is based on measured physiological parameters and results from blood samples, previous health status, and information about the ICU admission.[30]
6. The presence of monomicrobial growth of group A streptococci (GAS) in cultures.
   Tissue and blood samples taken at referring hospital and study hospital are used.[31]
7. The presence of monomicrobial growth of group B streptococci in cultures.
   Tissue and blood samples taken at both referring hospital and study hospital are used.[31]
8. The presence of monomicrobial growth of *Staphylococcus aureus* in cultures.
   Tissue and blood samples taken at both referring hospital and study hospital are used.[31]
9. The presence of polymicrobial flora in cultures.
   Tissue and blood samples taken at both referring hospital and study hospital are used.[31]
10. Affected body area at inclusion.
    Affected body area is defined as either head and neck, upper body (including upper extremities), abdomen and anogenital region, or lower extremities.
11. Any renal replacement therapy (RRT) within 90 days after inclusion.
    RRT is defined as either intermittent hemodialysis or continuous renal replacement therapy.

2.5.3 | Exploratory outcomes

Comparisons are made within the group of patients with diabetes type 1 and type 2:

1. Association between glycated hemoglobin (A1C) values before admission and all-cause mortality 90 days after inclusion.
2. All-cause mortality 90 days after inclusion in patients with diabetes type 1 compared to patients with diabetes type 2.
3. All-cause mortality 90 days after inclusion in patients with insulin-treated diabetes (type 1 and type 2) compared to non-insulin–treated patients with diabetes type 2.

4. Association between A1C values before admission and days alive and out of ICU at day 30 after inclusion.

5. Association between A1C values before admission and the presence of polymicrobial flora in cultures.

Tissue and blood samples taken at both referring hospital and study hospital are used.[31]

6. Association between A1C values before admission and affected body area at inclusion.

Affected body area is defined as either head and neck, upper body (including upper extremities), abdomen and anogenital region, or lower extremities.

7. Association between A1C values before inclusion and any RRT within 90 days after inclusion.

RRT is defined as either intermittent hemodialysis or continuous renal replacement therapy.

2.5.4 | Reported clinical data, comorbidities, risk factors for NSTI, and medication

-Sex;

-Age;

-Weight (before surgery; if not available then best estimate);

-Body mass index;

-Smoking habits (currently smoking/estimated package years);

-AIDS (HIV positive with clinical complications as Pneumocystis jiroveci pneumonia, Kaposi's sarcoma, lymphoma, tuberculosis, or toxoplasma infection);

-Other immunodeficiencies;

-Active malignancy;

-Metastatic carcinoma (proven metastasis by surgery, CT scan, or any other method);

-Hematologic cancer;

-Chronic obstructive pulmonary disease (COPD) or asthma;

-Current or previous cardiovascular disease (hypertension, myocardial infarction, angina pectoris, heart failure, apoplexy);

-Peripheral vascular disease;

-Diabetes type 1;

-Non-insulin–treated diabetes type 2;

-Insulin-treated diabetes type 2;

-Chronic kidney disease (s-creatinine > 100 before admission for NSTI or any kind of dialysis);

-Chronic liver disease (any history of chronic liver disease);

-Varicella;

-Rheumatoid disease;

-Previously admitted for necrotizing soft tissue infection;

-Surgery within the previous 4 weeks;

-Blunt trauma (a hit by a blunt object or a fall within the 4 weeks prior to diagnosis);

-Penetrating trauma (a hit by a sharp object penetrating the skin within the 4 weeks prior to diagnosis);

-Chronic wound or other skin disease;

-Intravenous drug use;

-Immunosuppressive drugs given for rheumatoid disease or malignant disease;

-Steroids.

2.6 | The INFECT project

Improving Outcome of Necrotizing Fasciitis: Elucidation of Complex Host and Pathogen Signatures that Dictate Severity of Tissue Infection (INFECT) is an international, prospective, multicenter observational research project. The goal is to improve the understanding of the pathogenesis, clinical characteristics, diagnosis, and prognosis of NSTIs.[32] Within the framework of INFECT, patients are recruited from 5 Scandinavian hospitals which are all referral hospitals for patients with NSTI.

INFECT is registered at ClinicalTrials.gov (NCT01790698).

2.7 | Study setting

Patients are recruited from intensive care departments in the following hospitals:

Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark.
Sahlgrenska University Hospital/Ostra, Gothenburg, Sweden.
Karolinska University Hospital, Stockholm, Sweden.
Blekinge County Council Hospital, Karlskrona, Sweden.
Haukeland University Hospital, Bergen, Norway.

2.8 | Eligibility

Patients arriving at a study hospital during the study period with a suspected NSTI are eligible for inclusion.

2.9 | Inclusion criteria

Adult (≥18 years) patients with confirmed NSTI treated in one of the participating hospitals who consent to participate in the study.

The diagnosis of NSTI is made by the surgeon during the primary operation. It is based on, but not restricted to, findings of necrosis, dissolved or deliquescent soft tissue, and fascial and muscular affection.

2.10 | Exclusion criteria

Denial or withdrawal of consent.
Later refuted NSTI diagnosis.

2.11 | Subject enrollment

Patients are included by local research staff at each hospital. Consent is obtained according to national ethical regulations.

The research staff ensures that the consenting party is given oral and written information approved by the respective ethical committee about the nature, purpose, possible risk, and benefit of the study.

2.12 | Group allocation (exposure/comparison)

Patients with an existing diagnosis of diabetes type 1 or type 2 in the medical records before inclusion in the study are allocated to the exposure group.

Patients without an existing diagnosis of diabetes type 1 or type 2 in the medical records before inclusion in the study are allocated to the comparison group.

2.13 | Timeline

The first patient was included in February 2013 and the last patient was included in June 2017. Patients' data registration is planned to be completed in March 2018.

2.14 | Data collection methods

Data are obtained from hospital medical records, hospital laboratories, and official death registries. Participation in the study results in additional blood sampling and extended collection of specimens for microbiological analysis.

2.15 | Registered variables

Description of clinical variables registered in the INFECT project including principles of bacterial categorization are available in a separate publication.[31]

Among patients with diagnosed diabetes, available A1C values from up to 6 months before inclusion are retrospectively collected from medical records.

Also in retrospect, patients with diabetes will be categorized as type 1, insulin-treated type 2, or non-insulin–treated type 2 based on information from medical records.

2.16 | Data management

All research data are handled confidentially and in accordance with national laws and regulations. Patients' data are anonymized, using an individual study code. Code lists are kept locked up and separate from registered study data. Original records will be retained at trial sites for the time specified by national regulations.

A common electronic database (eCRF) with all recorded data is kept in Copenhagen, Denmark (Danish Data protection Agency approval 30-0900). The trial database will be maintained for 15 years.

2.17 | Missing data

2.17.1 | Imputation

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 age, SOFA score at day 1, the presence of hematological cancer, presence of diabetes, presence of chronic obstructive pulmonary disease (COPD) or asthma, presence of current or previous cardiovascular disease, presence of chronic kidney disease, GAS found in cultures and 90-day mortality. The variables used in specific analyses will be excluded from the imputation. We will also present unadjusted, non-imputed analyses.

2.17.2 | SAPS II score

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

2.17.3 | SOFA score

If values from blood samples at day 1 are missing, values from day 2 will be used. Due to the nature of eCRF, day 1 can vary from 0 to 24 hours. If day 1 is <12 hours, 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.

2.17.4 | Diabetes type and A1C

If the amount of missing data exceeds 20%, the specific analysis will not be performed.

3 | STATISTICAL METHODS

3.1 | General analytical principles

For all analyses, the level of statistical significance (2-sided) is 0.05. P-values will be adjusted for multiple testing by the Benjamini-Hochberg method.

3.2 | Outcomes

3.2.1 | Primary outcome

The primary outcome (all-cause mortality 90 days after inclusion) expressed as dichotomous variable (0-1) will be analyzed by logistic regression with 90 days mortality as response, having diabetes (dichotomous variable) as regressor and considering age (in years) as a covariate. Data will be presented as relative risks (RR) including 95% confidence intervals (CI).

3.2.2 | Secondary outcomes

The secondary outcomes will be analyzed by Fisher's exact test for categorical variables (amputation, affected body area, and renal replacement therapy) and by Mann-Whitney-Wilcoxon test for the continuous variables (SOFA score and SAPS II). Specific considerations are mentioned below.

Number of days alive and out of hospital at day 90 and days alive and out of ICU at day 30 will be analyzed by Mann-Whitney-Wilcoxon test.

The presence of specific bacterial species in cultures of blood or tissue expressed as dichotomous variables (0-1) will be analyzed by logistic regression considering affected body area at admission as covariates. Data will be presented as RR including 95% CI.

3.2.3 | Exploratory outcomes

All-cause mortality at 90 days expressed as dichotomous variable (0-1) will be analyzed by logistic regression with mortality at 90 days as response and considering age (in years) as a covariate. Regressors will be either dichotomous variables (diabetes subgroups: type 1 and type 2 or insulin-treated and non-insulin–treated) or a continuous variable (A1C values before admission) depending on the outcome of interest. Data will be presented as RR including 95% CI.

The exploratory outcomes investigating association with A1C values before admission will be analyzed by Mann-Whitney-Wilcoxon test for continuous variables (number of days alive and out of ICU) and Fisher's exact test for categorical variables (affected body area, renal replacement therapy). Specific considerations are mentioned below.

The presence of specific bacterial species in cultures of blood or tissue expressed as dichotomous variables (0-1) will be analyzed by logistic regression considering affected body area at admission as covariates. Data will be presented as RR including 95% CI.

3.2.4 | Clinical data, comorbidities, risk factors for NSTI, and medication

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

3.3 | Sample size estimation and sensitivity analysis

A total of 400 patients or more are expected to be included. Estimating a diabetes incidence between 25% and 35%, the group sizes will range between $N_1 = 100$ and 140 for the diabetics and $N_2 = 300$ and 260 for the nondiabetics.

Sensitivity analysis was performed with G*Power.[33]

With a power of 0.8 and a confidence level $\alpha = 0.05$, the smallest difference in proportions estimated with a Fisher's exact test range between 7% and 12.5% both for $N_1/N_2 = 100/300$ and $N_1/N_2 = 140/260$.

For differences in mean, estimated with Mann-Whitney-Wilcoxon, the smallest estimable effect is 0.29 for $N_1/N_2 = 100/300$ and 0.27 for $N_1/N_2 = 140/260$.

For odds ratios calculated by estimating logistic regression, the estimable odds ratios are either smaller than 0.49 or higher than 1.80 for $N = 400$.

4 | DISCUSSION

Diabetes is a common disease worldwide. Its impact on outcome among patients with NSTI is reported to be negative. This study will provide clinical data regarding diabetes frequency and outcome for patients with diabetes in a prospective Scandinavian NSTI cohort study. The cohort will, compared to earlier studies, be large. The prospective design of the study is a strength, yet the observational design precludes causative conclusions.

An unknown number of patients will likely be misclassified as nondiabetics. They represent individuals who, before developing NSTI, would have fulfilled the diagnostic criteria for diabetes type 2, but had yet not received a diagnosis.

Metabolic control before hospitalization as well as type of diabetes (type 1 or 2) and treatment (insulin or non-insulin) might influence outcome in NSTI. There are numerous flaws connected to using retrospective A1C values. Nothing is known of the clinical circumstances when the A1C samples were collected. The time elapsed between A1C sample acquisition and hospital admission will be variable among patients. This, in combination with missing A1C values, makes estimation of blood glucose control before admission unreliable. Retrospective categorization of diabetes type and its treatment (insulin, non-insulin) for each included patient might be either not feasible or incorrect. Therefore, results from exploratory outcomes must be interpreted with caution.

Certain patient comorbidities, for example, peripheral vascular disease and chronic liver disease, are not clearly defined in the study protocol. This entails subjectivity and a risk of bias when registering patient data.

The use of 4 arbitrarily defined body areas to describe the infectious locus makes data compilation and statistical analysis easier. However, all categorization results in loss of information and reduced precision.

There is a relatively high number of secondary and exploratory outcomes. Statistical adjustment for multiple testing is used to reduce this risk of falsely positive findings. The amount of patients eligible for calculation of the exploratory outcomes will probably only number about 100-120, making statistical inference less precise.

Despite its limitations, this study will provide important data on patients with NSTI and diabetes.

ETHICS

The study is conducted in compliance with ICH Good Clinical Practice, national laws, applicable regulatory requirements and in accordance with the ethical principles in the Declaration of Helsinki. The study is approved by ethical committees in all participating countries (Danish ethical committee [1211709], Swedish ethical committee in Gothenburg [930-12], Regional committee for ethics in medical research in Western Norway [2012/2227]).

Patients get no direct benefit from participation in the study. Hopefully results from the study will lead to improved treatment for diabetic patients with NSTI in the future.

PUBLICATION

ACKNOWLEDGEMENTS

CONFLICTS OF INTEREST

AUTHORS' CONTRIBUTIONS

A. N.-T. is a coordinator of the INFECT project. A. R. drafted the first manuscript. F. B., E. S., and V. M. S. revised the statistical details of the statistical analysis plan. P. A., S. S., and O. H. are principal investigators and have contributed to study design and coordinated study conduct. M. B. M. is responsible for the eCRF. A. R., P. A., M. B. M., O. H., S. S., J. T., S. A., Y. K., M. N., and G. H. have contributed to patient inclusion and data collection. All authors contributed to the writing of this manuscript and approved the final version.

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APPENDIX 1

INFECT STUDY GROUP

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