Original Article

Neutrophil extracellular trap formation index predicts occurrences of deep surgical site infection after laparotomy

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Background: Deep surgical site infections (DSSIs) are serious complications after laparotomy. Neutrophil extracellular traps (NETs) play a vital role in the development of DSSI. Here, we focused on a new approach to predicting the occurrence of DSSI through the detection of the NET formation index (NFI), and compared its prediction ability with other clinical infection indicators.

Methods: Patients who received laparotomy were prospectively enrolled in this study. General information, APACHE II score, SOFA score, and serum infection indicators were recorded. The postoperative abdominal drainage fluid was collected within 3 days after the operation for quantification of the NFI.

Results: A total of 92 consecutive patients were included, with 22 patients were diagnosed with DSSI. The NFI in the DSSI group was 32.70%±19.33% while the corresponding index was 10.70%±8.25% in the non-DSSI group (P<0.01). The mean APACHE II and SOFA score had significant differences between the two groups. The NFI was positively correlated with the APACHE II score (P<0.01, r=0.269) and SOFA score (P=0.013, r=0.258). Patients with a high NFI (NFI >13.86%) had a higher risk of developing DSSI. According to the receiver operating characteristic (ROC) curve, the area under the ROC curve (AUC) of the NFI, C-reactive protein (CRP) and procalcitonin (PCT) were 0.912, 0.748 and 0.731, respectively.

Conclusions: In this cohort of surgical patients, the quantification of the NFI had a considerable predictive value for early identification of DSSI. The NFI in drainage fluid turned out to be a more sensitive and specific predictor of DSSI than serum infection indicators including CRP and PCT.

Keywords: Deep surgical site infections (DSSIs); neutrophil extracellular traps (NETs); NET formation index (NFI); abdominal drainage fluid

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Introduction

Surgical site infections (SSIs) are defined as infections that affect the incision, deep tissue as well as the organs or other body space within 30 days after operation (1). Deep SSIs (DSSIs) are the specific sites of SSIs, involving soft tissues of fascia and muscle infections (2). The DSSIs are becoming the most common healthcare-associated infections despite various guidelines for their prevention, and they continue to be a frequent cause of morbidity and mortality (3,4). Early source identification and control were considered fundamental to the treatment of most patients with DSSI, since early institution of appropriate therapy is associated with improved outcomes. In current clinical practice, an increasing number of surgical practitioners realize the paucity of laboratory studies regarding the clinical diagnosis and treatment of DSSI (5). It is imminent that a new biomarker should be discovered to predict local infection source early and accurately in patients after laparotomy.

An abdominal drainage tube after laparotomy is a window to detect complications, such as bleeding and fistulae, directly and promptly. After laparotomy, a large amount of fluid, which contains the residual blood, protein exudation, necrotic tissue cells, inflammatory factors or bacteria, is retained in the abdominal cavity. Indeed, by observing the color and properties of the abdominal drainage fluid, the occurrence of complications such as postoperative bleeding, pancreatic fistula or anastomotic fistula, can be judged early and treated in time (6,7). However, no direct evidence of evidence-based medicine has been presented that abdominal drainage fluid examination may be helpful in assisting the clinical diagnosis of DSSI. In addition, bacterial culture of the drainage fluid, which is unpractical and time consuming, cannot identify the infection source early (8).

Neutrophil extracellular traps (NETs), a new function of neutrophils, were first observed in 2004 (9). Various research studies have shown the complex composition of NETs, decondensed DNA conducts as the structural backbone, onto which a vast array of granular proteins, such as histones, myeloperoxidase (MPO), α-defensins and neutrophil elastase (NE), are dispersed (10,11). It is generally believed that NETs formation is triggered in response to a wide variety of microorganisms, calcium influx, immune complexes and inflammatory stimuli (11). Microbiota in the drainage fluid provide a chronic inflammatory stimulus that has an impact on NET formation (12). Currently, direct NET quantification has not yet been standardized, hampering its applicability in clinical trials (13). With the in-depth basic and clinical research, the NET formation index (NFI) and biomarkers of NETs, including cf-DNA, MPO, and citrullinated histone H3, were observed to indirectly reflect the level of NETs formation, which is linked to the episodes and severity of sepsis (14-16). These initial reports provided new enlightenment to the diagnostic or prognostic marker value for NET formation in the occurrence of postoperative infections.

In the current study, we hypothesized that the NFI of the postoperative abdominal drainage fluid can be used as an indicator for local infections. Thus, we proposed a novel method for the detection of the NFI by culturing neutrophils with postoperative abdominal drainage fluid, evaluating the predictive value of the NFI on the development of DSSI, and comparing it with that of other serum indicators. We present the following article in accordance with the STARD 2015 reporting checklist (available at https://dx.doi.org/10.21037/atm-21-1078).

Methods

Study design and study participants

We conducted a prospective study between September and December 2019 at Jinling Hospital. This study design was carried out according to the principles of the Declaration of Helsinki (as revised in 2013) and approved by the institutional ethics committee board of our institution (No.: 2020NZKY-011-01). The written consent was obtained from all patients and healthy volunteers prior to their inclusion in this study. This study focused on patients between the ages of 18 and 70 years. Patients who were scheduled for a laparotomy with placement of a drainage tube were considered eligible for the study. Patients with previous history of tumors or immune diseases and patients who took immunosuppressive drugs were excluded. Patients who received the application of negative pressure drainage device after the laparotomy were also excluded, since the drainage fluid would be diluted. Pregnant women, as well as patients whose records contained incomplete information and illegible handwriting were additional exclusion criteria.

Upon admission, patients received operations following the standard protocols of hospitalization. All surgical procedures were conducted according to institutional standards and followed a specific postoperative standardized clinical pathway. The postoperative treatment and nursing process did not have any reference to the test results of
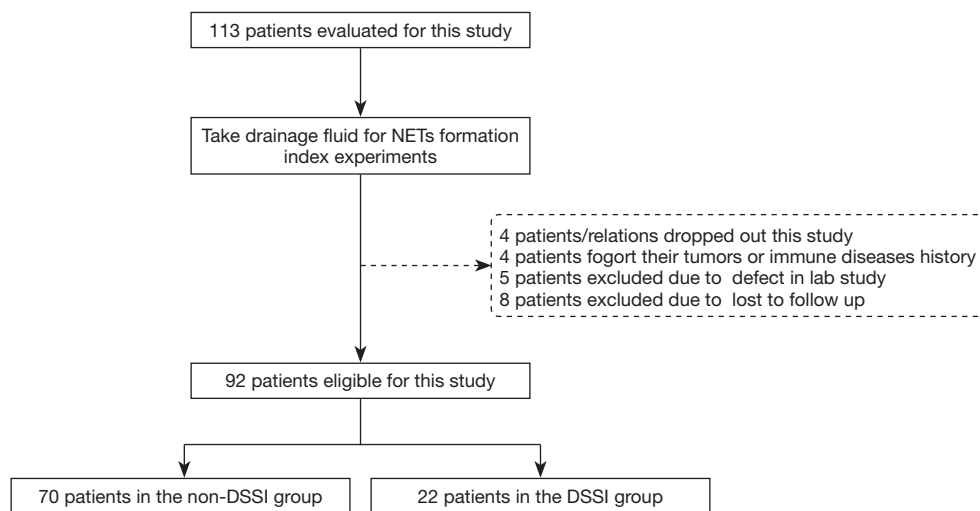
Figure 1 Study design flow chart. 113 patients evaluated for this study but 21 patients excluded due to different reasons. Finally, 92 patients were eligible for this study and divided into two groups. NETs, neutrophil extracellular traps; DSSI, deep surgical site infection.

the NFI. The SSI were classified according to the depth in: superficial, deep, and organ or space (17). In deep infections, the fascia or muscle is affected and must have at least one of the following criteria. Firstly, purulent drainage existed in the deep incision. Secondly, the deep incision spontaneously dehisced with positive culture, as well as the patient had body temperature greater than 38°C or had localized pain and tenderness. Thirdly, an abscess or other evidence of infection involving the deep incision was found. Deep surgical site infections (DSSIs) were diagnosed by a surgeon or attending physician (18).

Abdominal drainage fluid was drawn within 3 days after operation, centrifuged at 1,500 g at 4 °C for 15 min and the supernatant was taken for further experiments. At the same time, blood samples were collected. Markers such as complete blood count, CRP, and PCT were assessed. In addition, patient demographic information, the Acute Physiology and Chronic Health Evaluation (APACHE II) score and the Sequential Organ Failure Assessment (SOFA) score were recorded each day. The highest value for all indicators was selected for further analysis. The flow chart was shown in *Figure 1*.

Isolation of human peripheral neutrophils

Human peripheral neutrophils were isolated as described previously (19). Briefly, peripheral blood was drawn from healthy volunteers with K2EDTA blood collection tubes (Kangshi) under the ethics licenses Research Ethics

Committee (Jinling Hospital). Neutrophils from whole blood were isolated on Polymorphprep solution (Axis-Shield, Norway) according to the modified manufacturer's instructions. Neutrophils were removed into the centrifuge tube, and Red Blood Cell Lysis Buffer (Solarbio, China) was used to wash neutrophils. After two washes with PBS buffer, the neutrophils were resuspended at a density of 3×10^6/mL in RPMI 1640 (Sigma-Aldrich, USA) supplemented with HEPES buffer (10 mM, pH 7.2).

In vitro assay of the NFI

Cell-free DNA (cf-DNA) was reported as a marker for NET formation. We quantified the NET formation by detecting cf-DNA level as previously described (20). In general, SYTOX Green nucleic acid stain (ThermoFisher Scientific) was dissolved in RPMI 1640 at 5 µM. Background wells received 100 µL RPMI 1640, the TritonX-100 well received 50 µL 2% TritonX-100 and 50µl neutrophils (1×10^6/mL), the drainage fluid well received 50 µL abdominal drainage fluid and 50 µL RPMI 1640, and the test well received 50 µL abdominal drainage fluid and 50 µL neutrophils (1×10^6/mL). Each well was replicated in three duplicate wells. Then 100 µL SYTOX Green nucleic acid stain solution was added to each well. The 96-well plates were placed in a cell incubator for 4 hours at 37 °C in 5% CO_2. Test plates were centrifuged at 600 g for 15 min, and optical density (OD) in each well was determined at 485 and 535 nM through

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the FilterMax F3 Multi-Mode Microplate Reader. The cf-DNA concentration was calculated according to the standard curve. The NFI was calculated using the following formula: [C(Test) – C(Drainage Fluid)]/[C(TritonX-100)–C(Background)] ×100%.

Statistical analysis

Quantitative variables were expressed as the mean ± standard error of the mean (SEM) or median and interquartile range according to distributions after the Shapiro-Wilk test. The Student's t-test was used to compare the normally distributed data between the two groups. The Mann-Whitney U test was used to analyze non-normally distributed and heterogeneous data. The chi-squared test was used for comparing the proportions among groups. The Spearman's rank correlation test was used to analyze the correlation between two continuous variables. The relationship between the relevant indicators and abdominal infections was evaluated by binary logistic regression analysis. Receiver operating characteristic (ROC) curves were drawn for different indicators, and the areas under the ROC curves (AUC) of these scoring systems were compared. An indicator was regarded to have diagnostic value if its AUC was >0.5 and the difference was statistically significant when compared with 0.5. The statistical analysis was performed with the use of GraphPad Prism 26, and figures were performed with the use of GraphPad Prism 7. P<0.05 was considered to indicate a statistically significant difference.

Results

The NFI was positively correlated with the APACHE II and SOFA score

Overall, a total of 92 consecutive patients who received laparotomy were included in our study. Among them, 70 patients were included in the non-DSSI group, and 22 patients were included in the DSSI group. As shown in *Table 1*, the two groups of patients had significant differences in age, admission diagnosis, time of hospital stay, and ICU stay. Within three days after operation, the APACHE II score and SOFA score in the DSSI group was significantly higher than those in the non-DSSI group (P<0.01). The median and interquartile range of the APACHE II score in the DSSI group were 4.5 and [2–7] while those in the non-DSSI group were 7 and (2–11.75), respectively (P<0.01).

The median and interquartile range of the SOFA score in the DSSI group were 3 and (0.75–7) while those in the non-DSSI group were 0 and (0–1), respectively (P<0.01).

No significant difference was found in the infection indicators such as the white blood cell count, lymphocyte count, neutrophil count, bilirubin level, and platelet count, between the two groups. The present study speculated that some traditional serum infection indicators measured within three days after operation were not capable of predicting the incidence of further DSSI. However, patients in the DSSI group had higher procalcitonin and CRP levels than the non-DSSI group. It was interesting that the NFI showed significant differences between the two groups. The mean NFI in the DSSI group was 32.70%±19.33% while that in the non-DSSI group was 10.70%±8.25% (P<0.01) (*Table 1*). The correlation analysis between the NFI and the APACHE II/SOFA score suggested that the NFI was positively correlated with the APACHE II score (P<0.01, r=0.269) and SOFA score (P=0.013, r=0.258).

Occurrence rate of DSSI increased in patients with a high NFI

The results shown in *Table 1* were analyzed by multiple factor analysis statistically. We found that the NFI and the SOFA score were predictive factors of DSSI. The ROC curve was created for the NFI, CRP level, PCT level and white blood cell (WBC) level. Meanwhile, a comparison of the AUCs between these four indicators was made in the ROC curve. The AUC values of the NFI, CRP level, PCT level, and WBC level were 0.912, 0.748, 0.731, and 0.549, respectively (*Figure 2*).

According to the ROC curve, the corresponding cut-off value of the NFI was 13.86% (*Figure 2*). Consequently, low NFI was defined as the NFI less than or equal to 13.86%, and high NFI was defined as the NFI greater than 13.86%. Based on the NFI, 92 patients were divided into two groups: 55 patients were divided into the high-NFI group and 37 patients were divided into the low-NFI group. One patient in the low-NFI group (1.82%) and 21 patients in the high-NFI group (56.76%) were diagnosed with DSSI (P<0.001) (*Table 2*). From another perspective, in those 22 patients who were diagnosed with DSSI, 21 (95.45%) patients had a high NFI. The CRP and PCT levels in the high-NFI group were significantly higher than those in the low-NFI group (P<0.01). Similarly, there existed significant differences in the APACHE II score and SOFA score between two groups (*Figure 3*). These data supported the hypothesis

Table 1 Difference in clinical characteristics between patients with or without DSSI

Variable	Non-DSSI (n=70)	DSSI (n=22)	P value
NFI, mean ± SD	10.70%±8.25%	32.70%±19.33%	<0.001
APACHE II Score, median (IQR)	4.5 (2–7)	7 (2–11.75)	<0.001
SOFA Score, median (IQR)	0 (0–1)	3 (0.75–7)	<0.001
General information			
Age, years, mean ± SD	51.29±16.65	47.32±11.55	0.013
Male, n (%)	40 (57.14%)	15 (68.18%)	0.357
Time of hospital stay, days, median (IQR)	12 (9–17)	23 (18–40)	<0.001
Time of ICU stay, days, median (IQR)	0 (0–1)	6.5 (1.75–13)	<0.001
Mortality n (%)	0 (0%)	1 (4.54%)	0.073
Admission diagnosis, n (%)			
Pancreatic trauma	2 (2.86)	5 (22.72)	0.002
Multiple injuries	1 (1.43)	3 (13.64)	0.014
Splenic trauma	2 (2.86)	1 (4.55)	0.697
Choledocholithiasis	11 (15.71)	0 (0.00)	0.048
Gallstone	17 (24.29)	1 (4.55)	0.042
Hepatic cyst	8 (11.43)	0 (0.00)	0.097
Gastric perforation	4 (5.71)	2 (9.09)	0.576
Intestinal obstruction	15 (21.43)	2 (9.09)	0.193
Intestinal perforation	3 (4.29)	8 (36.36)	<0.001
Ischemic bowel disease	3 (4.29)	3 (13.64)	0.121
Acute appendicitis	7 (10.00)	0 (0.00)	0.123
Duodenal trauma	0 (0.00)	1 (4.55)	0.073
Laboratory examinations			
White blood cells, 10^9/L, median (IQR)	7.89 (6.63–10.02)	9.17 (5.71–12.84)	0.490
Lymphocytes, 10^9/L, median (IQR)	0.87 (0.65–1.28)	0.72 (0.55–1.12)	0.282
Neutrophils, 10^9/L, median (IQR)	6.39 (4.96–9.56)	6.94 (4.36–10.63)	0.960
Platelets, 10^9/L, median (IQR)	169 (140–205)	162 (124–205)	0.453
C-reactive protein, mg/L, median (IQR)	96.0 (33.2–136.6)	156.0 (97.5–230.5)	<0.001
Procalcitonin, µg/L, median (IQR)	1.53 (0.21–1.62)	2.21 (0.62–7.75)	0.001
Bilirubin, µm/L, median (IQR)	14.0 (10.2–19.1)	16.3 (12.8–25.7)	0.077

The 92 patients were divided into two groups according to whether or not developing the deep surgical site infection (DSSI). Values in table were reported as the frequency (number) with the percentage in parenthesis for categorical variables. Quantitative variables are expressed as the mean ± standard deviation (SD) or median and interquartile range (IQR). P value <0.05 revealed statistical significance. NFI, NET formation index; APACHE II score, acute physiology and chronic health evaluation score; SOFA score, sequential organ failure assessment score; ICU, intensive care unit.
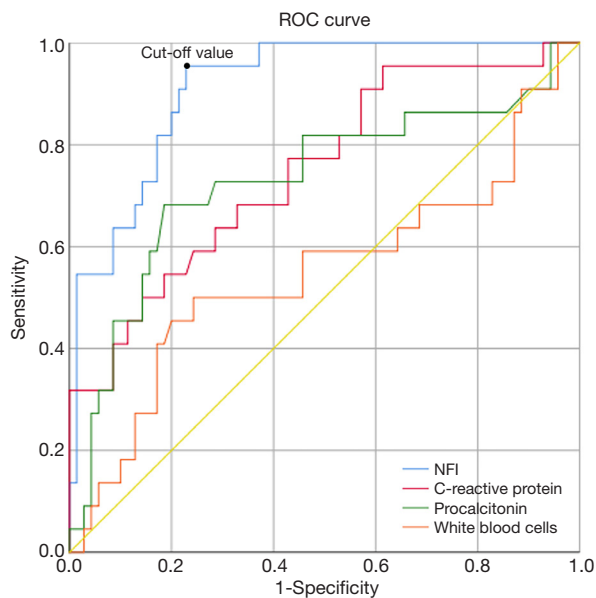
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Figure 2 ROC curves of the NFI, CRP level, PCT level and WBC level in predicting DSSI. The predictive power in the occurrence of DSSI was compared between the NFI in abdominal drainage fluid, as well as the CRP level, PCT level and WBC level in serum. The area under the ROC curve of the NFI, CRP level, PCT level and WBC level were 0.912, 0.748, 0.731, and 0.549, respectively. The cut-off value of the NFI was 13.86%. DSSI, deep surgical site infection; NFI, NET formation index; CRP, C-reactive protein; PCT, procalcitonin; WBC, white blood cell; ROC, receiver operating characteristic.

that the DSSI more likely occurred in patients with a high NFI within three days after laparotomy. Additionally, the occurrences of sepsis (P<0.01), bleeding (P=0.013), and pulmonary infections (P=0.045) were increased in patients with a high-NFI at a moderately significant level.

Peripheral neutrophil count impacted prediction ability of the NFI

Sixteen patients from the non-DSSI group were included to the high-NFI group, therefore, the further analysis was focused on the differences between these 16 patients and 54 patients in the non-DSSI group. Based on the NFI value and DSSI occurrences, patients were divided into four groups (*Table 3*). As shown in *Table 4*, no difference was found in the APACHE II score, the SOFA score and most of the laboratory examination results, while the plasma neutrophil count between the two groups was statistically

significant. The mean neutrophil count of the 16 patients included to the high-DSSI group was 16.38×10^9/L and that of the 54 patients in the non-DSSI group was 9.32×10^9/L (P<0.01) (*Table 4*); when it came to the comparison of the mean neutrophil count between these 16 patients and 21 patients in the high-NFI group, we found that the mean neutrophil count in these 16 patients was higher than that in 21 patients (7.62×10^9) too (P=0.004).

Discussion

This prospective study first proposed a promising method for early prediction of DSSI through the quantification of the NFI in drainage fluid. The NFI between patients with or without DSSI displayed a marked difference, and the NFI was positively correlated with the APACHE II score and SOFA score. Meanwhile, we classified the NFI as either as high or low, based on whether its value was larger than 13.86%, and significant differences existed in the occurrence of DSSI between patients with the high NFI and the low NFI. Furthermore, the AUC value of the NFI was significantly larger than that of CRP and PCT levels, which highlighted that the NFI turned out to be a more effective predictor of DSSI than CRP or PCT levels.

Previous studies had addressed the role of NETs in infections and characterized the clinical prediction potential for DSSI and sepsis. The NETs were rapidly formed (<20 min) once the host was infected (21). In the last two decades, the predictive power for the episodes of multiple organ dysfunction and sepsis, in the elevated levels of cf-DNA and other biomarkers of NETs in the plasma had been elucidated (22,23). In another study, normal neutrophils were incubated with patient plasma ex vivo, and then the NET formation capacity was detected. This assay indicated that the NET formation could be directly induced by patient plasma and was associated with clinically relevant information on disease severity, complications and outcome (24). Based on the previous research studies, a hypothesis was formulated that the NET formation could be directly induced by the abdominal drainage fluid and was relevant to the occurrence of the DSSI. Our findings were particularly appealing because biomarkers in the abdominal drainage fluid in the formation of DSSI were missing previously.

Abdominal surgery, which may involve multiple organs, was a serious stressor. DSSI was an important burden of the laparotomy, and the infections can spread to abdominal organs, and lead to shock, coma, and eventual death (25).

Table 2 Differences in clinical characteristics between patients with a low/high NFI

Variable	Low-NFI (n=55)	High-NFI (n=37)	P value
DSSI, n (%)	1 (1.82%)	21 (56.76%)	<0.001
APACHE II score, median (IQR)	4 (2–7)	7 (2–10.5)	0.006
SOFA score, median (IQR)	0 (0–1)	1 (0–4)	0.001
General information			
Age, years, mean ± SD	49.87±16.70	51.03±14.04	0.241
Male, n (%)	29 (52.73%)	26 (70.27%)	0.092
Time of hospital stay, days, median (IQR)	9 (12–16)	19 (13–24)	<0.001
Time of ICU stay, days, median (IQR)	0 (0–1)	3 (1–9.5)	<0.001
Laboratory examinations			
White blood cells, 10^9/L, median (IQR)	7.56 (6.39–9.95)	8.69 (6.72–12.13)	0.256
Lymphocytes, 10^9/L, median (IQR)	0.95 (0.61–1.33)	0.74 (0.59–1.19)	0.268
Neutrophils, 10^9/L, median (IQR)	6.17 (4.59–9.21)	7.32 (4.86–10.49)	0.366
Platelets, 10^9/L, median (IQR)	171 (145–206)	159 (120–195)	0.145
C-reactive protein, mg/L, median (IQR)	85.0 (20.8–133.5)	125.0 (92.6–184.5)	0.001
Procalcitonin, μg/L, median (IQR)	0.48 (0.15–1.34)	1.85 (0.46–4.50)	<0.001
Bilirubin, μm/L, median (IQR)	14.7 (10.2–18.1)	15.6 (12.2–22.5)	0.165
Other complications			
Sepsis	1 (1.82%)	9 (24.32%)	0.001
Bleeding	0 (0%)	4 (10.81%)	0.013
Pulmonary infections	4 (7.27%)	8 (21.62%)	0.045
Liver damage	10 (18.18%)	11 (29.73%)	0.196

The 92 patients were divided into two groups according to the NET formation index (NFI) value. Values in table were reported as the frequency (number) with the percentage in parenthesis for categorical variables. Quantitative variables are expressed as the mean ± standard deviation (SD) or median and interquartile range (IQR). P value <0.05 revealed statistical significance. DSSI, deep surgical site infection; APACHE II score, acute physiology and chronic health evaluation score; SOFA score, sequential organ failure assessment score; ICU, intensive care unit.

DSSI were also notoriously difficult to treat. It has been widely recognized that infection source identification and control as early as possible were critically important for the treatment of DSSI, as delays in administering appropriate treatment could precipitously affect the outcome (26,27). Currently, early diagnosis relied on blood tests for the detection of inflammation response related serum biomarkers, such as CRP and PCT (28,29). CRP had been used for many years, but its specificity had been challenged. PCT had been proposed as a more specific marker than CRP, although its specificity and sensitivity for the diagnosis of DSSI were relatively low (typically below 90%) (30,31).

Our data, which showed that 95.45% patients with DSSI had a high NFI, revealed the high sensitivity of the NFI in the prediction of DSSI. To gather further proof, the AUCs were compared. The AUC value of the NFI was 0.912, which was obviously higher than that of the CRP or PCT level.

Regarding the discrepancy in the predictive ability for the development of DSSI between the NFI of the abdominal drainage fluid and other serum biomarkers within three days after the laparotomy, the serum biomarkers were indicators of systemic inflammatory response rather than local infection response, offering limited diagnostic efficacy
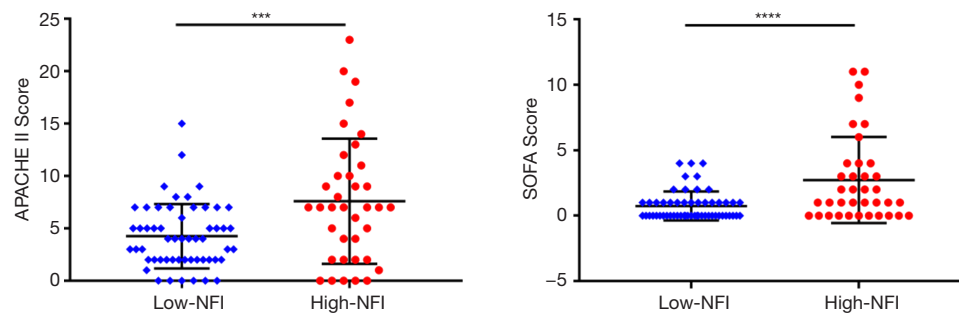
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Figure 3 Comparison of the APACHE II and the SOFA score in patients with a low/high NFI. The APACHE II and the SOFA sore were counted within three days after laparotomy. Both the mean APACHE II and SOFA score in the high-NFI group were higher. Long lines denote median values, short lines represent 25th to 75th percentiles. ***, P<0.001; ****, P<0.0001. APACHE, acute physiology and chronic health evaluation; SOFA, sequential organ failure assessment; NFI, NET formation index.

Table 3 Patients were divided into four subgroups in view of the NFI value and DSSI occurrence

	Low-NFI	High-NFI	n
DSSI	1	21	22
Non-DSSI	54	16	70
n	55	37	92

The 92 patients were divided into four subgroups according to the NET formation index (NFI) value and deep surgical site infection (DSSI) occurrence. DSSI occurred in 1 patient with low-NFI as well as 21 patients with high-NFI, 54 patients with a low-NFI as well as 16 patients with a high-NFI did not get DSSI.

(32,33). In addition, the serum biomarkers concentrations were rather erratic and their time course was not clearly in concert with the course of DSSI (34), prompting the discrepancy. The laboratory microbiological culture test remained a positive approach to identifying local infections after the laparotomy. However, the microbiological culture tests may take up to 72 hours, and their consistency remains an issue of controversy with false-negative results often observed (8). Although the biomarkers and microbiological culture tests would not predict the occurrence of DSSI within three days, they may require considerable time, effort and costs to measure. The measurement of the NFI in drainage fluid was a simple and prompt method to reflect local infections. This method took only few hours. Besides, the reagent and inspection equipment were easily obtained. Compared to pitfalls in serum biomarkers detection after venipuncture, the measurement of the NFI was painless and economical, avoiding the risk of spontaneous bleeding and phlebitis.

In spite of the great value of the NFI in the prediction of DSSI after laparotomy, no biomarker has been found to be greatly helpful to clinicians in clinical practice. In the present study, a high NFI was relative to the further occurrence of DSSI as well as the increased level of neutrophil count in serum. There may be a possible relationship between the neutrophil count in serum and the NFI in drainage fluid, but the details of the pathway and the underlying molecular mechanisms have not been deciphered. Different admission diagnosis may attribute to the difference in neutrophil count, but our data were not enough to prove the point. Studies speculated that a multitude of neutrophils will activate and then migrate to the surgical site and seroperitoneum in a short time (35). The more the number of activated neutrophils migrating to the surgical site, the more the number of NETs and cf-DNA forming in the drainage fluid (36). According to the calculation formula, exponential cf-DNA in the abdominal drainage fluid may lead to higher NFI. The OD value of the drainage fluid, which could reflect the concentration of cf-DNA in drainage fluid, was compared and no statistic difference was found in the OD value between four groups. In view of the complexity of DSSI, it is unlikely that a single ideal biomarker will ever be found. A combination of several biomarkers may be more effective, but this required further evaluation (34).

Some limitations of the present study need to be addressed. The PMN in this study was collected from the blood of healthy volunteers, the blood consumption was too high for general usage in clinical testing. MPO, citH3, or other NET formation biomarkers were not tested in this study, and the increased level of neutrophil count in serum contributed to a high NFI, making the

Table 4 Differences in laboratory examination results between patients with a low/high NFI, in the non-DSSI group

Variable	Non-DSSI with low-NFI (n=54)	Non-DSSI with high-NFI (n=16)	P
APACHE II score, median (IQR)	4 (2–6)	7 (4–9)	0.201
SOFA score, median (IQR)	0 (0–1)	1 (0–2)	0.108
Laboratory examinations			
White blood cells, 10^9/L, mean ± SD	8.84±3.88	8.76±2.14	0.798
Lymphocytes, 10^9/L, mean ± SD	1.05±0.57	0.87±0.38	0.371
Neutrophils, 10^9/L, mean ± SD	9.32±12.09	16.38±26.31	0.005
C-reactive protein, mg/L, mean ± SD	82.60±61.34	114.78±59.24	0.490
Procalcitonin, μg/L, median (IQR)	0.45 (0.14–1.35)	1.21 (0.43–1.83)	0.056

In this part, the 70 patients without deep surgical site infection (DSSI) were divided into two groups according to their NET formation index (NFI) value. A further analysis was focused on the differences in laboratory examination results between the two groups. Only the neutrophil count level between the two groups revealed statistical significance. Quantitative variables are expressed as the mean ± standard deviation (SD) or median and interquartile range (IQR). P value <0.05 reveals statistical significance. APACHE II score, acute physiology and chronic health evaluation score; SOFA score, sequential organ failure assessment score.

interpretation difficult. Additionally, the case samples and observation time were not enough, consequently, the link between the NET formation in the abdominal drainage fluid and mortality in patients after abdominal surgery was not analyzed. In future studies, MPO and citH3 levels will be tested, and we will collect PMN from blood of animals, so that this detection method can be widely used clinically. Meanwhile, whether the combination of various biomarkers increases the specificity of the prediction of DSSI occurrence, as well as the underlying molecular mechanisms between the neutrophil count and the NFI will be explored in detail. Furthermore, we wish to conduct further experiments and incorporate more single-disease cases in this research.

In summary, the present study primarily confirmed that the quantification of the NFI had considerable predictive values for early identification of DSSI. Our data highlighted that the NFI turned out to be a more rapid, sensitive and specific predictor of DSSI than traditional clinical infection indicators including CRP and PCT. Based on this research, further studies can be performed to elaborate the clinical value of the post-operative abdominal drainage fluid in the early diagnosis and prognosis of DSSI.

Acknowledgments

Footnote

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional ethics committee board of Jinling Hospital (No.: 2020NZKY-011-01) and informed consent was taken from all individual participants.

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