

Clinical Characteristics and Histopathology in Suspected Necrotizing Soft Tissue Infections

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Background. Necrotizing soft tissue infections (NSTIs) are severe diseases with high morbidity and mortality. The diagnosis is challenging. Several guidelines recommend tissue biopsies as an adjunct diagnostic in routine management, but neither biopsy sampling nor classification is standardized or validated. We studied the quality of tissue biopsy examination as part of routine diagnostics in NSTIs.

Methods. This was a retrospective cohort study of adult patients undergoing surgery due to suspected NSTIs in which tissue biopsy was taken as part of routine management. Clinical data were reviewed. The biopsies were evaluated according to a proposed histopathologic classification system and independently assessed by 2 pathologists. Interrater reliability and diagnostic accuracy were determined.

Results. Tissue biopsies from 75 patients were examined, 55 NSTIs and 20 non-NSTIs cases. The cohorts were similar in clinical characteristics. Interrater reliability for histopathologic staging was moderate (0.53) and fair (0.37) for diagnosis. The sensitivity of histologic diagnosis was 75% and the specificity 80%. The positive predictive value was 91% and the negative predictive value 53%. Necrotizing Infection Clinical Composite Endpoint (NICCE) success was associated with a more severe histological stage, achieved by 42% and 71% of the cases in stage 1 and 2, respectively ($P = .046$).

Conclusions. Our findings suggest that tissue biopsies have low clinical accuracy. The interrater reliability among experienced pathologists is only fair to moderate. A histopathologically more severe stage was associated with favorable outcome. These findings discourage the use of histopathologic evaluation as part of contemporary management of patients with suspected NSTI.

Keywords. histopathology; necrotizing soft tissue infections; prognosis; rapid diagnostic tool.

Necrotizing soft tissue infections (NSTIs) are rapidly spreading infections with severe morbidity and high mortality. NSTIs are heterogeneous and no set of diagnostic criteria has reached consensus [1–4]. Still, the diagnosis is based on clinical presentation and findings upon surgical exploration. Time to surgery and source control with debridement of all necrotic and infected tissue is associated with patient outcome [5]. At the same time, delayed diagnosis is a major challenge, as the clinical presentation can be vague, especially in early phases. Due to low incidence of NSTIs, few clinicians have extensive experience with the condition.

Common early symptoms and findings include localized edema, pain, and bruising of the skin, accompanied by general symptoms of infection [6]. In the largest study of NSTIs with a prospective patient enrollment, severe and out-of-proportion pain was restricted to half of the patients [7]. Approximately 50% of the cases have septic shock [7, 8]. Laboratory tests do not distinguish NSTI well from nonnecrotizing soft tissue infections (non-NSTIs). The Laboratory Risk Indicator for Necrotizing Fasciitis (LRINEC) score and other biomarker-based predictive models have been proposed, but external studies with a prospective enrollment of patients have yet to validate these [9–13]. New and promising biomarkers are identified recently, but none is externally validated or available in routine laboratories [14–16]. Ultrasonography, computed tomography, and magnetic resonance imaging may aid in discriminating NSTIs from non-NSTIs, but the specificity is limited and if used, they must not delay surgical intervention [4, 9].

The value of tissue biopsy as a useful adjunct in establishing an early, accurate diagnosis of NSTI (infectious gangrene) was first described by Stamenkovic and Lew in 1984 [17]. Since then, several experts and guidelines have suggested biopsy as a tool for establishing an NSTI diagnosis [2, 3, 12, 18, 19]. Nonetheless the evidence base is limited, as few studies have

Received 13 July 2022; editorial decision 19 October 2022; published online 3 November 2022

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been performed and with a low number of patients [5, 12, 18–22]. A histopathologic scoring system was proposed by Bakleh et al for prognostic use [21]. However, to date there is no standardized and validated procedures for biopsy sampling or clinical scoring systems for histopathology in the diagnostic management of NSTI. To explore the putative role of histopathologic sections in diagnosing NSTI, we studied patients with suspected NSTI, and compared surgically confirmed cases to cases with suspected NSTI, categorized as nonnecrotizing infections following surgical exploration. Additionally, we aimed to evaluate the sensitivity and specificity of histological diagnosis and to study the interrater reliability of histopathologic diagnosis, staging, and other findings. Last, we wanted to investigate associations between the histopathologic findings and patient outcomes.

METHODS

Study Design, Participants, and Clinical Parameters

Haukeland University Hospital (HUH) is a referral hospital in Health Region West in Norway. We conducted a single-center, retrospective cohort study of adult patients aged ≥ 16 years, admitted or transferred to HUH due to suspected NSTI, where tissue biopsies were obtained as a part of patient management. From 2011 to 2017, tissue biopsies were collected in 77 nonconsecutive cases undergoing surgery due to suspected NSTI. The biopsies were formalin-fixed, paraffin-embedded, sectioned, and stained with hematoxylin-eosin before examination by a pathologist as part of routine laboratory services. In this study, the biopsies were additionally stained with Gram and independently reevaluated by 2 experienced pathologists, blinded to the original diagnosis, and categorized according to the histopathologic classification scheme developed by Bakleh et al [21]. The biopsies were also reevaluated for diagnosis and for common histopathologic findings associated with NSTI as described by Solomon et al [23]. In 46 cases, the clinical variables were collected from a study database (the Improving Outcome of Necrotizing Fasciitis: Elucidation of Complex Host and Pathogen Signatures that Dictate Severity of Tissue Infection [INFECT] study [24]), and in the remaining cases through the electronic patient records.

NSTI was defined as peroperative findings of infection, spreading in any soft tissue layer (skin, subcutaneous tissue, superficial fascia, deep fascia, or muscles) with necrosis of the layer(s) involved, and hence requiring surgical debridement, according to Sartelli et al [4]. The definition encompasses different soft tissue infection categories such as necrotizing fasciitis, necrotizing cellulitis, necrotizing pyomyositis, infectious myositis, and gas gangrene. Non-NSTI was defined as pure abscesses, erysipelas, cellulitis, infected fistulas, and chronic dermatosis. The surgical variables and diagnosis were evaluated by an experienced plastic surgeon reviewing all surgical reports, and the surgical diagnosis

Table 1. Demographics of Patients With Necrotizing and Nonnecrotizing Soft Tissue Infections

Characteristic	NSTI (n = 55)	Non-NSTI (n = 20)	All (N = 75)	P Value
Demographics				
Male sex	37 (67)	10 (50)	47 (63)	.17
Age, y	51 (17–90)	49 (24–92)	50 (17–92)	.54
Transferred from another hospital	24 (44)	7 (35)	31 (41)	.50
Duration of symptoms, d	3 (2–21)	2 (0.5–14)	2.5 (0–21)	.12
Smoking	20 (36)	7 (35)	27 (36)	.88
Surgery past 4 wk	5 (9)	1 (5)	6 (8)	.56
Penetrating trauma	14 (26)	4 (20)	18 (24)	.63
Nonpenetrating trauma	8 (15)	2 (10)	10 (13)	.61
Underlying condition				
Diabetes mellitus	9 (16)	1 (5)	10 (13)	.20
Cardiovascular disease ^a	21 (38)	6 (30)	27 (36)	.50
Chronic kidney disease	5 (9)	2 (10)	7 (9)	.90
Chronic skin disease	3 (6)	2 (10)	5 (7)	.48
Hematologic cancer	2 (4)	1 (5)	3 (4)	.79
Metastatic carcinoma	0 (0)	1 (5)	1 (1)	.09
Other malignancy	2 (4)	2 (10)	4 (5)	.28
Immunodeficiency ^b	2 (4)	4 (20)	6 (8)	.02
Alcohol abuse ^c	3 (6)	0 (0)	3 (4)	.32
IDU	4 (7)	1 (5)	5 (7)	.73
1 or more comorbidities ^d	35 (64)	10 (50)	45 (60)	.29

Frequencies are given as No. (%) where percentages are calculated from the total No. in each column. Continuous data are given as median (range) unless otherwise indicated. Boldface indicates statistical significance ($P < .05$).

Abbreviation: IDU, intravenous drug use; Non-NSTI, nonnecrotizing soft tissue infection; NSTI, necrotizing soft tissue infection.

^aCardiovascular disease including hypertension.

^bImmunodeficiency (eg, hypogammaglobulinemia, human immunodeficiency virus) or immunosuppressive treatment including ongoing cytostatic treatment or steroids.

^cDefined as >14 units/week for women and >21 units/week for men.

^dAt least 1 of the comorbidities given above.

was used as a gold standard. The cases that did not fulfill NSTI criteria after evaluation were combined to create a control group. For detailed information about patient inclusion, categorization, and biopsy process, see the Supplementary Material. A definite bacteriological etiology was defined as microbes identified at species level in the Department of Microbiology following positive cultures from normally sterile sites as blood, deep tissue, or peroperative fluids, as detailed by Bruun et al [25]. The overall success rate was calculated using the Necrotizing Infection Clinical Composite Endpoint (NICCE) as proposed by Bulger et al, detailed in the present Supplementary Material [26]. NICCE is developed to overcome limitations of commonly used endpoints, and to compensate for the low number of patients often seen in trials with NSTIs. In this score system, sepsis is defined as infection and increase in modified Sequential Organ Failure Assessment score of 2 points or more, a definition used also in this study [26].

The study was approved by the Regional Ethics Committee of Health Region West of Norway (approval numbers 2010/1406 and 2012/2227).

Table 2. Clinical Characteristics of Patients With Necrotizing and Nonnecrotizing Soft Tissue Infections

Characteristic	NSTI (n = 55)	Non-NSTI (n = 20)	All (N = 75)	P Value
Location				
Head/neck	7 (13)	3 (15)	10 (13)	.80
Upper extremity	14 (26)	7 (35)	21 (28)	.42
Lower extremity	29 (53)	10 (50)	39 (52)	.83
Anogenital	6 (11)	1 (5)	7 (9)	.44
Truncus/abdomen	7 (13)	2 (10)	9 (12)	.75
TBSA%^a				
At admission	3 (0–10)	2 (0.5–10)	3 (0–10)	.21
At first revision	4 (0–20)	2 (0.5–9)	3 (0–20)	.11
Blood culture (positive)	23 (42)	4 (20)	27 (36)	.08
Deep tissue culture (positive)	28 (51)	9 (45)	37 (49)	.65
Peroperative fluid culture (positive)	28 (51)	7 (35)	35 (47)	.22
Causative etiology confirmed ^b	46 (84)	13 (65)	59 (79)	.08
Microbial etiology				
<i>Streptococcus pyogenes</i>	15 (27)	3 (15)	18 (24)	.51
<i>Streptococcus dysgalactiae</i>	10 (18)	1 (5)	11 (15)	.25
<i>Staphylococcus aureus</i>	5 (9)	3 (15)	8 (11)	.26
Gram-negative rods	7 (13)	1 (10)	8 (11)	.48
Obligate anaerobic bacteria	14 (7)	1 (5)	15 (20)	.10
Others	2 (4)	3 (15)	5 (11)	.10
Polymicrobial etiology	20 (36)	5 (25)	25 (33)	.75
Biochemistry/others				
mSOFA at admission	2.0 (0–10)	1.0 (0–5)	1.0 (0–10)	.75
mSOFA worst first 24 h	3 (0–12)	2 (0–7)	2.0 (0–12)	.17
Mean arterial pressure at admission	85 (40–126)	86 (38–113)	85 (38–126)	.70
WBC count ^c	14.7 (1–44)	17.0 (5–37)	14.8 (1–44)	.46
C-reactive protein ^e	217 (1–425)	124 (4–388)	201 (1–425)	.10
Lactate ^e	1.9 (0.7–15)	1.3 (0.3–4.5)	1.7 (0.3–15)	.10

Frequencies are given as No. (%) where percentages are calculated from the total No. in each column. Continuous data are given as median (range) unless otherwise indicated.

Abbreviations: mSOFA, modified Sequential Organ Failure Assessment score; Non-NSTI, nonnecrotizing soft tissue infection; NSTI, necrotizing soft tissue infection; TBSA, total body surface area with external signs of inflammation; WBC, white blood cell.

^aData are given in percentage according to the rule of nines.

^bPositive culture exclusively from normal, sterile environments (eg, blood, deep tissue, or peroperative fluid collection).

^cWBC count $4.1\text{--}9.8 \times 10^9/\text{L}$.

^dC-reactive protein $<5 \text{ mg/L}$.

^eLactate 0.3–1.4 mmol/L. Missing cases: NSTI, n = 24; non-NSTI, n = 7.

Statistical Methods

Data were analyzed using IBM SPSS Statistics for Windows software, version 24.0 (IBM, Armonk, New York). Continuous data are presented as median with interquartile range. The independent sample *t* test was used to analyze normally distributed continuous variables, and the 2-tailed Mann-Whitney *U* test for nonnormally distributed continuous variables and ordinal categorical variables. Categorical data are presented as frequencies with percentages and analyzed using

Table 3. Treatment and Outcome of Patients With Necrotizing and Nonnecrotizing Soft Tissue Infections

Treatment and Outcome	NSTI (n = 55)	Non-NSTI (n = 20)	All (N = 75)	P Value
28-day mortality	5 (9)	1 (5)	6 (8)	.56
Sepsis ^a	35 (64)	11 (55)	46 (61)	.50
Amputation	3 (5)	0 (0)	3 (4)	.29
Vasopressor	30 (55)	8 (40)	38 (51)	.27
Mechanical ventilation	25 (46)	5 (25)	30 (40)	.11
Renal replacement therapy	9 (16)	0 (0)	9 (12)	.05
Hyperbaric oxygen	14 (26)	1 (5)	15 (20)	.05
Immunoglobulins	2 (4)	0 (0)	2 (3)	.39
Clindamycin ^b	43 (78)	16 (80)	59 (79)	.87
Time to surgery, d ^c	3.75 (0.3–28)	2.5 (0.5–14)	3 (0.3–28)	.07
Time to surgery, h ^d	6.4 (0.7–85)	14.1 (2.5–42)	7 (0.7–85)	.11
Admitted ICU	35 (64)	7 (35)	42 (56)	.03
Days at ICU	4.5 (0–50)	0.0 (0–13)	2 (0–50)	.008
Days admitted	21.0 (1–64)	12.0 (4–34)	18 (1–64)	.003
TBSA removed, % ^e	0.22 (0–8)	0 (0)	0 (0–8)	.003
NICCE success	26/47 (55)	

Frequencies are given as No. (%) where percentages are calculated from the total No. in each column. Continuous data are given as median (range) unless otherwise indicated. Boldface indicates statistical significance ($P < .05$).

Abbreviations: ICU, intensive care unit; NICCE, Necrotizing Infection Clinical Composite Endpoint; Non-NSTI, nonnecrotizing soft tissue infection; NSTI, necrotizing soft tissue infection; TBSA, total body surface area.

^aSepsis defined as modified Sequential Organ Failure Assessment score ≥ 2 .

^bClindamycin as part of primary treatment.

^cTime to surgery from symptom debut.

^dTime to surgery from admission.

^eData given in percentage according to the rule of nines. Removed subcutaneous tissue or muscles not included.

χ^2 . Associations between preoperative cutaneous clinical findings and peroperative findings, and between histopathology and clinical outcomes were calculated with odds ratios (ORs). Cohen kappa (κ) was used to estimate interrater reliability in histopathologic staging and findings [27]. To estimate the accuracy of histopathologic diagnosis, sensitivity, specificity, and positive and negative likelihood ratios were calculated. For all analyses, a 2-sided *P* value $< .05$ was considered statistically significant. The figure was made using Matlab software (The MathWorks, Natick, Massachusetts).

RESULTS

Patient Characteristics and Outcome

A total of 77 patients were evaluated, and 75 were included. Two patients were excluded: In 1 case, only cytology was available, and in 1 case, the final diagnosis was pyoderma gangrenosum. Baseline demographics are presented in Table 1. NSTIs accounted for 73% and 27% were non-NSTIs, using surgical definition as a gold standard. Comorbidity was more common among NSTI cases, but immunocompromised state was the only condition demonstrating a significant difference between

the groups, although more frequent in the non-NSTI group (20% vs 4%). Clinical characteristics are presented in [Table 2](#). The lower extremity was the most commonly affected site in both groups, with 53% in the NSTI category and 50% in the non-NSTI category. Causative microbiological etiology was identified in 79% of the cases. Polymicrobial infection (33%) and monobacillary *Streptococcus pyogenes* (24%) were the most frequent etiologies. All cases with causative microbes, diagnosis, and anatomical localizations are listed in [Supplementary Table 1](#).

Treatment and outcome variables are presented in [Table 3](#). The overall 28-day mortality was 9% in the NSTI group and 5% in the non-NSTI group, a difference not reaching statistical significance. The amputation rate was low, and performed only in 3 cases in the NSTI group. The length of stay in the intensive care unit was significantly longer among patients in the NSTI cohort. Furthermore, in this category, the total length of hospital stay were longer. Regarding the clinical findings of edema, erythema, discoloration of the skin, and bulla formation, none could predict surgical findings specific to NSTIs ([Figure 1](#)).

Tissue Biopsies

The interrater reliability for histological staging and diagnosis in the NSTI cohort (surgical diagnosis) was moderate and fair, with a Cohen κ of 0.53 (95% confidence interval [CI], .31–.75) and 0.37 (95% CI, .10–.65), while the absolute agreement was 78% and 82%, respectively ([Table 4](#)). The subgroup analysis for the interrater reliability and absolute agreement of the individual histopathological components, and histopathologic staging and diagnosis, are presented in [Supplementary Tables 2–4](#). The sensitivity of histologic diagnosis was 75%, specificity 80%, positive predictive value 91%, and negative predictive value 53%. The positive likelihood ratio was 3.7 and the negative likelihood ratio was 0.32 ([Supplementary Table 5](#)).

We had complete data in 47 of 55 NSTI cases for assessing the overall NICCE success rate, which was 55% ([Table 3](#); [Supplementary Table 6](#)).

The biopsies in our cohort were only classified as either stage 1 or stage 2 disease. Notably, no biopsies were categorized as the most severe stage (ie, stage 3). When comparing the histological stages set by the pathologist to NICCE, we found that NICCE success was achieved by 42% and 71% of the cases in stage 1 and 2, respectively, with an OR of 3.4 (95% CI, 1.00–11.61; $P = .046$) ([Supplementary Table 7](#)).

DISCUSSION

In this study, we have explored the putative roles of histopathologic examination of tissue samples in routine NSTI management. We have evaluated the quality of histological diagnosis, interrater reliability in the staging of severity, and possible

associations of histopathologic findings and patient outcomes. We found an inadequate sensitivity, specificity, and negative predictive value of histopathology. The positive and negative likelihood ratios gave a small increase or decrease in the probability of disease, respectively. Furthermore, we found that in independent severity staging and diagnosis of the biopsies, the interrater reliability between the 2 experienced pathologists was only fair to moderate, with a large range of the absolute agreement for the different histopathologic variables. We also assessed the histopathologic staging in search for associations to clinical endpoints. Unexpectedly, we found a significantly higher probability for fulfilling NICCE with the more severe histopathological stage 2 compared to the less severe stage 1.

The role of tissue biopsies in NSTI diagnosis and prognosis is undetermined. Several guidelines and authors recommend or suggest it as part of the initial evaluation in NSTI, but the procedure is neither standardized nor validated [[3](#), [12](#), [18](#), [19](#)]. In addition, sampling errors of the biopsy may give a false-negative result. The use of frozen sections has been studied by Solomon et al, who demonstrated a lack of sensitivity and specificity using that method [[23](#)]. The clinical course and time to development of necrosis is variable in NSTIs [[28](#)]. Taking multiple biopsies from different locations may improve the sensitivity and specificity of histopathology, but not necessarily the interrater reliability.

The clinical differences between NSTI and non-NSTI patients may be subtle, particularly in the early phases. Classical signs such as discoloration of the skin, bulla formation, and skin necrosis are late signs [[6](#), [29](#)]. Our findings suggest that neither cutaneous, clinical, nor standard biochemical findings can discriminate between NSTI and non-NSTI in these instances, as previously noted in different studies and guidelines [[1](#), [3](#), [29](#)]. It is acknowledged that when NSTI is suspected, surgical exploration should be performed with as little delay as possible [[1](#), [4](#), [30](#)]. In everyday practice, clinicians require adjunct tools to aid management in ambiguous cases. As demonstrated by the data herein, the 2 cohorts were clinically difficult to distinguish at admission. This may be due to less severely ill NSTI cases than frequently reported, with lower mortality, fewer infections in the anogenital area, and more infections in the upper extremity [[7](#), [29](#), [31–34](#)]. On the other hand, the surgically categorized non-NSTI cases had high severity with need of organ supportive treatment, compared to other non-NSTI cohorts [[35](#), [36](#)]. Nevertheless, the total body surface area of tissue excised in the non-NSTI group equaled zero, confirming correct retrospective categorization of the cohort. Thus, we consider our non-NSTI cohort to be a relevant comparator group.

In NSTIs, there is a well-documented association between time to surgical debridement with source control and adverse patient outcomes [[29](#), [30](#), [32](#), [34](#), [37](#)]. The relatively low total mortality (8%) and few extremity amputations (4%) in our study could be explained by less severe cases, but also by short time to

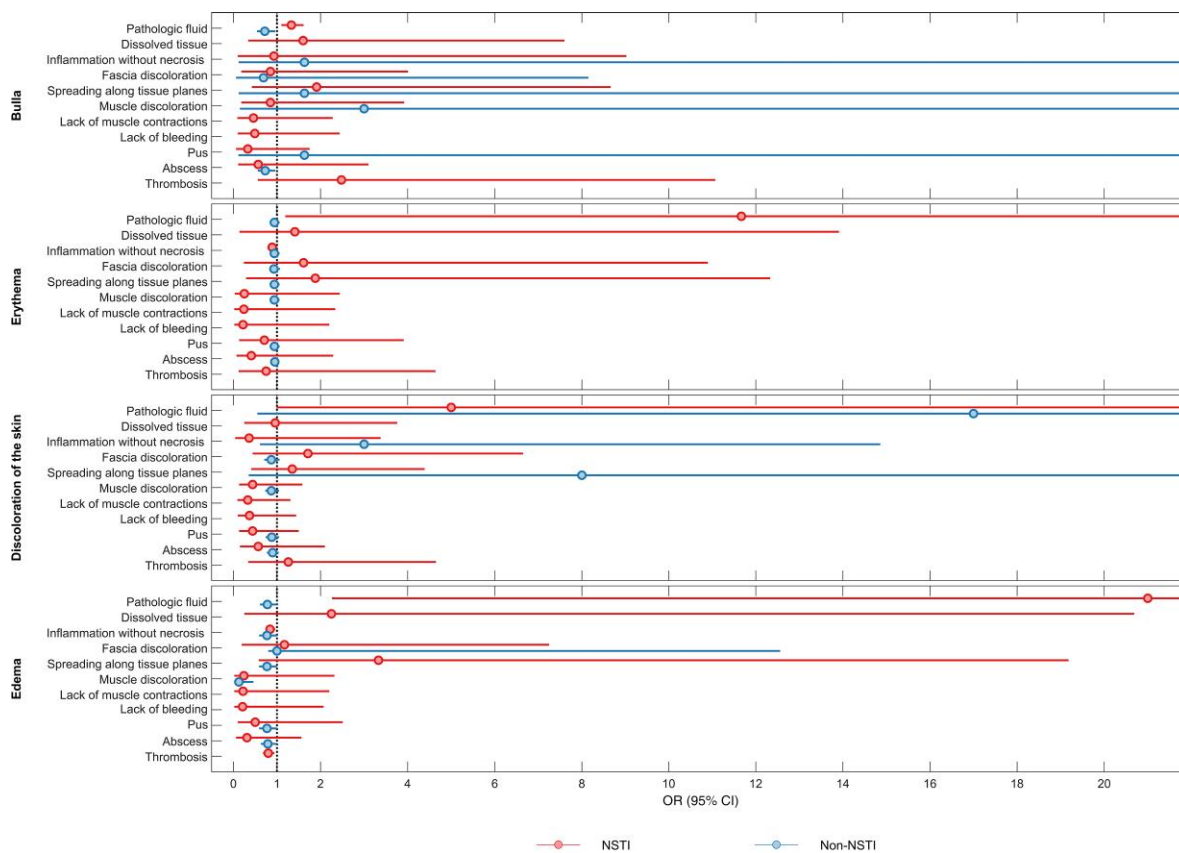


Figure 1. Clinical and surgical findings. The figure displays a Forest plot with the odds ratio and 95% confidence interval for surgical findings given clinical findings (bold). The only statistically significant finding is that bulla, erythema, and edema give a higher odds ratio for pathological fluid. Abbreviations: CI, confidence interval; Non-NSTI, nonnecrotizing soft tissue infection; NSTI, necrotizing soft tissue infection; OR, odds ratio.

surgery and possibly the practice of limb-sparing techniques and a dedicated group of skilled surgeons at our hospital. Our finding of an association between a more severe histopathological stage and a better outcome contrasts with the findings of Bakleh et al [21]. Their research demonstrated that patients categorized as stage 1 or 2 had a significantly lower risk of death than patients with stage 3 findings. They did, however, include both histological and surgically verified necrotizing fasciitis cases. In contrast, we exclusively included surgically verified NSTI cases. Our finding of a low accuracy of histopathology as a diagnostic test supports including only surgically confirmed cases in the NSTI group. In addition, Bakleh et al demanded moderate to severe sepsis caused by necrotizing fasciitis for inclusion. We categorized patients based on surgical exploration, and believe this resulted in a clinically more relevant selection. The median time to surgery was longer in the study by Bakleh et al, which may explain why more advanced cases and increased mortality risk were observed in their cohort.

Organization of services, priority, and resource use is of high importance when treating NSTIs. These infections are medical emergencies where diagnosis and subsequent surgical debridement is of paramount importance. Histopathology evaluation

requires a pathologist on call 24/7 to be an adjunctive in NSTI diagnosis, an on-call service that is resource demanding, and possibly impractical, for most hospitals. Finally, there is a risk that routine histopathologic service could contribute to treatment delay [4].

The results in this study shed light on a possible role for tissue biopsies as an adjunct diagnostic procedure in ambiguous NSTI cases. Our findings support other studies in that the benefit of routine use of histopathology is low [1, 4, 31, 38, 39]. The results obtained lead us to discourage the use of histopathology as part of emergency diagnostics in NSTIs. The diagnosis still relies on high awareness of typical clinical features, and are based upon surgical exploration and findings. Nevertheless, questions remain on the putative roles of biopsy in the everyday handling of this patient category. Consequently, we do encourage that biopsies are included in clinical studies with prospective patient enrollment, to answer novel research questions. There is still a need to bridge basic science, routine diagnostics, organization, and clinical handling of patients with NSTIs, in order to lower mortality rates and to improve survivors' clinical outcomes.

The major strengths of this study include the large number of tissue biopsies taken from patients with suspected NSTI, that

Table 4. Interrater Reliability of Histologic Variables

Variable	NSTI		Non-NSTI		All	
	Cohen κ (95% CI) (n = 55)	Absolute Agreement, %	Cohen κ (95% CI) (n = 20)	Absolute Agreement, %	Cohen κ (95% CI) (n = 75)	Absolute Agreement, %
Histologic stage	0.53 (.31–.75)	78	1.0 ^a	100	0.61 (.41–.8)	84
Degree of neutrophilic infiltration	0.11 (–.06 to .28) ^b	62	0.54 (.26–.82)	70	0.28 (.12–.44)	63
Bacteria present	0.53 (.31–.75)	78	1.0 ^a	100	0.61 (.41–.8)	84
Histologic NSTI diagnosis	0.37 (.10–.65) ^c	82	0.50 (.14–.86)	75	0.52 (.32–.72)	80

Abbreviations: CI, confidence interval; Non-NSTI, nonnecrotizing soft tissue infection; NSTI, necrotizing soft tissue infection.

^aDue to complete agreement, standard error = 0.

^bDue to only 2 cases with few or no neutrophilic infiltration in the NSTI group, they were included in the moderate neutrophilic infiltration to assess the Cohen κ .

^cDue to skewness in prevalence, the κ is misleading; see absolute agreement.

our hospital is a referral hospital for NSTIs, and that routines for the management of NSTIs are well established. During the study period, a team consisting of physicians from the Department of Plastic Surgery and Department of Medicine evaluated all cases. Also, due to centralized care, the involved surgeons are skilled and experienced in this field. Finally, this is one of the largest studies on the value of tissue biopsies in NSTIs to date.

The main limitation of this study is the retrospective design and the sampling routine, leading to nonconsecutive inclusion. A list of every patients who during the study period underwent surgery due to suspected NSTI, but in which a biopsy was not performed, is not available. Evaluation of symptom duration is unreliable, because of questionable data quality in electronic patient records at this point, a weakness shared by many retrospective studies on NSTI. Due to low sample size, we have not been able to assess differences in anatomical localizations or disease severity. Even though it is among the largest studies on tissue biopsies in NSTI in recent years, the number of patients is still quite low, and the statistical findings must be interpreted with caution.

In summary, our findings suggest that tissue biopsies in NSTIs are of low clinical accuracy. The interrater reliability among experienced pathologists is only fair to moderate. Unexpectedly, a favorable outcome was associated with a histopathologically more severe stage. Altogether, these findings discourage routine use of histopathologic evaluation as part of emergency management of patients with suspected NSTI.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. We thank clinical and research personnel in the Department of Pathology for providing routine diagnostics and the

Department of Microbiology for identification of bacterial etiologies, as well as the consenting patients and their relatives.

Author contributions. Conceptualization: I. M. G., T. B., K. A. M., S. S. Methodology: I. M. G., K. A. M., J. A., T. B., S. S. Patient inclusion: T. B., E. R., S. S. Funding acquisition: A. N.-T., S. S. Project administration: K. A. M., S. S. Supervision: K. A. M., T. B., S. S. Biopsy evaluation: E. B., H. K. H. Writing—original draft: I. M. G., S. S., K. A. M. Writing—review and editing: I. M. G., T. B., E. B., H. K. H., S. K. A., J. A., E. R., A. N.-T., S. S., K. A. M.

Patient consent. Written consent was obtained from all patients or representative relatives.

Financial support. The INFECT study was supported by the European Union's Framework Programme 7 (grant number FP7/2007-2013 305340); the Norwegian Research Council under the frame of NordForsk (project number 90456, PerAID); the Norwegian Research Council under the frame of ERA PerMed (project 2018-151, PerMIT); and the Swedish Research Council (2018-02475).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form of Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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