Risk factors and Predictors of Mortality in Streptococcal Necrotizing Soft-Tissue Infections: A Multicenter Prospective Study

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Summary: This prospective study of streptococcal necrotizing soft-tissue infections comprised 126 *Streptococcus pyogenes* (GAS) cases and 27 *Streptococcus dysgalactiae* cases. Among the GAS cases, several factors were associated with mortality, including age, septic shock and no administration of intravenous immunoglobulin.

Abstract

Background: Necrotizing soft-tissue infections (NSTI) are life-threatening conditions often caused by β-hemolytic streptococci, group A streptococcus (GAS) in particular. Optimal treatment is contentious. The INFECT cohort includes the largest set of prospectively enrolled streptococcal NSTI cases to date.

Methods: From the INFECT cohort of 409 adults admitted with NSTI to five clinical centers in Scandinavia, patients culture-positive for GAS or *Streptococcus dysgalactiae* (SD) were selected. Risk factors were identified by comparison with a cohort of non-necrotizing streptococcal cellulitis. The impact of baseline factors and treatment on 90-day mortality was explored using Lasso regression. Whole-genome sequencing of bacterial isolates was used for *emm* typing and virulence gene profiling.

Results: The 126 GAS NSTI cases and 27 cases caused by SD constituted 31% and 7% of the whole NSTI cohort, respectively. When comparing to non-necrotizing streptococcal cellulitis, streptococcal NSTI was associated to blunt trauma, absence of pre-existing skin lesions, and a lower BMI. Septic shock was significantly more frequent in GAS (65%) compared to SD (41%) and polymicrobial, non-streptococcal NSTI (46%). Age, male sex, septic shock, and no administration of intravenous immunoglobulin (IVIG) were among factors associated with 90-day mortality. Predominant *emm* types were *emm1*, *emm3* and *emm28* in GAS and *stG62647* in SD.

Conclusions: Streptococcal NSTI was associated with several risk factors, including blunt trauma. Septic shock was more frequent in NSTI caused by GAS than in cases due to SD. Factors associated with mortality in GAS NSTI included age, septic shock and no administration of IVIG.

Key words: Streptococcus pyogenes; Group A streptococcus; Streptococcus dysgalactiae; Necrotizing fasciitis; Intravenous immunoglobulin G.

INTRODUCTION

Necrotizing fasciitis and other necrotizing soft-tissue infections (NSTI) are rare, devastating conditions causing destruction primarily of subcutaneous tissue and/or muscle [1-3]. Historically, mortality is high, although a slight reduction has been reported [4]. Delayed diagnosis and controversies regarding optimal treatment are still major challenges and barriers towards improved survival [1-3]. The etiology is often polymicrobial, but since the upsurge of invasive *Streptococcus pyogenes* (group A streptococcus; GAS) infection in the industrialized world in the 1980s and 1990s, this pathogen has been a particularly feared cause of NSTI. Cases also among healthy individuals and high rates of shock, need for intensive care, and death were features highlighted by case series and surveillance studies at that time [5-9]. More recent surveillance studies on invasive GAS disease largely confirm this pattern [10, 11]. In addition, some reports have shown that *Streptococcus dysgalactiae* (SD), a species closely related to GAS and an emerging cause of invasive infections, also have the ability to cause NSTI [12, 13]. However, apart from two retrospective single-center studies, contemporary detailed data on streptococcal NSTI are scarce [13, 14]. Risk factors for streptococcal NSTI compared to more superficial streptococcal disease are still largely unknown. The predictive power of systemic toxicity and local findings in discerning streptococcal NSTI from non-streptococcal cases is also uncertain. Furthermore, treatment controversies persist due to lack of randomized trials. Apart from early surgery and clindamycin treatment, which are measures strongly suggested by several observational studies, the optimal treatment of streptococcal NSTI is debated [1-3]. In particular, the role of intravenous polyspecific immunoglobulin G (IVIG) is controversial.

The present study is part of the INFECT project which was initiated to advance the understanding of the pathophysiological mechanisms, diagnosis and prognosis of NSTI [15]. The INFECT cohort, which consists of NSTI cases prospectively enrolled at five different Scandinavian hospitals, includes the largest clinical cohort of streptococcal NSTI cases to date. The purpose of focusing on the streptococcal cases of the cohort is to provide a comprehensive overview of the characteristics of NSTI unique to GAS and SD and explore the impact on mortality of baseline factors, early clindamycin treatment, early surgery, and IVIG.

METHODS

Study design, Sites and Participants

The INFECT study is a multicenter, prospective observational cohort study registered at ClinicalTrials.gov (NCT01790698). Design, study sites, inclusion criteria, and ethics of the INFECT cohort are previously described [16]. In short, patients (age ≥ 18 years) with NSTI confirmed by peroperative signs of wide destruction spreading along tissue planes, were enrolled in five Scandinavian referral centers for NSTI between February 2013 and June 2017. The method for assignment of microbial causes in each case of the cohort is described in the Supplementary Methods. The β-hemolytic streptococcal cohort was defined by cultures positive for GAS or SD in blood or tissue samples obtained before or within 48 hours of diagnosis. To identify factors predisposing to NSTI, the streptococcal NSTI cases were compared with a previously described Norwegian cohort of prospectively enrolled non-necrotizing cellulitis cases with GAS or SD etiology confirmed by serology and/or culture [17]. Lastly, patients with streptococcal NSTI were compared to patients with polymicrobial, non-streptococcal NSTI.

Clinical parameters

The clinical and laboratory parameters collected are described elsewhere [16]. Data were registered prospectively using an electronic case report form and included demographics, comorbidities, preceding trauma or surgery, preoperative findings, antibiotic treatment, surgery, supportive care, hyperbaric oxygen therapy (HBOT), IVIG therapy, and details on outcome. Systemic severity was measured at inclusion using Simplified Acute Physiology Score (SAPS) II, Sequential Organ Failure Assessment (SOFA) score and registering the absence or presence of septic shock. The most abnormal biochemistry values obtained prior to the first surgery were also registered, including measurements performed at referring hospitals. The Laboratory Risk Indicator for Necrotizing Fasciitis (LRINEC) score, a laboratory based diagnostic tool for necrotizing fasciitis, was calculated based on preoperative measurements [18].

Microbiology

Identification of the bacteria and resistance testing was performed as part of the routine diagnostic service at each hospital. Streptococcal species identification was confirmed on all

stored isolates by whole-genome-sequencing (WGS). Details on WGS and our use in *emm*-typing, MLST-typing and screening for virulence genes are provided in the Supplementary Methods. The genomic sequences were deposited in the European Nucleotide Archive (ENA database) under the BioProject PRJNA524111.

Statistical analyses

Categorical data were analyzed using the χ^2 or Fisher's exact test. Continuous data were compared using the Mann–Whitney U test. All reported statistical tests were 2-sided, and *P* values <.05 were considered statistically significant. A multivariate model of predictors of mortality was developed for GAS cases only, as the SD cases were considered to include a more heterogeneous, less clearly defined population. Factors previously found to be associated with death were included, representing predisposing conditions, acute disease severity and major treatment modalities. HBOT is in general associated with a high risk of selection bias primarily due to limited accessability. In this study, all study hospitals were referral centers for NSTI with HBOT available. Many patients were probably transferred in order to have this treatment option. Decisions not to provide HBOT to patients in these settings probably involved strong selection mechanisms related to low or high severity. HBOT was therefore included in the multivariate model primarily to control for confounding. Logistic lasso regression was used, due to a high number of independent variables compared to the number of deaths, and the accompanying risk of severe overfitting when using ordinary logistic models [19]. As the risk factors for streptococcal NSTI compared to cellulitis are largely undocumented, an explorative approach was used to include covariates in the adjusted logistic regression model of risk factors, selecting covariates with a *P* value < .10 in univariate analysis. Details concerning the statistical methods are provided in the Supplementary Methods.

RESULTS

Microbial etiology

In the cohort of 409 NSTI patients there were 126 cases with GAS and 27 with SD, in total 37% of the cohort (Supplementary Figure 1). In addition, one case with *S. equi* [20] and 15 cases with *S. agalactiae* (Group B streptococcus; GBS) were identified. *S. aureus* was found together with GAS in eight cases and with SD in a single case. Bacteremia was significantly

more common in GAS than in SD cases (56% (69/126) vs. 30% (8/27); P= 0.014). Relevant antimicrobial resistance was rare (Supplementary Table 1).

Risk factors

Comorbidities and other possible risk factors were more common among SD compared to GAS cases, malignancy and blunt trauma in particular (Table 1). Among GAS cases, more than a third was without known comorbidity. In total, 57% (87/153) of GAS and SD cases had no preceding event in the form of surgery, trauma, wound, chronic skin disease or intravenous drug use. When compared to non-necrotizing streptococcal cellulitis, streptococcal NSTI was associated to a history of blunt trauma, absence of chronic wound and skin disease, and a lower BMI (Table 2).

Clinical features

As shown in Table 3, septic shock was more common in GAS disease, including the 12 cases involving co-pathogens of which 9 had septic shock. Among registered preoperative findings, severe pain was prevalent in both GAS and SD cases, but more than half of the patients did not receive opioid drugs before their first surgery. A LRINEC score below 6, previously associated with low risk of NSTI [18], was found in 15% (19/128) of the GAS and SD cases overall.

Selected clinical features of GAS NSTI were compared to polymicrobial non-streptococcal NSTI. Among polymicrobial NSTI cases without β-hemolytic streptococci, general comorbidity (as defined in Table 1) was more frequent than in GAS cases (77% (128/166) vs. 63% (79/126); P = .007), but the age distribution was similar (median 61 vs. 60 years; P = .28). The rate of septic shock in the polymicrobial cases was 46% (77/166) compared to 65% (82/126) for GAS (P = .001), and median SOFA score day 1 was 8 vs. 9 (P < .005). Regarding bacteremia, the proportions were 23% (38/166) for polymicrobial and 56% (70/126) for GAS cases (P < .005). A post hoc analysis of time between hospital admission and first surgery showed earlier surgery for the GAS cases (median 16 vs. 18 hours, P= 0.013).

Streptococcal virulence profiles

Among 82 available GAS isolates, the predominant *emm* type *was emm1* (Figure 1). In total *emm1*, *emm3* and *emm28* comprised 72 %. In SD, *stG62647* was the predominant *emm* type among 11 available SD isolates. Virulence gene profiles of GAS were closely linked to *emm* types (Supplementary Table 2). In a few isolates, the recently characterized superantigen

genes *SpeQ* and *speR* were identified [21]. In SD isolates, many had *speG*, but no other superantigen genes were detected.

Treatment, outcome and factors associated with mortality

Eighty-nine percent (136/153) of the GAS and SD cases were transferred from other hospitals. The great majority (96%) of the cases received standard antibiotic treatment with a betalactam antibiotic and clindamycin (Table 3). Three-quarters of the GAS cases received IVIG and a similar proportion was treated with HBOT.

The frequency of amputations was 15% and particularly low among the SD cases (Table 3). The 90 day case-fatality rate of 10% for GAS was significantly lower compared to polymicrobial non-streptococcal cases (20%; P = .02). Lasso regression analysis of the GAS cases identified increased age (odds ratio (OR) 1.15, per year), male sex (OR 5.09), septic shock (OR 1.96) and IVIG not administered (OR 3.15) as independent variables associated with mortality (Table 4). The variable with the highest odds ratio was HBOT, but this was included in the model to control for confounding (see Methods section). The area under the curve (AUC) of the Lasso model was 0.97 after correcting for optimism. Due to the possible effect of IVIG on mortality, the groups receiving IVIG or no IVIG were compared in a post-hoc analysis (Supplementary Table 3). No clear differences regarding severity or treatment that had not been adjusted for in the Lasso model were found, except more pain, fewer with mechanical ventilation, and longer intervals between surgeries in the non-IVIG group. These three parameters were not associated to mortality in an exploratory, univariate analysis in survivors vs. non-survivors (Supplementary Table 4). Time to first surgery or clindamycin not given prior to admittance to the study hospital were not associated to increased 90-day mortality either, as opposed to high age, high SAPS and SOFA scores, and late clinical NSTI signs. No clear patterns relating *emm* types or other virulence genes to septic shock or mortality were observed (Figure 1, Supplementary Figures 2 and 3).

DISCUSSION

In this study, the largest prospectively enrolled cohort of streptococcal NSTI to date is presented. The data provide a contemporary and detailed picture of risk factors, severity and specific features of streptococcal NSTI. Important predictors of mortality are described.

GAS outnumbered SD by a factor of 4.7, and by 7.6 for the monomicrobial cases. Still, this study confirms that SD plays a significant role as a cause of NSTI. The rigorous procedure applied for review of all microbiology results, aiming at avoiding inclusion of probable contaminants and colonizers, may explain why the proportion of polymicrobial cases were lower than in some other studies.

Possible predisposing factors were frequent, particularly in NSTI caused by SD, but the ability of GAS to cause NSTI in previously healthy individuals was confirmed. Obesity has previously been identified as a risk factor for invasive GAS infection [22]. It was common also in this cohort, but it was even more frequent in streptococcal cellulitis and therefore not a risk factor specific to NSTI. Skin disease, antecedent surgery or other wounds were rare, confirming streptococcal NSTI as a disease that often occurs without an obvious portal of entry [2]. Blunt trauma is recognized as an important preceding event in many streptococcal NSTI cases [2]. Our study affirms this association, by comparing with cellulitis.

Detailed data on clinical severity, including frequency of septic shock, are scarce, and the accuracy of such data from surveillance studies has been questioned [11]. The comparison of GAS and SD NSTI was limited by the number of SD cases, but when comparing with polymicrobial non-streptococcal NSTI, GAS NSTI clearly stands out with high rates of septic shock, organ failure and bacteremia. This accords with major differences in the pathogenesis of streptococcal versus polymicrobial infections, as recently demonstrated in a comparative, molecular study of selected cases from the INFECT cohort [23]. Septic shock was more frequent with higher age rather than younger, contrary to studies of invasive GAS linking Streptococcal Toxic Shock Syndrome (STSS) and younger age[9, 11, 24]. Lower mortality rate in GAS cases compared to SD and polymicrobial cases could be related to less comorbidity, as previously discussed [16]. Our finding that some comorbidities were inversely related to mortality illustrate the complexity of the host-pathogen-relationship, however. Earlier diagnosis and more aggressive management may have contributed to lower mortality in GAS disease, as suggested by the post hoc analysis revealing earlier surgery for this group. Systemic severity and the substantial number of patients that were previously healthy highlight the particular virulence potential of GAS. In addition to the strong

association of streptococcal NSTI and extremity location [16], these characteristics may also give diagnostic clues on probable etiology even before results of any rapid test is available. With respect to the LRINEC score, it had suboptimal sensitivity among the streptococcal cases, as shown also for the whole cohort [16]. This underscores the questionable value of this score in our setting, in line with a recent systematic review [25].

The observed predominance of *emm*1 accords with a rise in this *emm* type in Scandinavia [26, 27]. Even though several other *emm* types where identified, the fact that more than 70% of the isolates belonged to only three *emm* types add to the evidence that some *emm* types have a particular ability to cause severe infections [11, 28, 29]. In SD, *stG62747* was the predominant *emm* type, in accordance with a previous report from our region [30]. The virulence gene profiles of GAS largely reflected the *emm* type distribution, with a high proportion with *speA* and *speG* consistent with the predominance of *emm1* and *emm3* types. We did not find any clear association of these genes or *emm* types with severity, however, but the numbers for most *emm* types and virulence gene profiles were low.

As expected, systemic disease severity had an impact on mortality. Not receiving clindamycin before inclusion or undergoing surgery later than after 24 hours after admission was not associated with increased mortality in this cohort, possibly reflecting rapid identification and initiation of appropriate treatment for the most severely sick patients, as indicated by shorter time to surgery among patients with shock (Supplementary Table 3) and the relatively low mortality for GAS NSTI overall (10 % at 30 days). Mortality was lower in IVIG-treated patients, and it was also associated with lower odds of death in the adjusted model. Previous observational studies addressing this issue have shown conflicting results. Several observational studies suggest an effect of IVIG in STSS [31-34], whereas others do not [35, 36]. The only randomized trial of IVIG in STSS was prematurely terminated due to low inclusion, but a favorable, non-significant effect was observed [37]. Some of the patients in our study were co-enrolled in a recent randomized trial of IVIG in NSTI of all microbiological etiologies; in which no effect of IVIG was found on physical quality of life [38]. Possibly, the lack of effect in the latter study, as well as in the large observational study by Kadri et al [36], was the consequence of not restricting these studies to streptococcal NSTI. A propensity to offer IVIG as a last resort might also obscure beneficial effects. However, a recent meta-analysis of clindamycin-treated STSS patients from one randomized and four non-randomized studies found that IVIG was significantly associated with increased survival [39]. Our study suggests such an association also in GAS NSTI. All the observational studies performed are vulnerable to confounding, however. This includes our own study, but

interestingly, the report by Bergsten et al (revised manuscript also submitted to CID) demonstrated a dose-related toxin inhibition in plasma of our patients, providing a mechanistic correlate that also support a possible benefit of IVIG. Taken together, these studies encourage renewed efforts to conduct a sufficiently powered randomized clinical trial restricted to GAS NSTI.

The major strength of the study is its size and the prospective, multicenter design. The study was able to accurately stratify patients according to a wide range of clinical parameters, more so than retrospective studies and surveillance studies. However, since all study centers are academic referral hospitals for NSTI that offer HBOT, and transferred patients constituted the majority of cases, the present patient cohort may not represent streptococcal NSTI of all degrees of severity. Some patient categories are probably often not transferred, primarily those without a fair chance of survival as well as stable patients with smaller lesions. When analyzing factors associated to mortality, it is difficult to fully adjust for the lower propensity to provide costly treatment to patients with very high or low chance of survival. Furthermore, clinical details and the exact timing of initial treatment at the primary hospital were not registered, and incidence could not be calculated. In the study of risk factors there were differences regarding inclusion periods, study sites and microbiological methods for the cellulitis and NSTI cohorts, but the analyzed populations were well matched. The exploratory parts of the study, with many univariate comparisons between different groups and no correction for multiple testing, demands caution when interpreting low p-values.

In summary, this large prospective multicenter cohort study supports blunt trauma as an important risk factor for streptococcal NSTI. A high frequency of septic shock was observed, particularly in GAS disease. IVIG was among several factors associated with increased survival.

NOTES

Author contributions. ANT is project coordinator of the INFECT study. TB and SS conceived the streptococcal part of the study. TB drafted the first manuscript. OH, SS, PA, YK, and MN are national investigators and have contributed to study design and coordinated study conduct. MBM is responsible for the database and contributed to patient inclusion and data collection, as did TB, ER, MBM, PA, YK, MN, OO and SS. OO, AB and AI performed streptococcal typing and virulence factor screening. TB, ER, MBM, OO, FB, OH, ANT and SS contributed to analysis or interpretation of data or both. All authors contributed to the writing of this manuscript and approved the final version.

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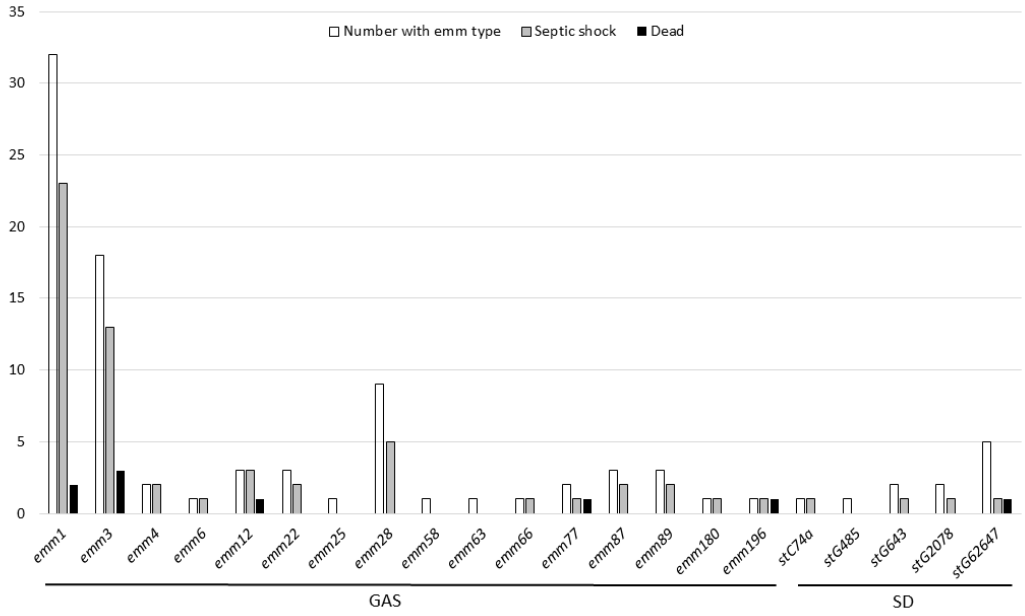
Figure legend

Figure 1. *emm* type distribution with rates of septic shock and 90-day mortality in 82 GAS and 11 SD cases of necrotizing soft-tissue infection. Forty-four GAS isolates and 16 SD isolates were not available for analysis.



Abbreviations: GAS, group A streptococcus; SD, *Streptococcus dysgalactiae*.

Table 1. Demographics and pre-existing factors in NSTI caused by GAS or SD

	GAS N=126	SD N=27	*P* value
Age (years)	60 (46-69)	63 (52-71)	0.183
Male sex	67 (53)	18 (67)	0.200
BMI[a] (kg/m^2)	26 (23-31)	28 (24-34)	0.239
Currently smoking[b]	25 (24)	7 (30)	0.491
Excessive Alcohol intake[c]	13 (14)	5 (23)	0.333
Comorbidities			
Active malignancy	8 (6)	6 (22)	**0.019**
Peripheral vascular disease	5 (4)	2 (7)	0.607
Cardiovascular disease[d]	48 (38)	13 (48)	0.333
Immunodeficiency/ immunosuppression[e]	25 (20)	4 (15)	0.545
No general comorbidity[f]	47 (37)	6 (22)	0.135
Preceding events or skin breaches[g]			
Blunt trauma[h]	17 (14)	9 (33)	**0.022**
Penetrating trauma[h]	12 (10)	2 (7)	1.000
Surgery[h]	5 (4)	4 (15)	0.052
Chronic wound or skin disease	15 (12)	2 (7)	0.739
Intravenous drug use	3 (2)	1 (4)	0.544
None of the factors listed above	75 (60)	12 (44)	0.151

Data are presented as median (inter-quartile range) or No./No. evaluated. (%).
Boldface indicates statistical significance (*P* < .05).
Abbreviations: NSTI, necrotizing soft tissue infection; GAS, Group A streptococcus; SD, *Streptococcus dysgalactiae*; BMI, Body mass index.
[a]Data were missing for 3 patients. Obesity (BMI ≥ 30) was registered in 29% (44/150) overall.
[b]Data were missing for 20 GAS and 4 SD patients.
[c]Defined as >14 units of alcohol/week for women and >21 units/week for men. Data were missing for 33 GAS and 5 SD patients.
[d]Includes, but is not limited to, hypertension, myocardial infarction, angina pectoris, heart failure, apoplexia.
[e]Innate immunodeficiencies, HIV, use of steroids or other immunosuppressant drugs, other acquired immunodeficiencies.
[f]None of the following: Active malignancy, chronic obstructive pulmonary disease or astma, current or previous cardiovascular disease, diabetes mellitus, chronic kidney failure, chronic liver disease, rheumatoid disease, Immunodeficiency/ immunosuppression.
[g]No cases of antecedent varicella was registered
[h]In a period of 4 weeks before NSTI diagnosis

Table 2. Risk factors in streptococcal NSTI vs. streptococcal cellulitis[a]

			Univariate model			Adjusted model[b] (n=192)		
	NSTI[c]	Cellulitis[c]	OR	95% CI	P value	OR	95% CI	P value
Age (years)	42 (38-60)	54 (43-67)	1.008	(0.989, 1.026)	0.410			
Sex (m)	32/66 (49)	92/146 (63)	0.552	(0.307, 0.995)	**0.047**	0.794	(0.344, 1.833)	0.590
BMI	26 (23-30)	28 (25-34)	0.933[d]	(0.886, 0.982)	**0.008**	0.921	(0.851, 0.996)	**0.027**
Currently smoking	12/61 (20)	25/146 (17)	1.185	(0.552, 2.545)	0.663			
Excessive alcohol intake[e]	7/50 (14)	7/144 (5)	3.186	(1.058, 9.593)	0.052	3.919	(0.987, 15.568)	0.058
Active malignancy	3/66 (5)	13/146 (9)	0.487	(0.134, 1.771)	0.401			
Peripheral vascular disease	1/66 (2)	7/146 (5)	0.305	(0.037, 2.535)	0.440			
Cardiovascular disease[f]	26/66 (39)	60/146 (41)	0.932	(0.515, 1.687)	0.815			
Immunodeficiency/ immunosuppression[g]	10/66 (15)	12/146 (8)	1.994	(0.815, 4.881)	0.125			
Any comorbidity[h]	41/66 (62)	90/146 (62)	1.020	(0.561, 1.857)	0.947			
Blunt trauma[i]	11/66 (17)	6/146 (4)	4.667	(1.645, 13.236)	**0.002**	5.489	(1.295, 23.269)	**0.016**
Penetrating trauma[i]	8/66 (12)	20/146 (14)	0.869	(0.362, 2.089)	0.753			
Chronic wound or skin disease	10/66 (15)	60/146 (41)	0.256	(0.121, 0.541)	**<0.005**	0.377	(0.142, 1.006)	**0.041**
Intravenous drug use	0/66 (0)	4/144 (3)	0.680	(0.059, 4.913)	0.311			

Boldface indicates statistical significance ($P < .05$).

Abbreviations: NSTI, necrotizing soft tissue infection; BMI, Body mass index; OR, odds ratio; CI, confidence interval.

[a]NSTI caused by *Streptococcus pyogenes* or *Streptococcus dysgalactiae* vs. cellulitis with these microbes confirmed by serology (anti-streptolysin O or anti-DNAse B) or culture in blood or normally sterile tissue. See ref. [17] for criteria. To analyze an NSTI population comparable with the cellulitis cohort, the following NSTI cases were excluded: polymicrobial cases with gram-negative or anaerobic bacteria, postoperative cases (cases with surgery the previous 4 weeks), and cases from Denmark (due to a substantially higher background level of alcohol consumption).

[b]Factors with a *P* value below 0.10 in the univariate analyses (performed also for chronic liver disease and diabetes mellitus) were included in the multivariable model. Adjustment for infection site was performed. A total of 49 NSTI patients and 143 cellulitis controls were included in the adjusted analysis.

[c]Data are presented as median (inter-quartile range) or No./No. evaluated. (%).

[d]Data were missing for 1 NSTI and 1 cellulitis patient.

[e]Defined as >14 units/week of alcohol for women and >21/week for men.

[f]Includes hypertension and peripheral vascular disease

[g]Innate immunodeficiencies, HIV, use of steroids or other immunosuppressant drugs, other acquired immunodeficiencies.

[h]None of the following: Active malignancy, chronic obstructive pulmonary disease or asthma, current or previous cardiovascular disease, diabetes mellitus, chronic kidney failure, chronic liver disease, rheumatoid disease, immunodeficiency/ immunosuppression

[i]In a period of 4 weeks before diagnosis

Table 3. Clinical features, treatment and outcome in NSTI caused by GAS or SD

	GAS N=126	SD N=27	*P* value
Preoperative symptoms/signs			
Pain treated with opioids	55/122 (45)	10/24 (42)	0.758
Skin bullae	47/124 (38)	6/26 (23)	0.150
Purple/black skin discoloration	47/125 (38)	12/26 (46)	0.416
Skin bruising	78/124 (63)	15/25 (60)	0.785
Skin anaesthesia	6/94 (6)	1/23 (4)	1.000
Preoperative biochemistry[a]			
CRP (mg/L)[b]	295 (193-370)	261 (148-339)	0.131
Leukocytes (x 10^9/L)[c]	14.2 (8.2-22.2)	16.9 (11.2-23.7)	0.393
Creatinine (µM)[d]	178 (109-266)	103 (73-181)	**0.001**
Severity			
Septic shock[e]	82/126 (65)	11/27 (41)	**0.019**
SAPS II (0-163)[f]	44 (34-57)	44 (36-58)	0.758
SOFA score day 1 (0-20)[g]	9 (7-12)	8 (6-11)	0.129
LRINEC score (0-13)[h]	8 (7-10)	8 (6-9)	0.148
LRINEC score ≥ 6[h]	90/104 (87)	19/24 (79)	0.352
Muscle affected[i]	83/126 (66)	14/27 (52)	0.170
Antibiotic treatment			
Clindamycin before inclusion[j]	89/126 (71)	23/27 (85)	0.121
Betalactam + clindamycin[k]	122/126 (97)	25/27 (93)	0.286
IVIG[l]	95/126 (75)	16/27 (59)	0.088
Hyperbaric oxygen at any time	94/126 (75)	21/27 (78)	0.729
Mechanical ventilation[k,m]	116/126 (92)	24/27 (89)	0.702
Surgery[n]			
Time from first admission to first surgery (hrs)	16 (6-29)	22 (6-42)	0.242
Time from first admission to admission at referral hospital (hrs)	18 (6-36)	14 (5-31)	0.441
Time between 1st and 2nd surgery (hrs)	11 (6-20)	9 (5-14)	0.165
Number of operations	4 (3-5)	4 (3-5)	0.636
Outcome			
Amputation[n]	20/126 (16)	3/27 (11)	0.809
30-day CFR	13/125 (10)	5/27 (19)	0.320
90-day CFR	13/125 (10)	6/27 (22)	0.110

Data are presented as median ((inter-quartile range) or No./No. evaluated (%). Boldface indicates statistical significance ($P < .05$).

Abbreviations: NSTI, necrotizing soft tissue infection; GAS, Group A streptococcus, *Streptococcus pyogenes*; SD, *Streptococcus dysgalactiae*; SAPS, simplified acute physiology score. SOFA, sequential organ failure assessment; LRINEC, Laboratory risk indicator for necrotizing fasciitis; IVIG, intravenous polyspesific immunoglobulin G; CFR, case-fatality-rate.

[a]Highest values observed.

[b]Data missing for 5 patients.

[c]Data missing for 7 patients.

[d]Data missing for 9 patients.

[e]Defined by use of vasopressor or inotropic agents and lactate > 2 mmol/l.

[f]SAPS II is calculated the first 24 hours in the Intensive care unit/high-dependency unit from 17 variables; scores range from 0 to 163, with higher scores indicating more severe disease. Data were missing for 16 patients.

[g]SOFA score includes sub scores ranging from 0 to 4 for each of five components (circulation, lungs, liver, kidneys, and coagulation). Aggregated scores range from 0 to 20, with higher scores indicating more severe organ failure. The scoring was modified because cerebral failure was not assessed. Data were missing for 3 patients.

[h]Data were missing for 25 patients.

[i]Assessed during surgery in the primary hospital or during first 7 days of stay at intensive care unit or high-dependency unit at specialized hospital

[j]Clindamycin given before admission to intensive care unit/high-dependency unit of the study hospital.

[k]During first 7 days of stay at intensive care unit or high-dependency unit

[l]Any dosage. The median number of doses among those receiving IVIG was 3 (inter-quartile range 2-3).

[m]Intubated patient or continuous non-invasive ventilation

[n]At primary hospital or during first 7 days of stay at intensive care unit or high-dependency unit at study hospital. Any body part.

Table 4. Factors associated with 90-day mortality in NSTI caused by GAS

	Univariate model (*n* = 125)		Adjusted model[a] (*n* = 125)		Lasso regression[b] (*n* = 125)
Characteristic	OR	*P*-value	OR	*P*-value	OR
Age (years)	1.06	0.015	1.32	<0.0005	1.15
Sex (male)	3.33	0.066	145	0.002	5.09
CVD	0.69	0.765	0.02	0.006	0.22
Active malignancy	0.89	1.000	<0.01	0.008	0.04
Septic shock[c]	1.93	0.541	25.7	0.048	1.96
Initial surgery >24 hours after admission[d]	0.33	0.216	0.30	0.422	0.95
No clindamycin before inclusion[e]	0.40	0.342	0.06	0.166	0.36
IVIG not given	2.98	0.086	7.60	0.125	3.15
HBOT not given	23.81	<0.0005	1220	<0.0005	78.80

Abbreviations: NSTI, necrotizing soft tissue infection; GAS, Group A streptococcus, *Streptococcus pyogenes*; OR, odds ratio; CVD, Cardiovascular disease (including hypertension and peripheral vascular disease); IVIG, treatment with polyspecific immunoglobulin G (any dose); HBOT, hyperbaric oxygen treatment.

[a]Logistic regression analysis

[b]Lasso regression is a shrinkage method, which gives us a more reliable model by shrinking the coefficient estimates (compared to the logistic model). Variables with little or no predictive value will be shrunken to zero (an odds ratio of 1). P values or confidence intervals can not be calculated (see also Supplementary Methods)

[c]Septic shock the first 24 hours after admission to intensive care unit/high-dependency unit, defined as lactate >2 mmol/l and use of vasopressor or inotrope.

[d]Initial surgery performed > 24 hours after the first admission to hospital.

[e]Clindamycin not given before admission to intensive care unit/high-dependency unit of the study hospital.