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Th1/Th2 cytokine levels: A potential diagnostic tool for patients with necrotizing fasciitis

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ABSTRACT

Introduction: Necrotizing fasciitis (NF) has emerged as rare but rapidly progressive, life-threatening severe skin and soft tissue infection. We conducted a study to investigate whether Th1/Th2 cytokines could serve as biomarkers to distinguish NF from class III skin and soft tissue infections (SSTIs).

Methods: A retrospective review was performed for 155 patients suffering from serious skin and soft tissue infections from October 2020 to February 2022. Th1/Th2 cytokines were obtained from peripheral blood and wound drainage fluid samples. Data on demographic characteristics, causative microbiological organisms, Th1/Th2 cytokines, c-reactive protein, procalcitonin and white blood cell (WBC) were extracted for analysis. Factors with statistical difference($p < 0.1$) were included in the multivariate logistic regression model. The clinical differential diagnostic values of interleukin-2(IL-2), IL-6, IL-10, tumor necrosis factor-α (TNF-α) and interferon-r (IFN-r) were analyzed by receiver operating characteristic (ROC) curve.

Results: Among the 155 patients, 66(43%) patients were diagnosed as NF. We found no significant difference for sex, age, location of infection, coexisting condition, predisposition, duration of symptoms before admission and micro-organisms, WBC, procalcitonin and c-reactive protein in NF and class III SSTIs group. NF had higher levels of IL-6 in serum (50.46 [24.89, 108.89] vs. 11.87 [5.20, 25.32] pg/ml; $p < 0.01$), IL-10 in serum (3.45 [2.03, 5.12] vs. 2.51 [1.79, 3.29] pg/ml; $p < 0.01$), IL-2 in wound drainage fluid (0.89 [0.49, 1.33] vs. 0.63 [0.14, 1.14] pg/ml; $p = 0.02$), IL-6 in wound drainage fluid (5000.84 [1392.30, 13287.19] vs. 1927.82 (336.65, 6759.27) pg/ml; $p < 0.01$), TNF-a in wound drainage fluid (5.20 [1.49, 22.97] vs. 0.96 [0.12, 3.21] pg/ml; $p < 0.01$) and IFN-r in wound drainage fluid (1.32 [0.47, 4.62] vs. 0.68 [0.10, 1.88] pg/ml; $p = 0.02$) as compared to the class III SSTIs. Multivariate logistic regression analyses showed that IL-6 in serum, IL-10 in serum and TNF-a in wound drainage fluid exhibited independently significant associations with diagnosis of NF($p < 0.05$).

Abbreviations: NF, necrotizing fasciitis; SSTIs, skin and soft tissue infections; WBC, white blood cell; IL, interleukin; TNF-α, tumor necrosis factor-α; IFN-r, interferon-r; ROC, receiver operating characteristic; SEWS, standardized early warning score; AUC, area under curve; LRINEC, laboratory risk indicator for necrotizing fasciitis; ELISA, enzyme linked immunosorbent assay

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In ROC curve analysis of IL-2, IL-6, IL-10, TNF-a and IFN-r for diagnosis of NF, the area under the curve (AUC) of IL-6 in serum could reach to 0.80 ($p < 0.001$). Using 27.62 pg/ml as the cut off value, the sensitivity was 74% and the specificity was 79% in IL-6 in serum.

Conclusions: Th1/Th2 cytokines, IL-6 in serum in particular, are potential biomarkers for the diagnosis of NF in the early stage. However, larger patient populations with multiple centers and prospective studies are necessary to ensure the prognostic role of Th1/Th2 cytokines.

1. Introduction

Necrotizing fasciitis (NF) has emerged as rare but rapidly progressive, life-threatening severe skin and soft tissue infection (SSTI). It affects around four in every 100,000 people per year in the world [1]. Overall mortality risk of NF was 12.6% in a US study [2]. In our previous study, we confirmed that the mortality rate in patients with NF and septic shock could exceed 30% [3]. Furthermore, NF is one of the most lethal skin and soft tissue infection diseases, and poses as a worldwide public health issue. NF can be caused by a single species of bacteria (type II), or by different species of bacteria (type I). Early identification are essential as early aggressive surgical debridement may be necessary to save life [4,5]. However, a key limitation in NF management is that early NF diagnosis is mainly based on clinical findings vulnerable to subjective assessments. Clinical manifestation displays low specificity because of the fact that multiple factors can affect judgment of clinical manifestation, such as clinical experience, and clinical manifestation is not able to accurately distinguish NF from other skin and soft tissue infection [6].

T-helper lymphocytes belong to CD4 T cells and perform an important role in human immunological diseases. T-helper lymphocytes are classified into Th1 and Th2 subsets based on the pattern of cytokine production. The Th1 subset produces interleukin-1(IL-1), IL-2, IL-12,tumor necrosis factor-α (TNF-α) and interferon-r (IFN-r),participates in cellular immunity and plays a role in delayed hypersensitivity and T cell differentiation. The Th2 subset produces IL-4, IL-6 and IL-10, participates in humoral immunity and plays a role in immediate allergic and anti-parasite reactions [7,8]. Infectious diseases may cause Th1/Th2 cytokine imbalances in human body. In a previous investigation, it has been shown that IFN-r, TNF- α and IL-6 levels increased significantly in the sera of patients with Epstein Barr virus infection [9]. In patients with Middle East Respiratory Syndrome, the levels of IL-10 and IL-4 increased in the sera, however no difference in the level of IFN- r [10]. Y. Tang reported that infection would cause high serum level of IL-4, IL-6, IL-10, TNF-α and IFN-r in children with hematological diseases [11]. In a study by Jiu-ling Zhao et al., it was demonstrated that plasma levels of IL-6 and TNF-α were significantly higher in patients with mycoplasma pneumonia than in controls, and a severity index using cytokines has been suggested for clinical evaluation of mycoplasma pneumonia [12].

In recent years, some studies regarding Th1/Th2 cytokines and NF were published. Bucaretchi et al. reported that, cytokines (IL-6, IL-10 and TNF-α) were increasing in patient with forearm NF caused by ricin injection [13]. IL-33/ST2 axis had a fairly good protective effect on Group A Streptococcus induced NF [14]. Flaherty et al. found that IL-1β was significantly upregulated in patient with Group A Streptococcus skin infection [15]. Th1/Th2 cytokines played a key determinant in regulating the immune response in NF patients [16,17]. However, in the previous study, we could not establish whether Th1/Th2 cytokines levels could be a significantly better diagnostic marker than c-reactive protein, procalcitonin, white blood cell(WBC) for the diagnosis of NF [18]. What's more, the rate of under-diagnosis is high in patients with NF in the early stages [19]. Given its rapid progression, any delay in the diagnosis of NF can cause death. Studies are still lacking that identify factors that may accurately distinguish NF from other skin and soft tissue infections(SSTIs). The aims of this study were to evaluate the clinical diagnostic value of Th1/Th2 cytokines for NF in the early period.

2. Materials and methods

2.1. Subjects

Between October 2020 to February 2022, 155 consecutive patients who were hospitalized in the First Affiliated Hospital of Wenzhou Medical University and met the requirements were enrolled in the study(Fig. 1). The study was performed according to the principles of the Declaration of Helsinki and was approved by the local ethics committee (Professional ethics committee of clinical research, the First Affiliated Hospital of Wenzhou Medical University, China). Inclusion criteria were: 1) patients older than 18 years; 2) willingness to participate; 3) Class III(SSTIs severity classification) or NF. 4) duration of symptoms before admission was less than or equal to 7 days. 5) early period appearance: redness, swelling, heat, pain, bloody blister or ecchymosis. Exclusion criteria were: 1)refused to join the experiment; 2) patients with underlying immune system diseases, such as HIV; 3) pregnant or breastfeeding woman. Diagnosis of NF was based on intraoperative findings(necrosis of the subcutaneous tissue, undermining of the skin, thrombosis of the superficial veins). The judgements were independently done by two experienced doctors(medical work more than 10 years) above the attending level.

2.2. SSTIs severity classification and correlation definition

SSTIs severity were evaluated using different categories. Class I: no recorded significant co-morbidity (peripheral

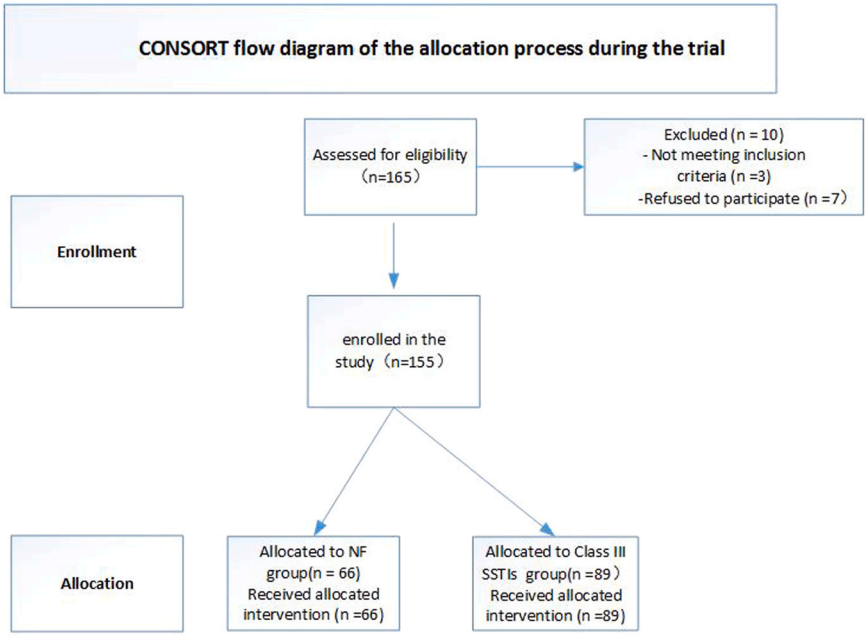


Fig. 1 – CONSORT flow diagram of the allocation process during the trial.

vascular disease, chronic venous insufficiency or morbid obesity), no sepsis and standardized early warning score (SEWS) < 4;Class II: documentation of one or more significant co-morbidities, but no sepsis and SEWS < 4; Class III: sepsis but SEWS < 4 [20].

Sepsis was defined using the criteria from the international sepsis definitions conference 2016, also known as the 'Sepsis 3' definition [21]. SEWS was calculated using six physiological parameters: 1) respiratory rate, 2) oxygen saturation, 3) temperature, 4) systolic blood pressure, 5) heart rate and 6) level of consciousness. According to the degree of abnormality, the abnormal values scored from one to three. We summed up all the scores of six physiological parameters and calculated SEWS [22].

2.3. *Cytokines determination*

A total of 155 patients were included for determination of IL-2,IL-4,IL-6,IL-10,TNF-a and IFN–r. Wound drainage fluid samples were extracted with syringes and stored in sterile test tubes. Serum samples pertaining to peripheral blood and wound drainage fluid samples were taken from patients at admission and centrifuged at 1000 rpm at 20 °C for 20 min. The supernatants were collected and the levels of Th1/Th2 cytokines were detected using the BD FACSCanto flow cytometry (BD Biosciences, San Jose, CA, USA). The concentrations of cytokines were measured using a Human Th1/Th2 Cytokine Kit (Saiji Biotechnology Co.LTD, Hangzhou, China). Standard curves were established, the maximum concentration was 5000 pg/ml. The samples would be diluted when the maximum concentration had been reached [23]. Blood and wound drainage samples were obtained for clinical reasons (assessment of inflammatory status) and subsequently used for experimental purposes following approval by the ethics committee.

2.4. *C-reactive protein, procalcitonin and white blood cell*

Determination of C-reactive protein, procalcitonin, WBC and wound culture were performed at admission. The level of c-reactive protein was detected by nephelometry using the automatic protein analyzer (Siemens BN II, Germany). Procalcitonin was detected by electrochemiluminescence using the automatic biochemical immune analyzer (Roche Cobas8000 , Switzerland). WBC was detected by fluorescein staining technique and laser flow analysis technology using automatic blood analyzer(SYSMEX XE-2100 L, Japan).

2.5. *Statistical analyses*

The data were analyzed using SPSS software (version 22.0; SPSS, Inc., Chicago, IL, USA). Continuous data were assessed for normality using the Kolmogorov-Smirnov test. Data were presented as mean ± standard deviation for normally distributed variables and as median (interquartile range) for nonnormal distribution. Between group differences in continuous data were tested using either the Student t test or the Mann-Whitney U test, as appropriate. For categorical variables, descriptive analysis was based on percentages and frequencies. Categorical variables were analyzed using either the chi-square test or the Fisher's exact test. Factors with statistical difference ($P < 0.1$) by analysis mentioned earlier (shown in Table 2) were included in the multivariate logistic regression model. The receiver operating characteristic (ROC) curve analysis was calculated to evaluate the diagnostic ability in suspected NF using Th1/Th2 cytokines. Larger areas under the ROC curve (AUC) indicated higher diagnostic value. All tests were two-sided and values of $P < 0.05$ were considered statistically significant.

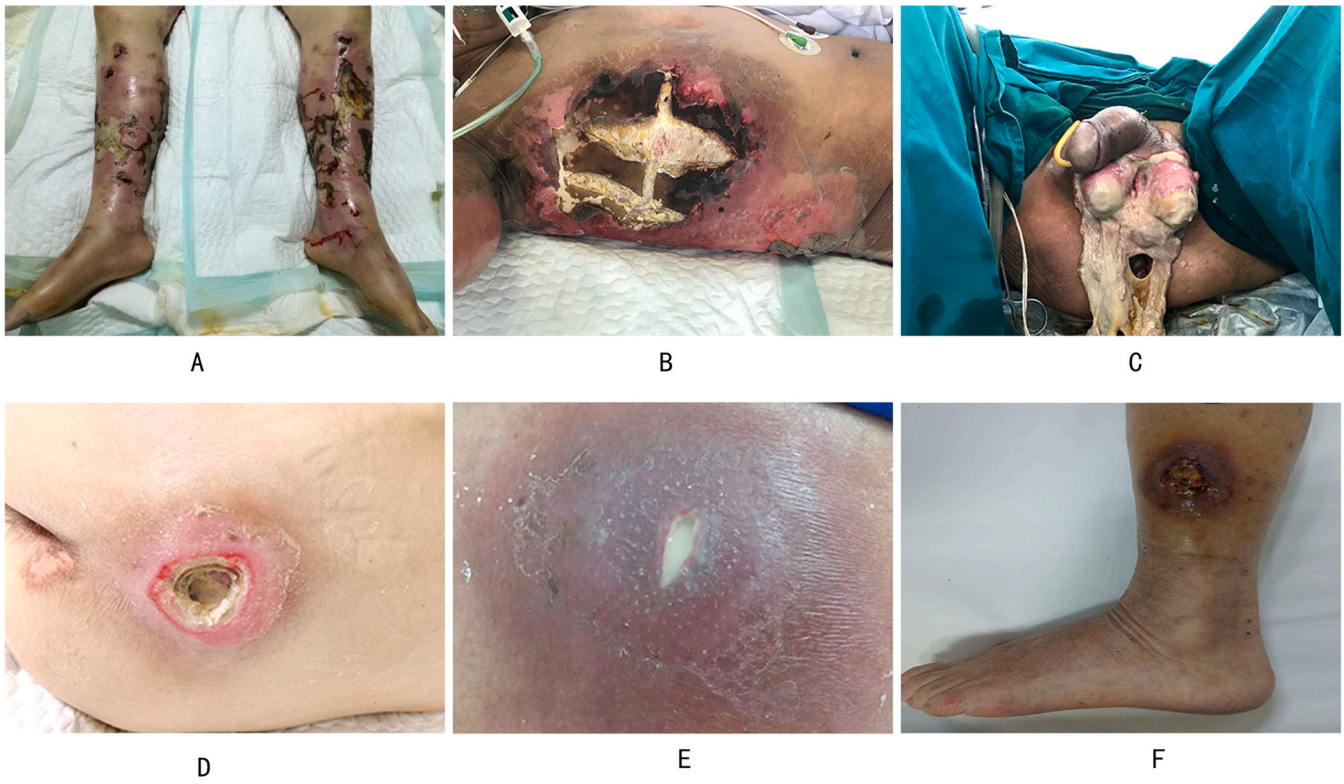


Fig. 2 – Representative wound photographs in NF group (A-C) and Class III SSTIs group (D-F). (A) Type II NF(caused by staphylococcus); (B) Type II NF(caused by pyogenic streptococcus); (C) Type I NF(caused by different species of bacteria); (D) Pressure ulcer on sacrococcygeal region; (E) Abscess on the back; (F) Skin necrosis on a leg.

3. Results

3.1. *The grouping of patients*

155 enrolled subjects were split into two groups(NF group and Class III SSTIs group) based on SSTIs severity classification and intraoperative findings. Diagnosis of NF was based on intraoperative findings as shown in Fig. 2A,B,C(necrosis of the subcutaneous tissue, undermining of the skin, thrombosis of the superficial veins). If patients had the signs of NF, they were allocated to NF group. If patients had sepsis, no signs of NF and SEWS < 4, they were allocated to Class III SSTIs group, and included conditions like pressure ulcer , abscess , extensive skin necrosis(Fig. 2D,E,F). Of 155 enrolled subjects, 66(43%) patients were allocated to NF group and 89(57%) patients were allocated to Class III SSTIs group. The judgements were independently done by two experienced doctors(medical work more than 10 years) above the attending level.

3.2. *Demographics and clinical parameters*

- The mean age of the included 155 patients was 59(47,70) years old (range 18–94 years old) , 73.5% of the patients were male. The infective sites were lower limb (n = 96, 61.9%) , upper limb (n = 14, 9.0%) , trunk (n = 14, 9.0%) , perineum (n = 12, 7.7%) , buttock (n = 10, 6.5%) , head and neck (n = 9, 5.8%) . The length of hospital stay was 20(14,33) days. Trauma in Table 1 include: traffic accident (n = 16), fall (n = 8) ,burn (n = 13) ,puncture (n = 11) ,crush injury (n = 4), scratch (n = 1) . The results of positive wound culture were as followed: polymicrobial infection (n = 57,36.8%), monobacterial gram positive infection (n = 41,26.5%), monobacterial gram negative infection (n = 34,21.9%), fungal infection (n = 6,3.9%). Staphylococcus (24.5%) was the most often identified organism among gram positive bacteria. Among gram negative bacteria, Escherichia coli (9.7%) were the most common organisms. Comparisons of the baseline characteristics between NF group and Class III SSTIs group were shown in Table 1. Briefly, these two groups did not statistically differ in sex, age, location of infection, coexisting condition, predisposition, duration of symptoms before admission and micro-organisms($P > 0.05$). The length of hospital stay was much more longer in NF group than Class III SSTIs group ($P < 0.01$) , because NF was a more complex and serious disease.

3.3. *Th1/Th2 cytokines, c-reactive protein, procalcitonin and WBC in NF group and Class III SSTIs group*

We compared the levels of Th1/Th2 cytokines, c-reactive protein, procalcitonin and WBC of the participants between NF and Class III SSTIs. The levels of IL-6 in serum, IL-10 in serum, IL-2 in wound drainage fluid, IL-6 in wound drainage fluid, TNF-a in wound drainage fluid and IFN-r in wound

Table 1 – Demographic characteristics and micro-organisms involved in skin and soft tissue infection.

	Overall (n = 155)	NF group (n = 66)	Class III SSTIs group (n = 89)	P
Sex, male, n	114	52	62	0.20
Age(years) †	59(47,70)	56.5(46,67.25)	64(47,71.5)	0.08
Location of infection,limbs, n	110	47	63	0.95
Coexisting condition, n				
Diabetes mellitus	66	29	27	0.08
Hypertension	59	23	36	0.48
Hepatic disorders	18	11	7	0.09
Cerebral vascular disease	8	3	5	0.77
Cardiac disease	15	7	8	0.74
Nephropathy	21	10	11	0.62
Malignancy	8	3	5	0.77
Predisposition				
None	85	41	44	0.11
Trauma	53	17	36	0.06
Postoperative incision infection	7	2	5	0.70
Prolonged bed rest	3	2	1	0.79
Application of Chinese herbs	7	4	3	0.69
Duration of symptoms before admission (d)†	3(2,5)	3(2,5)	3(2,5)	0.29
Hospital stay (d)†	20(14,33)	30.5(21.75,42)	15(9.5,21)	< 0.01
Positive wound culture, n	138	60	78	0.52
Polybacterial infection, n	57	28	29	0.21
Monobacterial Gram positive, n	41	18	23	0.84
Monobacterial Gram negative, n	34	14	20	0.73
Gram positive				
Staphylococcus, n	38	17	21	0.76
Streptococcus, n	16	9	7	0.24
Enterococcus, n	16	8	8	0.53
Gram negative				
Vibrio, n	2	2	0	0.18
Proteus, n	13	6	7	0.79
Escherichia coli, n	15	7	8	0.74
Klebsiella, n	14	7	7	0.56
Pseudomonas, n	6	2	4	0.96
Serratia marcescens, n	7	3	4	1.00

drainage fluid were significantly increased in NF patients compared with Class III SSTIs patients ($p < 0.05$). However, there were no significant differences between NF patients and Class III SSTIs patients with regard to other Th1/Th2 cytokines, c-reactive protein, procalcitonin and WBC ($p > 0.05$).The results were clearly presented in Table 2.

3.4. *Multivariate analysis for the major predictors of NF*

To further investigate the prognostic factors for NF, multivariate logistic regression analyses were conducted. Factors with statistical difference ($P < 0.1$) by analysis mentioned earlier (shown in Table 2) were included in the multivariate logistic regression model. The results showed that IL-6 in serum, IL-10 in serum and TNF-a in wound drainage fluid exhibited independently significant associations with diagnosis of NF (odds ratio: 0.99, 95% CI: 0.97–0.99, P = 0.003; odds ratio: 0.77, 95% CI: 0.61–0.98, P = 0.03; odds ratio: 0.96, 95% CI: 0.93–0.99, P = 0.02).

3.5. *Receiver operating characteristic curve*

Results of ROC curve analysis and selected cutoff points for IL-6 in serum, IL-10 in serum,IL-2 in wound drainage fluid,IL-6 in wound drainage fluid, TNF-a in wound drainage fluid and IFN-r in wound drainage fluid were presented in Table 3. ROC curves were presented in Table 4. The AUC of IL-6 in serum was 0.80 (95% CI=0.73–0.87, $p < 0.001$), suggesting sufficient accuracy and specificity.

3.6. *Th1/Th2 cytokines between types I and II NF*

We compared the levels of IL-6 in serum, IL-10 in serum,IL-2 in wound drainage fluid,IL-6 in wound drainage fluid,TNF-a in wound drainage fluid and IFN-r in wound drainage fluid of the participants between type I and type II NF, but here were not significant differences (Fig. 3).

4. Discussion

In the past 10 years, reports about NF had been increasing year after year [24,25]. Due to the rapid progression of disease and the subtlety of early signs and symptoms, NF is difficult to diagnosis in the early stage and have a high mortality. Early diagnosis, surgical exploration and thorough debridement play an important role during the treatment of NF. Delayed diagnosis and intervention contribute to increased mortality rates. The findings of a past study showed that debridement time had a direct correlation with the prognosis of NF. The longer it took to start debridement, the poorer the

Table 2 – Th1 /Th2 cytokine and inflammatory factor levels in different groups.

Variable	NF group (n = 66)	Class III SSTIs group (n = 89)	P value
C-reactive protein (mg/l)	73.00(32.88, 138.20)	56.00(13.05, 110.50)	0.05
Procalcitonin (ng/ml)	0.27(0.08, 1.29)	0.11(0.05, 2.55)	0.19
WBC ($X10^9$/l)	9.44(7.37, 14.95)	8.48(6.81, 12.43)	0.19
Neutrophil ($X10^9$/l)	7.68(5.12, 10.96)	5.80(4.08, 10.32)	0.08
Eosinophil ($X10^9$/l)	0.07(0.03, 0.12)	0.1(0.04, 0.21)	0.09
Monocyte ($X10^9$/l)	0.69(0.45, 1.02)	0.59(0.44, 0.80)	0.18
Lymphocyte ($X10^9$/l)	1.27(0.99, 1.73)	1.45(1.10, 1.83)	0.22
Basophil ($X10^9$/l)	0.02(0.01, 0.03)	0.02(0.01, 0.03)	0.28
Th1 /Th2 cytokine in serum samples			
IL-2 (pg/ml)	0.73(0.17, 0.61)	0.40(0.10, 0.61)	0.43
IL-4 (pg/ml)	0.34(0.10, 0.52)	0.37(0.1, 0.69)	0.77
IL-6 (pg/ml)	50.46(24.89, 108.89)	11.87(5.20, 25.32)	< 0.01
IL-10 (pg/ml)	3.45(2.03, 5.12)	2.51(1.79, 3.29)	< 0.01
TNF-a (pg/ml)	0.57(0.1, 1.42)	0.58(0.25, 1.07)	0.71
IFN-r (pg/ml)	0.44(0.10, 1.10)	0.35(0.10, 0.62)	0.13
Th1 /Th2 cytokine in wound drainage fluid samples			
IL-2 (pg/ml)	0.89(0.49, 1.33)	0.63(0.14, 1.14)	0.02
IL-4 (pg/ml)	0.37(0.10, 0.85)	0.45(0.12, 0.73)	0.58
IL-6 (pg/ml)	5000.84(1,392.30, 13,287.19)	1927.82(336.65, 6,759.27)	< 0.01
IL-10 (pg/ml)	1.82(0.47, 17.23)	0.90(0.41, 2.70)	0.09
TNF-a (pg/ml)	5.20(1.49, 22.97)	0.96(0.12, 3.21)	< 0.01
IFN-r (pg/ml)	1.32(0.47, 4.62)	0.68(0.10, 1.88)	0.02

prognosis ultimately became [26]. If NF patients got surgery within 6 h after infection, it significantly improved the survival rate of this disease [27]. However, in clinical practice , there is some difficulty in early diagnosis. As a result of confusing NF with Class III SSTIs (erysipelas, cellulitis, abscess, etc.), operative time will be delayed and cause death [28]. Therefore, we explored early diagnostic value of Th1 / Th2 cytokines in NF. Fortunately, our studies not only revealed that some Th1/Th2 cytokines had the ability to distinguish between NF and Class III SSTIs, but also showed the clinical acceptability of IL-2,IL-6, IL-10, TNF-a and IFN-r in the diagnosis of NF in early period.

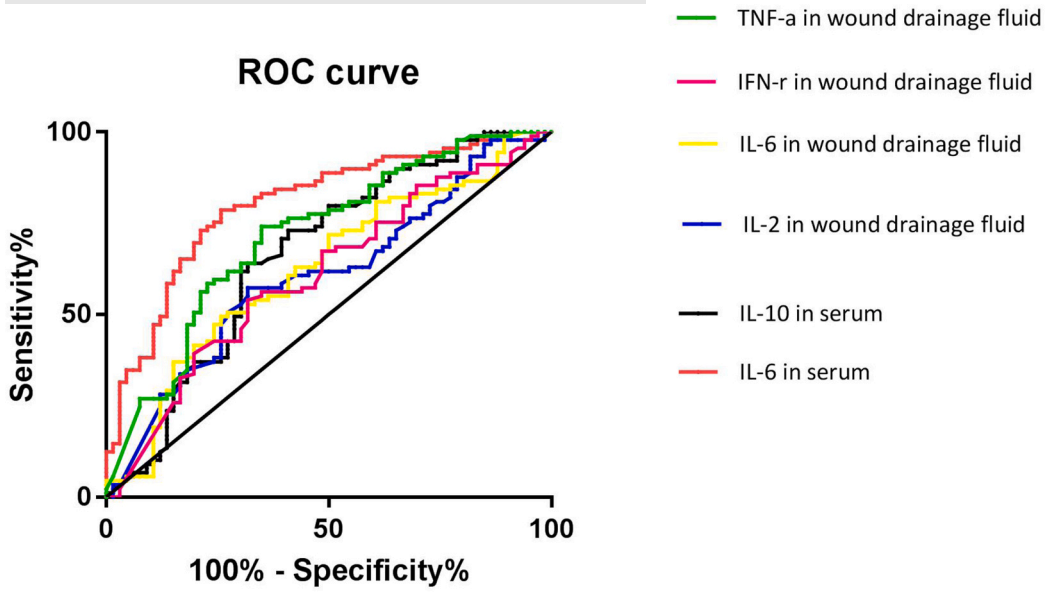
Class III SSTIs affected any layer of the soft tissue and is easily confused with NF. Such confusion often bedeviled the surgeons in clinic. Studies has explored ways for early recognition of NF and Class III SSTIs. Diagnostic techniques of imaging include X-ray, computed tomography and b-ultrasonics. X-ray could show subcutaneous pneumatosis, computed tomography indicated subcutaneous edema, subcutaneous pneumatosis and intense fascia signal, b-ultrasonics demonstrated that subcutaneous tissue became thicker. Nevertheless, the specificity of imaging examination was not satisfactory [29]. At present, LRINEC(Laboratory Risk Indicator for Necrotizing Fasciitis) is a score system which is commonly used in NF.LRINEC score greater than 6 indicates a likelihood of NF. However, the score system has limitations, including the low detection rate of NF and high misdiagnosis rate [30]. The traditional clinical model for diagnosing NF was for surgeons to make the diagnosis. Clinically, intraoperative wound appearance is the gold standard for NF, and the white blood cell, C-reaction protein and procalcitonin are widely used to assess the severity of SSTIs presently. Nevertheless, the specificity and sensitivity of these indicators are low for early diagnosis of NF.

Our studies revealed that IL-2,IL-6, IL-10, TNF-a and IFN-r had the ability to distinguish between NF and Class III SSTIs, and IL-6 in serum had the best diagnostic accuracy (AUC=0.80). Multivariate logistic regression analyses showed that IL-6 in serum, IL-10 in serum and TNF-a in wound drainage fluid exhibited independently significant associations with diagnosis of NF.There has been a few reports about Th1/ Th2 cytokines and severe bacterial infection diseases.

Table 3 – The differential diagnostic value between NF and Class III SSTIs.

Variables	IL-6 in serum	IL-10 in serum	IL-2 in wound drainage fluid	IL-6 in wound drainage fluid	TNF-a in wound drainage fluid	IFN-r in wound drainage fluid
AUC	0.80	0.67	0.61	0.62	0.72	0.61
P value	< 0.001	< 0.001	0.02	0.008	< 0.001	0.02
Youden index	0.53	0.32	0.26	0.24	0.39	0.22
Cutoff value (pg/ml)	27.62	2.94	0.75	1691.08	2.88	0.85
Sensitivity	0.74	0.68	0.68	0.74	0.65	0.68
Specificity	0.79	0.64	0.57	0.49	0.74	0.54
PPV	0.78	0.65	0.61	0.59	0.71	0.60
NPV	0.75	0.67	0.64	0.65	0.68	0.63
Agreement rate	0.77	0.66	0.63	0.62	0.70	0.61

Table 4 – ROC analyses for determining the optimal cutoff value of IL-2, IL-6, IL-10, TNF-a and IFN-r to differentiate NF from Class III SSTIs. ROC=receiver operating characteristic.



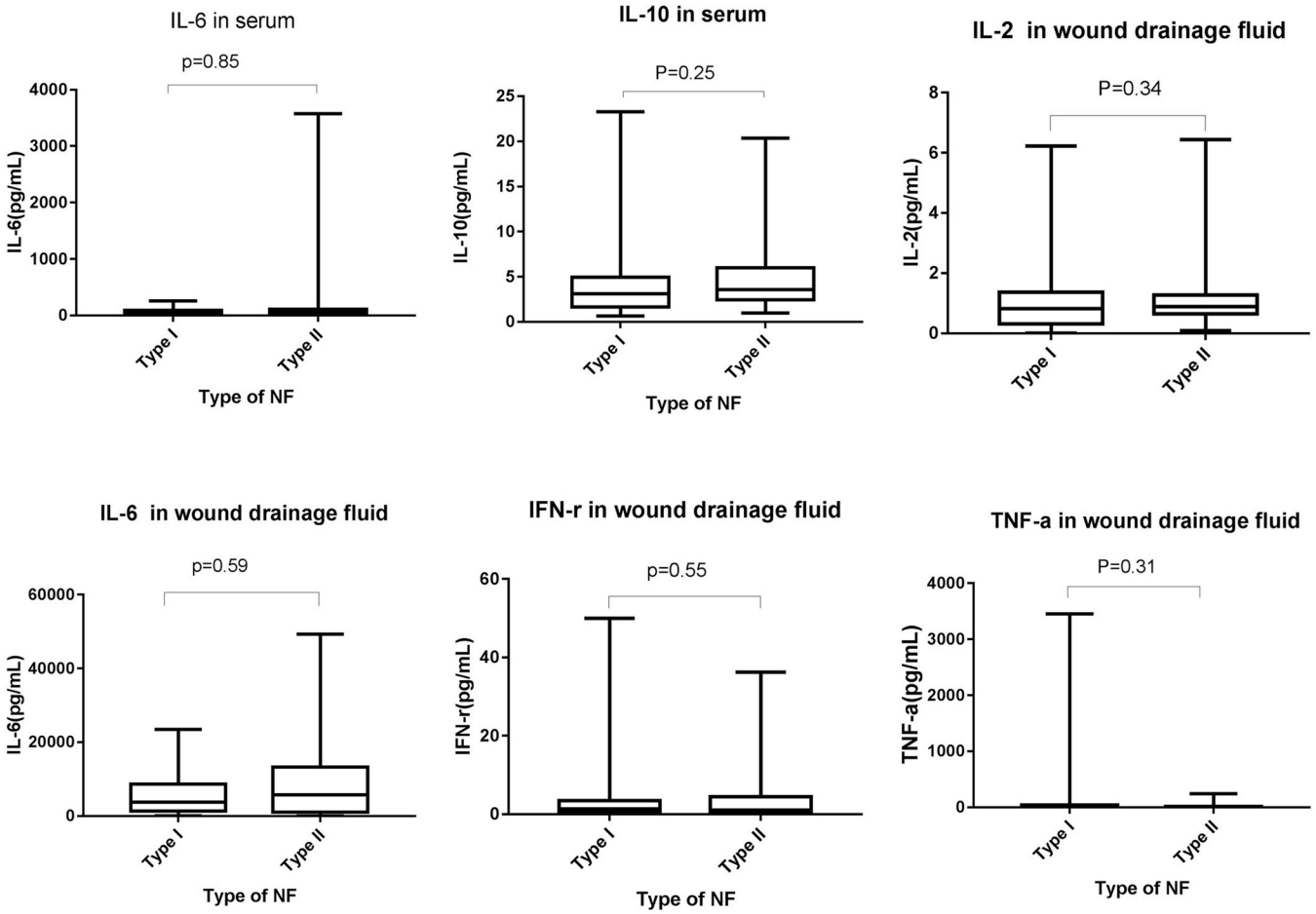


Fig. 3 – Comparison of cytokine levels between types I and II NF.

Yongmin Tang had shown ability of IL-6, IL-10 and TNF-a in serum to distinguish between gram negative and gram positive bacterial diseases. What's more, IL-6,IL-10 and TNF-a in serum could also estimate the severity of bacterial infection in children with hematological disorders [31]. Jingjing Guan found that IL-6 and IL-10 in the serum were closely associated with the severity of bacterial bloodstream infections, and could help to distinguish between gram-positive infection and gram-negative infection [32]. Hansen reported that IL-6 could be used to early assessment of necrotising soft tissue infection and IL-1β could be used for prognostic evaluation [33]. Th1/Th2 cytokines are produced in the host tissues, and they play an important role in the stimulation and regulation of cellular and humoral immune responses to establish the inflammatory reaction. We have demonstrated that interleukin-6 (IL-6), a key cytokine regulating human innate immunity activity, was highly expressed in NF tissues (5000.84 pg/ml). Its wound drainage fluid level was elevated in NF patients compared to class III SSTIs (5000.84 pg/ml vs 1927.82 pg/ml). IL-6 participates in the host-bacteria response, and as an example of diagnostic use, it has been proposed a diagnostic criterium for prosthetic joint infection of IL-6 ≥ 12.55 ng/L [34]. In our study, the patients having positive serum IL-6(≥ 27.62 pg/ml)were almost certain to have the correct diagnosis of NF. IL-6 measurement will be a new type of tool for NF diagnosis, and it might supplement traditional diagnostic methods and become an important diagnostic tool.

Clinical samples in our study were tested using flow cytometry, flow cytometry is much better than protein microarray technology and enzyme linked immunosorbent assay (ELISA) in detecting Th1/Th2 cytokines. It can detect multiple cytokines at the same time and has many other advantages, such as: rapid detection, small size of the sample [35].

The limitations were as follows: (1)There was still not sufficient data to show whether Th1/Th2 cytokines can aid in the diagnosis of NF. Our patients were from a single medical center in Wenzhou, China. Larger patient populations with multiple centers are necessary to ensure the diagnostic role of Th1/Th2 cytokines. (2) This was a retrospective study, it is strongly needed to investigate the clinical diagnostic value of Th1/Th2 cytokines for NF by prospective studies. (3) In our study, we found there were no significant differences between type I and type II NF patients with regard to these Th1/Th2 cytokines. These analyses were possibly underpowered, which should be commented.

5. Conclusions

In patients with NF, the value of WBC, c-reaction protein and procalcitonin for early diagnosis of NF is limited. When the early diagnosis of NF is crucial for a definitive decision regarding therapeutic options in patients with NF, our data suggestted that Th1/Th2 cytokines could possibly be used as an adjunctive tool for the diagnosis of NF in the early stage. We systematically tested the application of IL-2, IL-6, IL-10, TNF-a and IFN-r to recognize early NF, and finally we found that IL-6 in serum had the best diagnostic value for NF. However, larger patient populations with multiple centers and prospective studies are necessary to ensure the prognostic role of Th1/Th2 cytokines.

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Conflict of interest

The authors declare no conflict of interest.

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