ORIGINAL RESEARCH

Plasma Fibrinogen as a Diagnostic Marker of Infection in Patients with Nonunions

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Background: The timely and accurate diagnosis of infected nonunion is challenging, and there is a need for more efficient biomarkers. Previous studies have shown that fibrinogen plays an important role in mediating inflammation in bacterial infections and, therefore, could be a valuable biomarker for infected nonunion. The purpose of this study was to evaluate and compare the performance of plasma fibrinogen and other traditional blood markers for the diagnosis of infected nonunion.

Materials and Methods: We retrospectively studied 146 patients who underwent surgery for primary nonunion between January 2018 and January 2020. The patients were divided into those with infected nonunion (n = 55) and those with aseptic nonunion (n = 91). The preoperatively analyzed parameters were plasma fibrinogen, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) level, and white blood cell (WBC) count. Receiver operating characteristic (ROC) curve analysis was used to assess the sensitivity and specificity of the biomarkers, and Youden's index was calculated to determine their optimal cut-off values.

Results: The plasma fibrinogen values were significantly higher (p < 0.001) in the patients with infected nonunion than in those with aseptic nonunion. ROC curve analysis showed that plasma fibrinogen had a high value of area under the curve (0.816), which indicated that it had good diagnostic ability. Further, at the optimal threshold value of 2.75 g/L, plasma fibrinogen had the highest sensitivity (78.2%; 95% CI = 64.6–87.8) and good specificity (82.4%; 95% CI, 72.7–89.3).

Conclusion: In comparison to the traditional markers of infection, plasma fibrinogen showed good diagnostic ability for the detection of infected nonunion. It may have potential as a practical and cost-efficient biomarker for the diagnosis of infected nonunion.

Keywords: infected nonunion, diagnostic test, plasma fibrinogen, blood biomarkers

Introduction

Infected nonunion is a catastrophic complication that often occurs after open reduction and internal fixation; it can delay healing, lead to permanent functional loss, or even necessitate amputation of the affected limb.[1] The management of infected nonunions is usually difficult, as it goes beyond the technical aspects of fracture fixation or the features of the bone.[2–4] The first priority is to treat and eradicate the infection, and only then is definitive nonunion treatment attempted; therefore, the key factor affecting treatment is whether the nonunion is infected.[5] However, there has been a decrease in the number of patients with typical clinical manifestations of infection and an increase in the number of patients with quiescent infections. Therefore, the diagnosis of infected nonunion is difficult, and it remains

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one of the most challenging musculoskeletal complications in trauma surgery that can have devastating consequences if not managed properly.

Serological examination is always the first choice among clinicians for the diagnosis of infected nonunion and assessment of its invasiveness and spread. Among the various types of tests available, tests that measure blood biomarkers, including white blood cell count (WBC), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP), are simpler and more practical than others. However, these blood biomarkers have limitations and are often challenging.[6] Therefore, it is essential to identify additional laboratory tests that can help in the diagnosis of infected nonunions.

Recent studies have shown that systemic and local infections result in fibrinolytic activity, and therefore, coagulation-related indicators, including D-dimer and fibrinogen, have been investigated for their potential use as markers of infected nonunions.[7,8] For example, D-dimer or fibrinogen levels have been proven to be diagnostic markers for periprosthetic joint infection (PJI).[9,10] Further, Wang et al[11] demonstrated that serum D-dimer was a promising marker for the preoperative diagnosis of infected nonunion, and it had better sensitivity and specificity than WBC, ESR, and CRP. Fibrinogen also plays a key role in activating and mediating the inflammation process,[12,13] but to the best of our knowledge, no study has evaluated the use of this indicator for the diagnosis of infected nonunion. It might be beneficial to investigate its diagnostic potential for infected nonunion, as fibrinolysis analysis is feasible at most institutions and does not incur a high cost. Therefore, the aim of the present study was (1) to investigate the diagnostic value of the preoperative markers plasma fibrinogen, WBC, CRP, and ESR in bone nonunion patients with suspected infection, and (2) to compare the sensitivity and specificity of these blood biomarkers.

Materials and Methods
Patients

This study included a total of 179 consecutive patients who were surgically treated for primary nonunion between January 2018 and January 2020. Patients with inflammation related to factors other than orthopedic infection, viral infection, rheumatic disease, history of hypercoagulation disorders, heavy smoking, malignancies, cardiovascular and cerebrovascular disease, and liver diseases, were

excluded. Patients were also excluded if they received antibiotics before surgery or if the findings of their examinations were incomplete. Ultimately, 146 patients (114 males and 32 females) were included in the study. Informed consent was obtained from the participants (or their guardians). Ethical approval was obtained from the Clinical Research Ethics Committee of The Affiliated Drum Tower Hospital of Nanjing University Medical School. The methods were carried out in accordance with the relevant guidelines and regulations.

A nonunion was defined as radiographic evidence of non-progression of healing for at least 3 months, or lack of healing at 9 months after the initial injury.[14] Infected nonunion was defined according to the AO/ASIF criteria.[15] First, multiple gross tissue specimens (≥5 samples) were obtained intraoperatively and cultured. Infection was considered to be present if at least two cultures of the intraoperative sample were positive for the same organism. Based on whether infection was present, the patients were divided into Group A (infected nonunion, n = 55) and Group B (aseptic nonunion, n = 91).

The medical records of the included patients were well documented and carefully reviewed, and included baseline demographics (ie, sex and age), body mass index, and the involved location. The patients' fasting venous blood samples were collected on the day of admission, and within 1–2 h, the samples were sent to our hospital's clinical laboratory for blood routine examination (the measured variables included plasma fibrinogen levels, ESR, CRP, and WBC count). Antibiotics were administered after intraoperative specimens were collected, unless the patient needed anti-infective therapy urgently. Patients who were administered antibiotic therapy before the procedure were excluded, as explained earlier.

Statistical Analysis

Parametric data were expressed as mean ± SD (standard deviation) and analyzed using Student's t-test. Categorical variables were expressed as absolute numbers and analyzed using the chi-squared test. Non-parametric data were presented as median and analyzed using the Mann–Whitney U-test. A p value of <0.05 was considered to indicate statistical significance.

The diagnostic value of each marker for the assessment of infected nonunion was determined by receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC) and 95% confidence interval (CI) were calculated. Based on the AUC value, the discriminatory

capacity was interpreted as excellent (0.9 to 1), good (0.8 to 0.89), fair (0.7 to 0.79), or poor (0.6 to 0.69), and values of 0.5–0.59 indicated that the marker had no discriminatory capacity. The optimal threshold value of each marker for the diagnosis of infected nonunion was determined by calculating the Youden J index (J = [sensitivity + specificity] − 1). Further, the sensitivity, specificity, positive predictive value, and negative predictive value of the markers were calculated. All statistical analyses were performed using STATA version 18.0 (SPSS Inc., Chicago, IL, USA).

Results

The characteristics of the enrolled patients are depicted in Table 1. There was no statistically significant difference between the patients with infected nonunion (Group A) and aseptic nonunion (Group B) with regard to age, gender, BMI, and involved location (p > 0.05). With regard to the laboratory parameters, the median WBC counts were comparable between the two groups. In contrast, the median CRP, ESR, and plasma fibrinogen were significantly higher in group A than in group B (Table 2). ROC curve analysis showed that ESR had the highest AUC (0.885), and it was followed by plasma fibrinogen (AUC = 0.816). The AUCs of both these markers were higher than 0.8, which indicates good diagnostic value. The AUC for CRP was 0.716, which indicates fair diagnostic value. However, the WBC count had the lowest AUC of 0.585, which indicates poor diagnostic value (Figure 1).

The optimal threshold values of each marker for the diagnosis of infected nonunion are shown in Table 3. ESR was found to have a sensitivity of 69.1% (95% CI, 55.0–80.5) and a specificity of 92.3% (95% CI, 84.3–96.6). The sensitivity of CRP and WBC was 54.5% (95% CI, 40.7–67.8%) and 38.2% (95% CI, 25.7–52.3%), respectively,

Table 1 Patient Characteristics of the Two Groups

	Group A (n = 55)	Group B (n =91)	P-value
No of women	4/51	15/76	0.109
Age (year, mean ± SD)	48.9 ± 15.0	46.5± 13.3	0.309
BMI (kg/m2, mean ± SD)	24.1±3.7	23.8±3.5	0.125
Nonunion site (Lower Extremity)	49/6	77/14	0446

Notes: Group A = infected nonunion; Group B = aseptic nonunion. *P<0.05 indicated significances.
Abbreviation: BMI, body mass index.

Table 2 Comparison of the Tested Markers in the Two Groups

	Group A (n = 55)	Group B (n = 91)	P-value
WBC (10^9/μL)			0.087
Median	6.5	6.4	
P25, P75	5.5 ~ 8.2	5.5 ~ 7.1	
CRP (mg/L)			<0.001*
Median	6.6	3.5	
P25, P75	3.6 ~ 17.5	2.6 ~ 5.1	
ESR (mm/hr, median)			<0.001*
Median	23.0	6.0	
P25, P75	13.0 ~ 34.0	4.0 ~ 9.0	
Plasma Fibrinogen (mg/L)			<0.001*
Median	3.3	2.4	
P25, P75	2.8 ~ 5.1	2.0 ~ 2.8	

Notes: Group A = infected nonunion; Group B = aseptic nonunion. *P<0.05 indicated significances.
Abbreviations: WBC, white blood cell; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

and their specificity was 86.8% (95% CI, 77.7–92.7%) and 85.7% (95% CI, 76.4–91.9%), respectively. Plasma fibrinogen had the highest sensitivity at 78.2% (95% CI,
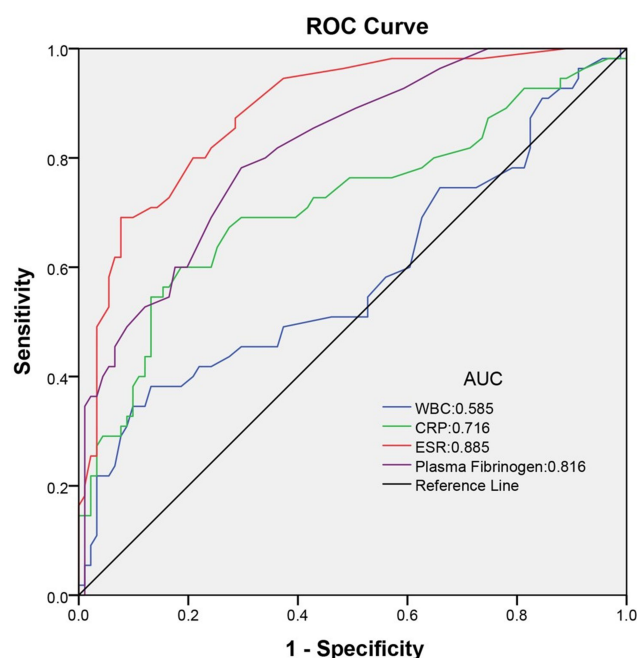

Figure 1 ROC curve shows the infected nonunion predictive value of WBC, CRP, ESR, and plasma fibrinogen.
Abbreviations: WBC, white blood cell; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

Table 3 The Diagnostic Value of Tested Markers in Patients

Variables	AUC(95% CI)	Optimal Cutoff Value	Sensitivity	Specificity	PPV	NPV
WBC	0.585 (0.484–0.685)	7.65×10^9/L	0.382	0.857	0.618	0.696
CRP	0.716 (0.624–0.807)	6.35 mg/L	0.545	0.868	0.714	0.612
ESR	0.885 (0.830–0.940)	17.5 mm/h	0.691	0.923	0.783	0.674
Plasma Fibrinogen	0.816 (0.747–0.885)	2.75 g/L	0.783	0.824	0.729	0.862

Abbreviations: WBC, white blood cell; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; AUC, areas under the curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

64.6–87.8%), and it also had good specificity (82.4%; 95% CI, 72.7–89.3%) (Table 3).

Discussion

In this study, our data demonstrated that compared with traditional inflammatory markers (WBC count, CRP, and ESR), plasma fibrinogen had the highest sensitivity (78.2%) and good specificity (82.4%) at a cut-off value of 2.75 g/L.

Plasma fibrinogen is a basic indicator that can easily be tested and applied preoperatively. It is an acute-phase protein, and its primary function is to stop excessive bleeding by forming a fibrin-based blood clot to occlude blood vessels, after enzymatic conversion to fibrin (catalyzed by thrombin) in tissue and at the sites of vascular injuries.[16] Some studies showed that fibrinogen plays a key role in activating and mediating the inflammation process,[17] and is a useful predictive marker for a variety of inflammation-related pathologies such as appendicitis,[18] periodontitis,[19] malaria,[20] and sepsis.[21] In addition, Li et al[22] performed a multicenter retrospective study of 565 patients and demonstrated that plasma fibrinogen levels were significantly higher in patients with PJI than in patients with aseptic failure (median: 4.82 g/L vs 3.11 g/L, respectively). At an optimal threshold value of 4.01 g/L based on Youden's index, plasma fibrinogen was found to have good sensitivity and specificity for the diagnosis of PJI, as it had values similar to those of classical markers, including CRP and ESR. Similarly, Klim et al[9] and Xu et al[23] also reported that fibrinogen was a practical biomarker for the detection of PJI. In our study, we found that the plasma fibrinogen level was significantly higher in the infected nonunion group than in the aseptic nonunion group (p < 0.05). The present findings corroborate the previous studies, as plasma fibrinogen had the highest sensitivity (78.2%; 95%

CI, 64.6–87.8) and good specificity (82.4%; 95% CI, 72.7–89.3). The optimal threshold value of plasma fibrinogen was 2.75 g/l, and the AUC for plasma fibrinogen was 0.816, which was the second-highest AUC value in our study and consistent with data from previous studies. Thus, plasma fibrinogen has potential as a diagnostic marker of infected nonunion.

WBC count, CRP, and ESR are the most commonly used biomarkers for the diagnosis of infected nonunion. Based on our data, the new optimal predictive cutoff for the traditional inflammatory markers WBC count, CRP, and ESR are 7.65×10^9/L (sensitivity, 38.2%; specificity, 85.7%), 6.35 mg/L (sensitivity, 54.5%; specificity, 86.8%), and 17.5 mm/h (sensitivity, 69.1%; specificity, 92.3%), respectively. However, their sensitivities were lower than the sensitivity of plasma fibrinogen.

There are several limitations to our study that should be taken into consideration when interpreting our findings. First, this study had a retrospective design, which has its inherent biases as a result of, for example, inaccurate data in the medical records. Second, we excluded patients with some diseases such as malignancy and autoimmune disease; therefore, the number of patients and subgroup analysis were limited. In the future, studies with larger samples should be conducted to obtain more robust evidence. Finally, the use of antibiotics and anticoagulants before admission was not recorded in the electronic records of some patients, and this might have affected our results.

Conclusions

In the present study, plasma fibrinogen levels were found to be significantly higher in patients with infected nonunion than those with aseptic nonunion. Further, compared

with traditional inflammatory markers, plasma fibrinogen was found to have high sensitivity and good specificity for the diagnosis of infected nonunion.

Abbreviations

AUC, area under the curve; CI, confidence interval; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; PJI, periprosthetic joint infection; ROC, receiver operating characteristic; SD, standard deviation; WBC, white blood cell.

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Disclosure

The authors report no conflicts of interest for this work. Xiang-Jin Wang and Zhen Wang are co-first authors for this study.

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