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INFECTION

Synovial fluid neutrophil extracellular traps could improve the diagnosis of periprosthetic joint infection

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Aims

This study aimed to explore the diagnostic value of synovial fluid neutrophil extracellular traps (SF-NETs) in periprosthetic joint infection (PJI) diagnosis, and compare it with that of microbial culture, serum ESR and CRP, synovial white blood cell (WBC) count, and polymorphonuclear neutrophil percentage (PMN%).

Methods

In a single health centre, patients with suspected PJI were enrolled from January 2013 to December 2021. The inclusion criteria were: 1) patients who were suspected to have PJI; 2) patients with complete medical records; and 3) patients from whom sufficient synovial fluid was obtained for microbial culture and NET test. Patients who received revision surgeries due to aseptic failure (AF) were selected as controls. Synovial fluid was collected for microbial culture and SF-WBC, SF-PNM%, and SF-NET detection. The receiver operating characteristic curve (ROC) of synovial NET, WBC, PMN%, and area under the curve (AUC) were obtained; the diagnostic efficacies of these diagnostic indexes were calculated and compared.

Results

The levels of SF-NETs in the PJI group were significantly higher than those of the AF group. The AUC of SF-NET was 0.971 (95% confidence interval (CI) 0.903 to 0.996), the sensitivity was 93.48% (95% CI 82.10% to 98.63%), the specificity was 96.43% (95% CI 81.65% to 99.91%), the accuracy was 94.60% (95% CI 86.73% to 98.50%), the positive predictive value was 97.73%, and the negative predictive value was 90%. Further analysis showed that SF-NET could improve the diagnosis of culture-negative PJI, patients with PJI who received antibiotic treatment preoperatively, and fungal PJI.

Conclusion

SF-NET is a novel and ideal synovial fluid biomarker for PJI diagnosis, which could improve PJI diagnosis greatly.

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Keywords: Periprosthetic joint infection, Neutrophil extracellular traps, Diagnosis

Article focus

- This study focused on the diagnostic value of a novel synovial fluid biomarker — neutrophil extracellular traps in periprosthetic joint infection (PJI) diagnosis.

Key messages

- This study showed that synovial fluid neutrophil extracellular traps (SF-NETs) have a good performance in PJI diagnosis, especially for culture-negative PJI, preoperative antibiotic treatment PJI, and fungal PJI. SF-NET is a novel and promising biomarker for PJI diagnosis.

Strengths and limitations

- This was the first study to explore the diagnostic efficacy of SF-NET in PJI, and showed that SF-NET could improve PJI diagnosis greatly.
- This was a single-centre study with a small sample size; a multicentre study is warranted to fully evaluate the diagnostic value of SF-NET.

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Introduction

Periprosthetic joint infection (PJI) is a devastating complication after joint arthroplasty; the incidence rate is about 1% to 2%.[1,2] Since there are growing numbers of patients who receive arthroplasty surgery, the number of PJIs is increasing year by year,[3] posing a challenge to orthopaedic doctors. Therefore, how to diagnose PJI precisely and treat it well is an important issue, which remains to be addressed.

Presently, there are still many challenges in diagnosing PJI, especially culture-negative PJI. Microbial culture is regarded as the 'gold standard' for PJI diagnosis, however due to the presence of biofilms, viable but non-cultural state organisms and fastidious bacteria, and the administration of antibiotics before sampling, the positive rates of microbial culture are far from satisfactory, varying from 47.1% to 80% in most centres according to previous reports,[4,5] and might be up to 90% after optimizing the culture methods in a few centres.[6-8] In the last few decades, the emergence of molecular diagnosis has initiated a new era for the microbial diagnosis of PJI,[9] especially next-generation sequencing (NGS), such as metagenomics next-generation sequencing (mNGS), which could detect all microorganisms in the sample at a time, including bacteria, fungi, viruses, and parasites, greatly improving the diagnosis of PJI. The sensitivity of mNGS in PJI diagnosis has been reported as up to about 92%.[10-13] However, due to its high testing cost and lack of availability in smaller institutions, the wide application of mNGS seems to be unfeasible. Moreover, the question of how to distinguish background bacteria from true pathogenic bacteria is another tough nut for microbiologists and surgeons to crack, especially for multiple bacterial PJI. Thus, it is urgent to develop a cheap, point-of-care test to diagnose PJI precisely.

Synovial fluid biomarkers have been proved helpful in PJI diagnosis.[14,15] Synovial fluid α defensin has been widely investigated in the diagnosis of PJI: it is secreted by neutrophils, playing a key role in bacterial clearance and infection control. Previous studies have showed that α defensin was a promising biomarker for PJI diagnosis;[16,17] the Synovial α Defensin Lateral Flow test (Synovasure; Zimmer-Biomet, USA) has been developed and was approved by the USA Food and Drug Administration (FDA). However, its diagnostic value in PJI is still controversial,[18,19] with a considerably large scope of sensitivities (from 67% to 100%) and specificities (from 68% to 100%) according to previous reports.[16,17,20] In recent research performed by Ivy et al,[18] the diagnostic value of synovial α defensin in PJI was not superior to that of synovial white blood cell (WBC) count and polymorphonuclear neutrophil percentage (PMN%), but its test costs were higher than synovial WBC and PMN% test. There are other synovial biomarkers, including interleukin (IL)-1β, IL-6, cysteine-rich with EGF-like domains 2 and calprotectin, etc,[15,21-25] but whether these really have a role in PJI diagnosis has not yet been established, as the sensitivities and specificities reported by previous studies are different.[15,21,22,25] Therefore, it is urgent to develop a novel biomarker to diagnose PJI precisely.

Neutrophils are effector cells of the innate immune system which play an important role in the process of infection defense. Neutrophils are recruited to the nidus after being activated by chemokines, and kill pathogens by means of phagocytosis or degranulation.

In recent years, it has been found that after being stimulated by pro-inflammatory factors, or pathogens, intracellular chromatin DNA and antibacterial granule proteins of neutrophils could be released and form a network, namely neutrophil extracellular traps (NETs), which could capture and kill pathogens, participating in the innate immune response.[26] It had been reported that NETs have an important role in the elimination of viral, bacterial, or fungal infections, and contribute to disease diagnosis and prognosis.[27-30] Previous studies have showed that the formation of NETs could be induced in the infections caused by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, etc, and their products,[31-34] which are among the microbiological profiles of PJI, and that NETs have a significant role in the defense of these infections. Thus, NETs might be involved in the pathogenesis of PJI and provide a novel synovial fluid biomarker for PJI diagnosis. The purpose of this study was to explore the value of synovial fluid NET in PJI diagnosis.

Methods

Patient selection. This study was approved by an Ethics Committee of First Affiliated Hospital, Fujian Medical University, and enrolled patients who received revision surgery due to aseptic failure (AF) or PJI in our centre from 2013 to 2021. Patients who were diagnosed as AF were selected as controls. The inclusion criteria were listed as follows: 1) patients who received revision surgery after arthroplasty in our centre; 2) patients with complete medical records, including CRP, ESR, synovial fluid WBC, PMN%, and so on; and 3) patients who have sufficient synovial fluid (SF) for microbial culture and SF-NET test. The exclusion criteria were: 1) patients who suffered from other inflammatory diseases at the same time, such as rheumatoid arthritis; 2) patients who suffered from other infectious diseases, such as pneumonia; and 3) patients with insufficient synovial fluid for NET test. The diagnosis of PJI was based on the Musculoskeletal Infection Society (MSIS) criteria.[35] The major criteria were: 1) a sinus tract communicating with the prosthesis, or 2) a pathogen isolated by culture from two separate tissue or fluid samples obtained from the affected prosthetic joint. The minor criteria were: 1) elevated ESR and CRP; 2) elevated synovial fluid WBC count; 3) elevated synovial fluid PMN%; 4) presence of purulence in the affected joint; 5) isolation of a microorganism in one periprosthetic tissue or fluid culture; and 6) > five neutrophils per high-powered field in five high-power fields observed from histological analysis of periprosthetic tissue at ×400 magnification. Patients

Table I. Demographic characteristics.

Parameters	PJI (n = 46)	AF (n = 28)	p-value
Sex, n (%)			0.677
Male	22 (47.83)	12 (42.86)	
Female	24 (52.17)	16 (57.14)	
Mean age, yrs (SD)	65.28 (11.0)	62.32 (10.91)	0.261†
Joint involved, n (%)			
Hip	23 (50.00)	18 (64.29)	0.231
Knee	23 (50.00)	10 (35.71)	
Median CRP, mg/l (IQR)	15.68 (9.78 to 33.85)	5.6 (3.68 to 9.30)	< 0.001‡
Median ESR, mm/h (IQR)	62 (28.5 to 89)	19.25 (17.05 to 22.83)	< 0.001‡
Median SF-WBC, 10^6/l (IQR)	3,416.5 (2,563.25 to 6,576)	1,585.5 (717 to 2,803)	< 0.001‡
Mean SF-PMN, % (SD)	74.82 (11.54)	52.71 (17.41)	< 0.001†

*Chi-squared test.
†Independent-samples *t*-test.
‡Mann-Whitney U test.
AF, aseptic failure; IQR, interquartile range; PJI, periprosthetic joint infection; SD, standard deviation; SF-PMN%, synovial fluid polymorphonuclear neutrophil percentage; SF-WBC, synovial fluid white blood cell.

who met one of the major criteria or at least four of minor criteria were diagnosed as having PJI.

Demographic characteristics. Overall, 60 PJI cases were enrolled in this study from 2013 to 2021 in our centre. Eight cases were excluded due to insufficient medical records, four cases were excluded since they were complicated with rheumatoid arthritis, and two cases were excluded since they refused to be included in this study; finally, 46 PJI cases were included, and 28 AF cases who received revision surgeries were selected as controls. The demographic characteristics are listed in Table I. There were 22 males and 24 females in the PJI group, and 12 males and 16 females in the AF group, with no difference in sex composition between the two groups (p = 0.68). The mean age of PJI cases was 65.28 years (standard deviation (SD) 11.0), and 62.32 years (SD 10.91) for AF cases.

Samples collection and microbial culture. Preoperative joint aspiration was performed routinely for pathogen detection, synovial fluid was used for WBC, PMN% test, and microbial culture; the rest of the synovial fluid was stored at -80°C for further experiment. Moreover, synovial fluid, synovium, and periprosthetic tissues were also collected intraoperatively. For microbial culture, synovial fluid was injected into aerobic and anaerobic BacT/AlertFN culture bottles (Becton Dickinson, Germany), and synovium and periprosthetic tissues were processed as in the previous study.[6] Briefly, tissues were transported to Eppendorf (Ep) tubes with 1 ml brain heart broth, vortexed and shaken for 15 minutes, and homogenized for 60 to 90 seconds in a fully automated rapid grinding instrument (JXFSTPRP-24, Jingxin Industrial, China) set at 40 Hz, then homogenates were inoculated on blood agar plates. To improve the positive rate of microbial culture, the culture time was extended to two weeks. Finally, the species identification and drug sensitivity test were performed via the Vitek II system (Biomerieux, USA).

Synovial fluid NET test. The NETs enzyme linked immunosorbent assay (ELISA) kit (Hu00596, BOSK biology engineering, China) was used for synovial fluid NET test. The NET test was performed according to manufacturer's instruction. In brief, 10 µl synovial fluid was diluted to 50 µl, 100 µl of horseradish peroxidase (HRP)-labelled antibody was added to each well, then the wells were sealed with a sealing membrane, and incubated at 37°C for 60 minutes. Afterwards, the reaction fluid was removed, and washed by washing liquid five times (one minute each). Then, 50 µl of substrate A and 50 µl of substrate B were added to each well, and incubated at 37°C for 15 minutes in the dark; 50 µl of stopping solution was added to each well to stop antigen-antibody reaction. The optical density (OD) values were obtained at 405 nm via microplate reader (iMark Microplate Reader; Bio-Rad Laboratories, USA). The linear regression curve was drawn according to the standard concentration and the corresponding OD value, and the concentration of each sample was calculated based the standard linear regression curve.

Statistical analysis. The quantitative data were expressed as mean and SD, and compared by independent-samples *t*-test if the data were normal distribution. These were expressed as median (interquartile range (IQR)) and compared by Mann-Whitney U test if the data were non-normally distributed. The qualitative data were expressed as absolute numbers (percentage), and compared by chi-squared test. All statistical analyses were performed on GraphPad Prism V7.0 (GraphPad Software, USA).

The receiver operating characteristic curve (ROC) of synovial NET, WBC, PMN%, and area under the curve (AUC) were obtained by MedCalc V19.6.4 to assess the diagnostic efficacies. The optimal cut-off value of NET was determined based on Youden's Index (sensitivity + specificity -1). The sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated, and the differences of Se and Sp between various methods were compared

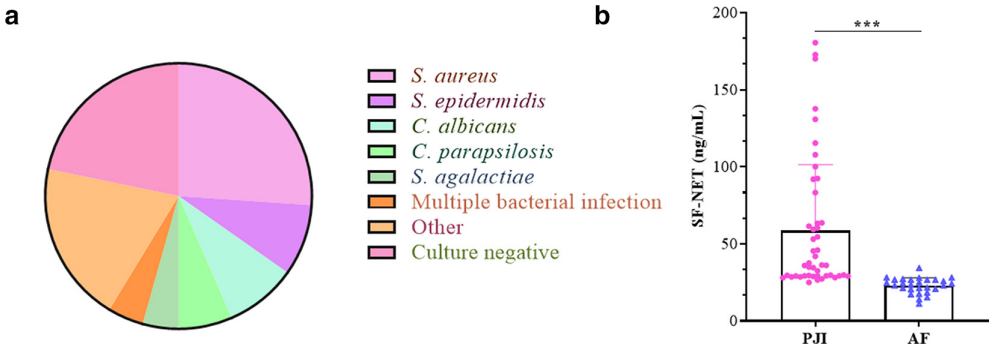

Fig. 1

a) The pathogenic microorganisms isolated for periprosthetic joint infection (PJI). *S. aureus, Staphylococcus aureus; S. epidermidis, Staphylococcus epidermidis; C. albicans, Candida albicans; C. parapsilosis, Candida parapsilosis; S. agalactide, Streptococcus agalactiae.* b) The levels of synovial fluid neutrophil extracellular traps (SF-NET) in PJI and aseptic failure (AF) patients. ***p < 0.001, Mann-Whitney U test.

by McNemar's test. A p-value < 0.05 was considered to be statistically significant.

Results

The median levels of CRP, ESR, SF-WBC, and SF-PMN% in PJI group (15.68 mg/l (IQR 9.78 to 33.85), 62 mm/h (IQR 28.5 to 89), 3,416.5 × 10^6/l (2,563.25 to 6,576), and mean 74.82% (SD 11.54%), respectively) were significantly higher than those in AF group (5.6 mg/l (3.68 to 9.30), 19.25 mm/h (17.05 to 22.83), 1,585.5 × 10^6/l (717 to 2,803), mean 52.71% (SD 17.41%) (p < 0.001, Mann-Whitney U test). *Staphylococcus aureus* was the most common pathogen isolated from PJI cases (12 cases), followed by *Staphylococcus epidermidis* (four cases). There were two PJI cases with multiple bacterial infection, and ten culture-negative PJI cases (Figure 1a).

The levels of synovial fluid NET in PJI group were significantly higher than those in AF group. To assess the potential role of NET in PJI diagnosis, the synovial fluid collected from PJI and AF cases was detected by enzyme-linked immunosorbent assay (ELISA). The results showed that the SF-NET of PJI group was significantly higher than that of AF group ((58.62 ng/ml (SD 6.33) vs (22.94 ng/ml (SD 0.95); p < 0.001) (Figure 1b). To further evaluate the diagnostic value of SF-NET in PJI, ROC curve analysis of SF-NET, CRP, ESR, SF-WBC, and SF-PMN% were used, and the area under the ROC curve (AUC) was calculated. The ROC curves were summarized in Figure 2. The AUC of SF-NET was 0.971 (SD 0.0177; 95% confidence interval (CI) 0.903 to 0.996), which was higher than that of CRP (0.83 (SD 0.480; 95% CI 0.725 to 0.908); p = 0.010), ESR (0.797 (SD 0.0547; 95% CI 0.687 to 0.881); p = 0.002), SF-WBC (0.839 (SD 0.0485; 95% CI 0.735 to 0.914); p = 0.010), and SF-PMN% (0.861 (SD 0.0469; 95% CI 0.761 to 0.930); p = 0.031). The above results showed that SF-NET might be a promising synovial biomarker for PJI diagnosis.

Comparison diagnostic efficacies of different diagnostic indexes for PJI. The optimal cut-off value of SF-NET was determined according to Youden's Index, with 28.03 ng/ml, then the diagnostic efficacy was calculated, including

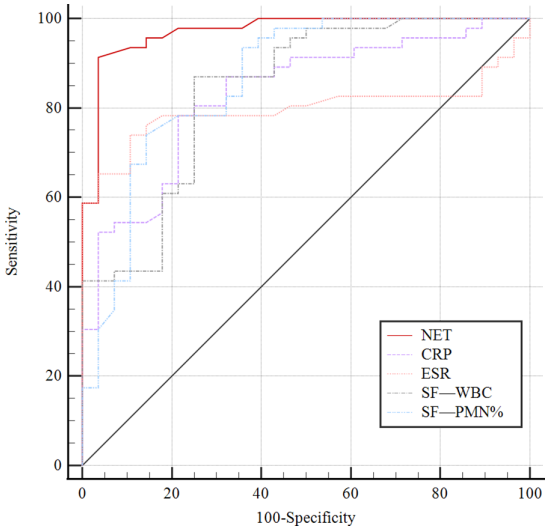

Fig. 2

The receiver operating characteristic curve of neutrophil extracellular traps (SF-NET), CRP, ESR, synovial white blood cell count (SF-WBC), and polymorphonuclear neutrophil percentage (SF-PNM%).

sensitivity (Se), specificity (Sp), accuracy (Ac), positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), and negative likelihood ratio (LR-), and compared with that of other diagnostic indexes. The results are summarized in Table II. The Se of SF-NET was 93.48% (95% CI 82.10% to 98.63%), Sp was 96.43% (95% CI 81.65% to 99.91%), Ac was 94.60% (95% CI 86.73% to 98.50%), PPV was 97.73%, NPV was 90%, LR+ was 26.17, and LR- was 0.068. Microbial culture (MC) was regarded as the 'gold standard' for PJI diagnosis, however the diagnostic efficacy of MC in this study was not so ideal, with 78.26% (95% CI 63.64% to 89.05%) Se, 82.14% (95% CI 63.11% to 93.94%) Sp, 79.73% (95% CI 68.78% to 88.19%) Ac, 87.80% PPV, 69.70% NPV, 4.38 LR+, and 0.27 LR-. The sensitivity was significantly lower than that of SF-NET (F value = 4; p = 0.039, McNemar's test). In summary, as Table II shows,

Table II. Comparison diagnostic efficacies of different indexes.

Parameters	Se (100%)	Sp (100%)	Ac (100%)	PPV (100%)	NPV (100%)	LR+	LR-
MC	78.26	82.14	79.73	87.80	69.70	4.38	0.27
CRP	71.74	78.57	74.32	84.62	62.86	3.35	0.36
ESR	73.91	85.71	78.38	89.47	66.67	5.174	0.30
SF-WBC	63.04	75.00	67.57	80.56	55.26	2.52	0.49
SF-PMN%	78.26	71.43	75.68	81.82	66.67	2.74	0.30
SF-NET	93.48	96.43	94.60	97.73	90.00	26.17	0.068

Ac, accuracy; LR+, positive likelihood ratio; LR-, negative likelihood ratio; MC, microbial culture; NPV, negative predictive value; PPV, positive predictive value; Se, sensitivity; SF-NET, synovial fluid neutrophil extracellular traps; SF-PMN, synovial fluid polymorphonuclear neutrophil percentage; SF-WBC, synovial fluid white blood cell; Sp, specificity.

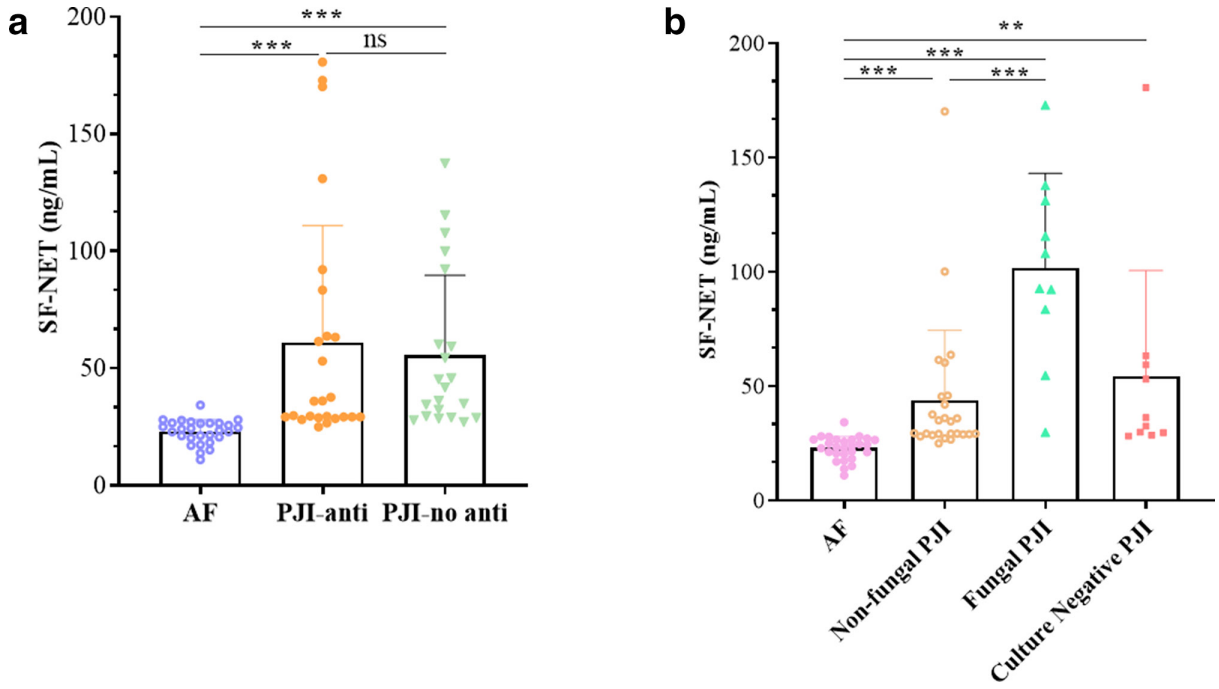

Fig. 3

a) Comparison of synovial fluid neutrophil extracellular traps (SF-NET) levels between aseptic failure (AF) group, periprosthetic joint infection (PJI) patients who received antibiotic treatment (PJI-anti), and those who did not (PJI-no anti). b) Comparison of SF-NET levels between AF group, fungal or non-fungal PJI, and culture-negative PJI. ** $p < 0.01$; ***$p < 0.001$. ns, not statistically significant.

compared with other PJI diagnostic indexes, SF-NET has a higher diagnostic efficacy in diagnosing PJI.

SF-NET could promote PJI diagnosis under certain conditions. It is a common phenomenon clinically that some suspected PJI patients have received antibiotics treatment before hospitalization or revision surgeries, which significantly lowers the positive rates of microbial culture and serum inflammatory indexes, finally leading to the missed diagnosis of PJI. To evaluate whether SF-NET could promote PJI diagnosis in this group of patients, the levels of SF-NET between PJI patients who have received and not received antibiotic treatment were compared. As Figure 3a shows, the levels of SF-NET were not significantly affected by the administration of antibiotics, and the levels of SF-NET in PJI cases who have received and not received antibiotic treatment were both significantly higher than those of AF cases ($p < 0.001$, Mann-Whitney U test). The Se and Sp of SF-NET was 92% and 96.43%, and 90.47% and 96.43%, in PJI patients who received or not received antibiotic treatment, respectively (Table III). Due to the administration of antibiotics, some PJI patients' microbial culture results might be negative. In order to estimate the value of SF-NET in diagnosing culture-negative PJI, we compared the level of SF-NET between culture-negative PJI cases and AF cases; the results showed that SF-NET in culture-negative PJI cases was higher than that of AF cases ($p = 0.001$, Mann-Whitney U test) (Figure 3b), the Se and Sp for SF-NET in culture-negative PJI diagnosis was 100% and 96.43%, respectively, with the optimal cut-off value of 28.1 ng/ml (Table III).

Interestingly, when we classified PJI as fungal PJI (ten cases) and non-fungal PJI (26 cases) according to the types of pathogenic bacteria, we found that the levels of SF-NET in fungal PJI group were significantly higher than

Table III. The diagnostic efficacies of synovial fluid neutrophil extracellular traps in different periprosthetic joint infection subgroups.

Parameters	Se (100%)	Sp (100%)	Ac (100%)	PPV (100%)	NPV (100%)	AUC
PJI-anti	92	96.43	94.34	95.83	93.10	0.96
PJI-no anti	90.47	96.43	93.88	95	93.10	0.98
Fungal PJI	100	96.43	94.87	90.91	100	0.99
Non-fungal PJI	84.62	96.43	90.74	95.65	87.10	0.96
Culture-negative PJI	100	96.43	94.87	90.91	100	0.98

Ac, accuracy; PJI-no anti, PJI patients who did not receive antibiotic treatment before hospitalization or revision surgeries; AUC, area under the curve; non-fungal PJI, PJI not caused by fungi; NPV, negative predictive value; PJI, periprosthetic joint infection; PJI-anti, PJI patients who have received antibiotic treatment before hospitalization or revision surgeries; PPV, positive predictive value; Se, sensitivity; Sp, specificity.

those of non-fungal PJI group (43.76 ng/ml (SD 6.02) vs 101.70 ng/ml (SD 13.07); $p < 0.001$, Mann-Whitney U test) (Figure 3b). All these cases were precisely diagnosed as PJI according to SF-NET with 28.03 ng/ml cut-off value, but only six, five, three, and eight cases were diagnosed as PJI by serum CRP, ESR, SF-WBC, and SF-PMN%, respectively. However, when we used optimal cut-off value determined by Youden's index in the subgroup analysis, the optimal cut-off value for fungal PJI was 28.84 ng/ml, the Se and Sp were 100% and 96.43%, respectively (Table III); the optimal cut-off value for non-fungal PJI was 28.31 ng/ml according to Youden's index, while the Se and Sp were 84.62% and 96.43%, respectively (Table III), indicating that SF-NET might be more prominent in the diagnosis of fungal PJI.

Discussion

PJI is a devastating complication after arthroplasty. In the last few decades, we have witnessed a significant increase in the number of PJIs, and this is projected to continue in the coming decades.[2] Presently, the precise diagnosis of PJI is still a challenge for surgeons, especially for culture-negative PJIs. Therefore, it is of great importance to find an ideal strategy to improve PJI diagnosis. Although microbial culture plays an important role in diagnosis and treatment, its positive rate is limited due to preoperative administration of antibiotics and the existence of biofilm, fastidious bacteria,etc. To tackle this limitation, molecular diagnosis was introduced; it is reported that 16 S polymerase chain reaction (PCR) could improve the diagnosis of PJI to some extent.[36] However, the diagnostic value of PCR in PJI has not yet been fully elucidated, and is limited to poly-microbial PJI diagnosis. The advent of NGS has taken molecular diagnosis to a new level, and appeals to surgeons and clinical microbiologists. But the presence of background bacteria is a frustrating problem for NGS; moreover, the costs of NGS are so high that many patients cannot afford the costs.

Synovial fluid biomarker detection is an effective and easy strategy in PJI diagnosis, which has aroused widespread interest. Synovial fluid IL-1β/6, and α-defensin, have been widely investigated, but there are disputes about the role of these biomarkers in diagnosing PJI,[15] and no unified biomarkers have been yet universally acknowledged for diagnosis. Thus, novel biomarkers should be explored to improve PJI diagnosis. In this study, we firstly explored the diagnostic value of a novel synovial biomarker-NET in PJI. We showed that the levels of SF-NET in PJI were significantly higher than that of the AF group, and performsedwell in PJI diagnosis. Recent studies have showed that other neutrophilic biomarkers, like neutrophilic gelatinase-associated lipocalin (NGAL) and leucocyte esterase (LE), also have a role in the diagnosis of PJI. Although diagnostic efficacy varied between different studies,[37,38] Dijkman et al[39] have evaluated these biomarkers comprehensively in a retrospective cohort study of 86 patients with suspected PJI after TKA, and showed that the diagnostic efficacy of NGAL (Se: 92%; Sp: 83%) was significantly higher than that of LE (Se: 39%; Sp: 88%), but not superior to that of SF-WBC (Se: 92%; Sp: 84%). We, however, showed that the SF-NET had a good performance in PJI diagnosis (Se: 93.48%; Sp: 96.43%), which was better than that of SF-WBC, and LE, NGAL compared with Dijkman et al[39] studies. Therefore, SF-NET might be provided as a novel and ideal synovial fluid biomarker for PJI diagnosis.

Our further analysis showed that SF-NET could promote the diagnosis of culture-negative PJIs, preoperative antibiotic treatment PJIs and, interestingly, fungal PJIs. Previous studies have shown that in fungal PJI, the levels of CRP and ESR were slightly elevated, even normal. Azzam et al[40] reviewed 31 fungal PJI cases from 1999 to 2006, and found that the mean ESR was 54 mm/hr (12 to 104 mm/hr), the mean CRP was 17.5 mg/l (0.6 to 73.9 mg/l), the mean SF-WBC of 11 patients was 8,761/ml (440 to 26,700/ml), and the mean SF-PMN% was 76% (19% to 94%) – these three indexes do not amount to much in fungal PJI diagnosis. LE was reported to be a potential synovial fluid biomarker for bacterial PJI, however measuring LE activity does not add any additional benefit because yeast cells cannot be differentiated from red cells or amorphous crystals.[41] Moreover, although α-defensin is a promising synovial fluid biomarker in diagnosing PJI, but there were few studies with regard to the value of α-defensin in fungal PJI diagnosis, and Deirmengian et al[42] showed that the levels of α-defensin did not vary from bacterial species.

Previous studies demonstrated that NET has an indispensable role in host defence against fungal infection.[43]

Fungal cell wall polysaccharides (mannan, β-dextran, etc.) and aspartic protease secreted by fungi could trigger NET to trap pathogens, limiting, eliminating, and further preventing the spread of infection.[43] For example, the extracellular killing of *Candida albicans* hyphae and yeast spores largely depends on NET, accounting for about 20% to 30% of *C. albicans* clearance.[29,44] In this study, all fungal PJIs were precisely diagnosed by SF-NET, with a sensitivity of 100% and specificity of 96.43%, indicating that SF-NET might have a promising role in fungal PJI diagnosis.

There were some limitations this study: first, this was a single-centre study with a small sample size, and a multicentre study with a larger sample size is warranted to fully evaluate the diagnostic value of SF-NET. Second, the present work has included many fungal PJI cases, and whether this have an impact on the results was not clear; further studies with fewer fungal PJI cases should be performed.

In conclusion, the present study showed that the level of SF-NET was increased in PJI, and had good diagnostic efficiency, especially for culture-negative PJI, preoperative antibiotic treatment PJI, and fungal PJI. Thus, SF-NET could be a novel and ideal synovial fluid biomarker for the diagnosis of PJI.

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