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ORIGINAL RESEARCH

A Clinical Diagnostic Study: Fibulin-2 is a Novel Promising Biomarker for Predicting Infection

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ABSTRACT

Introduction: Infection remains a major cause of morbidity and mortality in hospital. As uncontrolled early infection may develop into systemic infection and eventually progress to sepsis, it is important to address infection at an early stage. Furthermore, early detection and prompt diagnosis of infection are the basis of clinical intervention. However, as a result of the interference of complex aetiologies, including fever and trauma, problems regarding the sensitivity and specificity of current diagnostic indices remain, such as for C-reactive protein (CRP), procalcitonin (PCT), white blood cells (WBC), neutrophil ratio (NEU%), interleukin-6 (IL-6) and D-dimer. As a result, there is an urgent need to develop new biomarkers to diagnose infection.

Methods: From January to October 2021, consecutive patients in the emergency department (ED) were recruited to investigate the feasibility of fibulin-2 as a diagnostic indicator of early

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infection. Fibulin-2 concentrations in plasma were determined with enzyme-linked immunosorbent assay (ELISA). The performance of fibulin-2 for predicting infection was analysed by receiver operating characteristic (ROC) curves.

Results: We found that the plasma fibulin-2 level was elevated in patients with infection compared with those without infection. ROC curve analysis showed that the area under the curve (AUC) for fibulin-2 was 0.712. For all patients included, the diagnostic ability of fibulin-2 (AUC 0.712) performed as well as CRP (AUC 0.667) and PCT (AUC 0.632), and better than WBC (AUC 0.620), NEU% (AUC 0.619), IL-6 (AUC 0.561) and D-dimer (AUC 0.630). In patients with fever, fibulin-2 performed as well as PCT and better than the other biomarkers in infection diagnosis. In particular, fibulin-2 performed better than all these biomarkers in patients with trauma.

Conclusion: Fibulin-2 is a novel promising diagnostic biomarker for predicting infection.

Keywords: Infection; Fibulin-2; PCT; CRP; Biomarker

Key Summary Points

Detection of infection at an early stage is important. However, there are still some problems in the sensitivity and specificity of current diagnostic indices.

Secreted fibulin-2 was significantly upregulated in the plasma of patients with infection compared to uninfected volunteers. As fibulin-2 can be easily detected by enzyme-linked immunosorbent assay (ELISA), we speculated that it is likely to be a potential biomarker for infection.

The levels of fibulin-2 were significantly higher in the patients with bacterial, fungal or viral infection than in the patients without infection.

In all involved patients, the diagnostic ability of fibulin-2 performed as well as C-reactive protein (CRP) and procalcitonin (PCT) and better than white blood cells (WBC), neutrophil ratio (NEU%), interleukin-6 (IL-6) and D-dimer. In patients with fever, fibulin-2 performed as well as PCT and better than the other biomarkers in infection diagnosis. In particular, fibulin-2 performed better than all these biomarkers in patients with trauma.

INTRODUCTION

Infection can be local or systemic and is caused by the colonization, expansion and invasion of microorganisms, including bacteria, fungi, viruses and others [1]. Although considerable research has shed some light on the mechanism, diagnosis and treatment of infection, it remains a major cause of morbidity and mortality today [2, 3]. A common process of infection development is that early local infection develops into systemic infection and eventually into sepsis. Despite new advances in the treatment and prevention of infectious diseases, the incidence of sepsis is increasing. Approximately 48.9 million patients with sepsis were confirmed annually worldwide [4]. In particular, infectious COVID-19 has led to more than one million deaths in the first half a year of the pandemic [5]. Overall, early detection and prompt diagnosis of infection are important to inform clinical intervention to control and prevent infection at an early stage and ultimately reduce the morbidity and mortality of sepsis [6, 7].

In general, the initial signs and symptoms of infection are frequently nonspecific, which often leads to a late diagnosis, especially when coupled with the presence of interfering factors, such as different pathogenic microorganisms, fever and non-infection inflammatory responses caused by trauma. Indeed, over one-third of patients with infection presented to the hospital with vague symptoms not specific for infection. The diagnosis of infection and the

subsequent medicine administration are accordingly delayed in these patients [8]. Therefore, a rapid and reliable detection method to rule out infection will contribute to timely clinical decision-making and improve patient outcomes [7, 9]. At present, infection diagnosis is based on microbiological culture, biochemical methods and molecular techniques [10]. Although microbiologic culturing remains the gold standard for detecting infection, it is time-consuming and requires at least 1–2 days. Moreover, only 5–10% of blood cultures performed in hospitals show microorganisms, and negative cultures do not exclude the presence of infection [11, 12]. C-reactive protein (CRP), procalcitonin (PCT), white blood cells (WBC), neutrophil ratio (NEU), interleukin-6 (IL-6) and D-dimer are widely reported as immunologic biomarkers for diagnosing infectious diseases [13–17], but only CRP and PCT are commonly used as clinical indicators. Although PCT is a good negative predictive indicator, it lacks sensitivity to predict infection [18]. CRP is thought to be a sensitive biomarker soon after a microorganism infects the host human, but it has weak specificity [19]. Molecular approaches often require expensive technologies and equipment, and they may not be affordable for many hospitals [10]. As a result, it is still necessary to identify more economical, convenient and reliable diagnostic biomarkers as early indicators of infection [20].

As a member of the fibulin family of proteins, fibulin-2 is a calcium-binding extracellular matrix (ECM) glycoprotein that stabilizes and maintains ECM integrity and tissue architecture [21]. Fibulin-2 is widely expressed in many types of tissues, including tumour, heart, skin and bone tissue. Previous studies have found that fibulin-2 is upregulated during heart development, skin wound healing and cancer invasion and metastasis [22–24], though it is not thought to become elevated after infection. In our preliminary experiment, we found that secreted fibulin-2 was significantly increased in the plasma of patients with infection compared to volunteers without infection. Because fibulin-2 is a secreted protein that can be upregulated in the plasma at the onset of infection, and is easily detected by enzyme-linked immunosorbent assay (ELISA), it is likely to be a potential biomarker of infection.

To the best of our knowledge, no previous study has investigated the clinical significance of fibulin-2 as a biomarker of infection. In this study, we first identified that fibulin-2 is elevated in the plasma of infected patients, and it is a novel biomarker for the early diagnosis of infection. We also confirmed that fibulin-2 performed as well as CRP and PCT, and better than WBC, NEU%, IL-6 and D-dimer in predicting infection in all involved emergency department (ED) patients, especially for differential diagnosis of infection in patients with trauma or fever.

METHODS

Study Design and Setting

This was a clinical diagnostic accuracy study conducted at Daping Hospital in compliance with the principles outlined in the Declaration of Helsinki of 1964 and its later amendments. The study was approved by the Clinical Ethics Committee of Daping Hospital (approval number: Medical research review (2021) NO 07). We enrolled patients with infection and uninfected control patients admitted to the ED of Daping Hospital, a university teaching hospital with approximately 80,000–100,000 ED admissions per year. From January to October 2021, consecutive patients who agreed to participate in this study were enrolled. All participants or guardians signed a written informed consent form prior to participating in this study.

Inclusion and Exclusion Criteria

Two criteria were required for study eligibility. The first was admission to the Daping Hospital with a clinical diagnosis of infection, as suspected by the acquisition of clinical data at first admission by the primary ED team, performed independently and blinded to the study. The second group included patients without infection who volunteered to provide blood samples within 12 h of admission. Exclusion criteria

were as follows: patients with tumours, stroke or acute myocardial infarction; less than 18 years old; incomplete medical records; ambiguous diagnosis; and pregnancy. Patients were divided into an infected group and a non-infected group according to the presence or absence of infection on admission.

Determination of Infection, Trauma and Fever

Infection in our study was clinically defined on the basis of clinical signs, laboratory detection and radiographic evidence. All final patient classifications were determined using a majority rule among three senior doctors, all blinded to the fibulin-2 results. The designation "infected" included those with clinically relevant positive microbiological cultures collected within 12 h of enrolment. For primary analysis, these cultures included sputum, blood, urine, cerebrospinal, pleural, peritoneal and wound exudate cultures. Of note, those patients showing strong evidence of bacterial infection in the absence of positive cultures were also included in the "infected" group. These cases included findings such as radiographic evidence (computed tomography, X-ray, etc.) or physical exam findings strongly suggestive of infection in the absence of positive cultures. All other subjects were classified as "non-infected". The diagnosis of trauma was made by ED trauma physicians, according to the history of injury. Eligible patients with fever were those who presented with an axillary temperature of greater than 37.3 °C on admission.

Data Collection

In addition to fibulin-2 measurements, relevant demographic data were collected, including age, sex, reasons for admission, medical history, presence of comorbidities, vital signs, both source and aetiology of infection, and laboratory values, such as proalbumin, albumin, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, bilirubin, WBC, NEU%, platelets, D-dimer, creatinine, glomerular filtration, CRP, PCT, IL-6 and erythrocyte sedimentation rate. Data were collected by trained abstractors using standardized data collection forms and entered into Excel 2016 (Microsoft Corporation, Redmond, Washington). All data were reviewed by another trained researcher to assess the data collection validity, who corrected any inconsistencies.

Detection of Fibulin-2

Using the Daping Hospital electronic medical record, the investigating team was notified daily of all available blood samples associated with informed consent. Venous blood samples from within the same time frame in the Daping Hospital clinical laboratory were obtained in the ED; the blood samples were collected within 12 h after admission to the ED. Blood was also collected at another time during disease progression from willing volunteers. The blood samples were collected in tubes containing heparin, centrifuged at 4000*g* for 5 min to obtain plasma, and stored at − 80 °C. Fibulin-2 levels were measured within 7 days after collection using an Enzyme-linked Immunosorbent Assay Kit for Human Fibulin-2 (CloudClone Corp, Wuhan, China), with a normal reference range of 0.625–40 ng/mL. All measurements were repeated twice, and the average for each sample was taken. The operators were unaware of the related clinical information.

Sample Size Calculation

A main goal of this study was to assess whether the performance of fibulin-2 as a biomarker of infection is better than that of other biomarkers, including CRP, PCT, WBC, NEU%, IL-6 and D-dimer. Assuming an expected AUC of 0.710 for fibulin-2, 0.614 for WBC, 0.611 for NEU%, 0.623 for D-Dimer, 0.559 for IL-6, 0.620 for PCT and 0.640 for CRP, as determined in our preliminary study, we used PASS software version 11 to estimate that a sample size of 707 patients was needed. A sample of 257 from the positive group and 450 from the negative group achieved 90% power to detect a difference of 0.070 between the AUC under the null hypothesis of 0.640 (assumed AUC of CRP) and

an AUC under the alternative hypothesis of 0.710 (assumed AUC of fibulin-2) using a two-sided Z test at a significance level of 0.05, comprising continuous response data. The AUC was computed between false-positive rates of 0.000 and 1.000. The ratio of the standard deviation of the responses in the negative group to the standard deviation of the responses in the positive group was 1.000. Given the aforementioned preliminary data, we anticipated a data collection duration of approximately 10 months. Finally, we recruited 992 volunteers and involved 722 patients for analysis.

Statistical Analysis

GraphPad Prism version 8 (GraphPad Software Inc., La Jolla, CA, USA), SPSS Statistics version 25 (SPSS Inc. Chicago, IL, USA) and MedCalc version 20.022 (MedCalc Software Ltd, Ostend, Belgium) were used for statistical analysis. All continuous variables are presented as the mean ± SD; categorical variables are presented as frequency (percentages). Paired and unpaired t tests and analysis of variance (ANOVA) tests were applied to compare variables between groups. Percentages were compared with the chi-square or Fisher's exact test. ROC curves were calculated to measure the sensitivity and specificity of biomarkers for infection. The Z test was used to compare the difference of ROC curves among various biomarkers. Logistical regression was used to assess the association between different biomarkers and infection. A probability of $p < 0.05$ was considered the threshold of significance.

RESULTS

Characteristics of the Participants

As shown in the flow chart of the study (Fig. 1), 992 patients were screened and enrolled from January to October 2021. In total, 270 patients were excluded because they did not meet the inclusion criteria and because of inadequate information collection or screening errors. Then, 722 patients (non-infection group 461, infection group 261) were involved in ROC curve analysis of different biomarkers, including fibulin-2, WBC, NEU%, D-dimer, IL-6, CRP and PCT. Because age and sex appeared to be uneven between the infection group and non-infection group, we performed data matching according to age and sex in a 1:1 ratio between them. The subsequent analysis included 494 patients to compare the fibulin-2 level between the infection group ($n = 247$) and the non-infection group ($n = 247$). The demographic characteristics of the patients after data matching are shown in Table 1. There were 138 men and 109 women in each group, and the age was 58.190 ± 20.078 years in the non-infection group and 58.267 ± 20.337 years in the infection group. Race, medical history and comorbidities were not significantly different between the two groups ($P < 0.05$). The site of infection was examined for those in the infection group, including 22 upper respiratory tract, 88 lower respiratory tract, 28 urinary tract, 24 abdominal, 1 central nervous system, 19 limbs, 2 heart, 12 blood, 10 others and 41 multiple sites. Among these patients, 107 bacterium-, 8 fungus-, 13 virus-, 3 mycoplasma- and 19 multiple microorganism-infected individuals were confirmed and 97 patients had undetermined infection types. Levels of fibulin-2, WBC, NEU%, D-dimer, IL-6, CRP and PCT were significantly higher in the

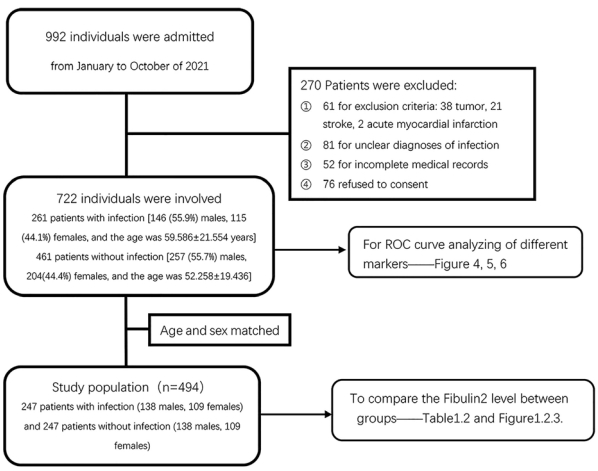

Fig. 1 Flow chart for the study population. Data are presented as the mean ± SD. *ROC* receiver operating characteristic

Table 1 Characteristics of the participants after data matching

	Control	Infection	Statistical value
n	247	247	
Age (years)	58.190 ± 20.078	58.267 ± 20.337	$t = 0.042$, $P = 0.996$
Sex, male (female) (*n*)	138 (109)	138 (109)	$X^2 = 0.000$, $P = 1$
Weight (kg)	63.169 ± 12.454	59.717 ± 11.299	$t = -1.958$, $P = 0.052$
Race, *n* (%)			
Han	245 (99.2%)	244 (98.8%)	$P = 1$
Ethnic minority	2 (0.8%)	3 (1.2%)	
Medical history, *n* (%)			
Smoking	30 (12.1%)	43 (17.4%)	$X^2 = 2.716$, $P = 0.099$
Alcohol consumption	20 (8.1%)	33 (13.3%)	$X^2 = 3.572$, $P = 0.059$
Allergy	3 (1.2%)	5 (2.0%)	$P = 0.724$
Surgery	21 (8.5%)	33 (13.3%)	$X^2 = 2.994$, $P = 0.084$
Comorbidities, *n* (%)			
Diabetes mellitus	31 (12.6%)	46 (18.6%)	$X^2 = 3.462$, $P = 0.063$
Hypertension	59 (23.9%)	72 (29.1%)	$X^2 = 1.756$, $P = 0.185$
Coronary heart disease	14 (5.7%)	22 (8.9%)	$X^2 = 1.918$, $P = 0.166$
Dyslipidaemia	10 (4.0%)	5 (2.0%)	$X^2 = 1.719$, $P = 0.294$
COPD	9 (3.6%)	6 (2.4%)	$X^2 = 0.619$, $P = 0.431$
Vital signs and mental status at time of admission			
Body temperature (°C)	36.548 ± 0.357	36.742 ± 0.628	$t = 3.337$, $P = 0.001$
Respiratory rate (breaths/min)	19.573 ± 1.544	20.103 ± 2.129	$t = 2.057$, $P = 0.041$
Pulse rate (times/min)	82.528 ± 15.498	88.410 ± 15.605	$t = 2.845$, $P = 0.005$
Systolic blood pressure (mmHg)	130.326 ± 20.340	130.487 ± 23.421	$t = 0.054$, $P = 0.957$
Mean arterial pressure (mmHg)	93.764 ± 13.209	93.466 ± 15.085	$t = -0.156$, $P = 0.877$
Disturbance of consciousness, *n* (%)	5 (2.0%)	9 (3.6%)	$X^2 = 1.176$, $P = 0.278$
Glasgow Coma Scale score	14.777 ± 1.120	14.519 ± 1.758	$t = -1.440$, $P = 0.151$
Positive microorganism culture, *n* (%)		30 (12.1%)	
Site of infection, *n* (%)			
Upper respiratory tract		22 (8.9%)	
Lower respiratory tract		88 (35.6%)	
Urinary tract		28 (11.3%)	
Abdominal		24 (9.7%)	
Central nervous system		1 (0.4%)	

Table 1 continued

	Control	Infection	Statistical value
Limbs		19 (7.7%)	
Heart		2 (0.8%)	
Blood		12 (4.9%)	
Other		10 (4.0%)	
Multiple sites		41 (16.6%)	
Type of microorganism, *n* (%)			
Bacterium		107 (43.3%)	
Fungus		8 (3.2%)	
Virus		13 (5.3%)	
Mycoplasma		3 (1.2%)	
Multiple microorganisms		19 (7.7%)	
Indeterminate		97 (39.3%)	
Laboratory values, mean ± SEM			
Proalbumin	164.310 ± 104.857	150.738 ± 94.800	$t = -0.784$, $P = 0.434$
Albumin	39.711 ± 7.080	38.169 ± 15.061	$t = -1.188$, $P = 0.236$
Alanine aminotransferase	38.076 ± 87.372	63.973 ± 327.777	$t = 0.963$, $P = 0336$
Aspartate aminotransferase	44.760 ± 74.294	49.453 ± 89.807	$t = 0.526$, $P = 0.599$
Lactate dehydrogenase	472.211 ± 464.076	515.938 ± 415.907	$t = 0.870$, $P = 0.385$
Bilirubin	14.925 ± 10.138	16.674 ± 14.768	$t = 1.083$, $P = 0.280$
White blood cells, 10^9/L	8.089 ± 3.371	9.604 ± 4.566	$t = 3.994$, $P = 0.000$
NEU%	72.702 ± 11.260	75.534 ± 13.503	$t = 2.402$, $P = 0.017$
Platelets, 10^9/L	225.700 ± 84.408	216.504 ± 95.244	$t = -1.080$, $P = 0.281$
D-dimer (ng/mL)	527.649 ± 1022.195	1040.05 ± 2662.422	$t = 2.229$, $P = 0.026$
Creatinine, mg/dL	102.545 ± 153.828	108.960 ± 126.403	$t = 0.452$, $P = 0.651$
Glomerular filtration (mL/min/1.73 m^2)	122.507 ± 46.605	119.082 ± 58.016	$t = -0.631$, $P = 0.528$
C-reactive protein (mg/L)	10.221 ± 21.718	32.638 ± 50.526	$t = 5.736$, $P = 0.000$
Procalcitonin (ng/mL)	0.395 ± 1.601	1.733 ± 6.875	$t = 1.988$, $P = 0.049$
Interleukin-6 (pg/mL)	42.303 ± 68.490	268.325 ± 925.001	$t = 2.428$, $P = 0.017$
Erythrocyte sedimentation rate	28.926 ± 28.264	40.303 ± 33.712	$t = 1.397$, $P = 0.168$
Fibulin-2 (ng/mL)	3.987 ± 1.846	5.435 ± 2.323	$t = 8.509$, $P = 0.000$

infection group than in the non-infection group ($P < 0.05$).

Performance of Fibulin-2 for Detecting Infection

Figure 2a illustrates each value for the plasma fibulin-2 concentration of all involved patients, showing increases in patients with infection (5.435 ± 2.323 ng/mL) compared to non-infection controls (3.987 ± 1.846 ng/mL) ($P < 0.05$). Figure 2c shows that the fibulin-2 concentration was decreased in patients after they recovered from infection on discharge (3.816 ± 1.421 ng/mL) compared to the time when they were admitted to the hospital as a result of infection (4.744 ± 2.522 ng/mL) ($P < 0.05$). However, the level was the same in the control group (3.957 ± 1.519 ng/mL on discharge vs. 3.788 ± 0.928 ng/mL on admission) (Fig. 2b). In addition, the elevated blood fibulin-2 concentration correlated with the onset of infection (6.497 ± 3.301 ng/mL for infection vs. 3.588 ± 1.499 ng/mL for preinfection), which reverted to the baseline concentration following successful therapy (4.357 ± 1.844 ng/mL) (Fig. 2e). In the non-infection group, fibulin-2 remained constant and did not change with disease progression (3.201 ± 0.314 ng/mL on admission vs. 3.420 ± 0.893 ng/mL at hospitalization vs. 2.352 ± 0.776 ng/mL on discharge) (Fig. 2d). The AUC for infection was 0.721 [95% confidence interval 0.676–0.776] for fibulin-2 (Fig. 2f).

The cut-off value of 4.3428 ng/mL provided optimum diagnostic power by balancing the ability to detect infection (sensitivity 67.60%) and case controls (specificity 69.20%) and had the highest Youden index (0.368).

Levels of Fibulin-2 in Patients with Different Infection Types and Performance of Fibulin2 in Different Diagnosis of Infection

As infection can be caused by various microorganisms and may have pathological consequences at different sites in the host, it is necessary to determine the fibulin-2 level in different populations. As depicted in Fig. 3a, the level of fibulin-2 was significantly higher in the bacterial (5.539 ± 2.218 ng/mL), fungal (6.359 ± 2.186 ng/mL), viral (5.828 ± 2.557 ng/mL) and indeterminate (5.419 ± 2.498 ng/mL) infection groups than in the non-infection group (3.987 ± 1.846 ng/mL). However, there was no significant difference between the mycoplasma (3.988 ± 1.763 ng/mL) and multiple microorganism (4.502 ± 1.763 ng/mL) groups and the control group (3.987 ± 1.846 ng/mL). The fibulin-2 level was also higher in the respiratory tract (6.062 ± 2.462 ng/mL for the upper respiratory tract and 5.746 ± 2.572 ng/mL for the lower respiratory tract), urinary tract (5.938 ± 2.658 ng/mL), abdominal (4.949 ± 1.842 ng/mL), central nervous and heart (6.862 ± 3.178 ng/mL), blood (5.840 ± 2.736 ng/mL) and multiple site (4.951 ± 1.761 ng/mL) infection groups than in the non-infection group (3.987 ± 1.846 ng/mL). There was no significant difference in the limbs (4.135 ± 1.254 ng/mL) or others (4.183 ± 0.987 ng/mL) as sites of infection when compared to controls (Fig. 3b).

Then, the ROC curves of fibulin-2 for the differential diagnosis of bacterial infection (Fig. 3c), fungal infection (Fig. 3d) and viral infection (Fig. 3e) are presented. The results showed that fibulin-2 has no predictive ability for different diagnosis of bacterial infection (AUC 0.535, $P = 0.346$, 95% Cl 0.463–0.607), fungal infection (AUC 0.632, $P = 0.203$, 95% Cl 0.465–0.799) or viral infection (AUC 0.577, $P = 0.351$, 95% Cl 0.422–0.732) from all clinical infection.

Comparison of the Performance of Fibulin-2 with Other Biomarkers for Infection Detection in All Patients

As the comparisons of ROC curves among different biomarkers were carried out in the same populations, there was no need to perform data matching anymore and we compared the ROC curves in all involved patients ($n = 722$). Performances of fibulin-2, CRP, PCT WBC, NEU%, D-dimer and IL-6 for the diagnosis of infection in all patients are presented in Fig. 4. The ROC curves of fibulin-2 (AUC 0.712, $P = 0.000$,

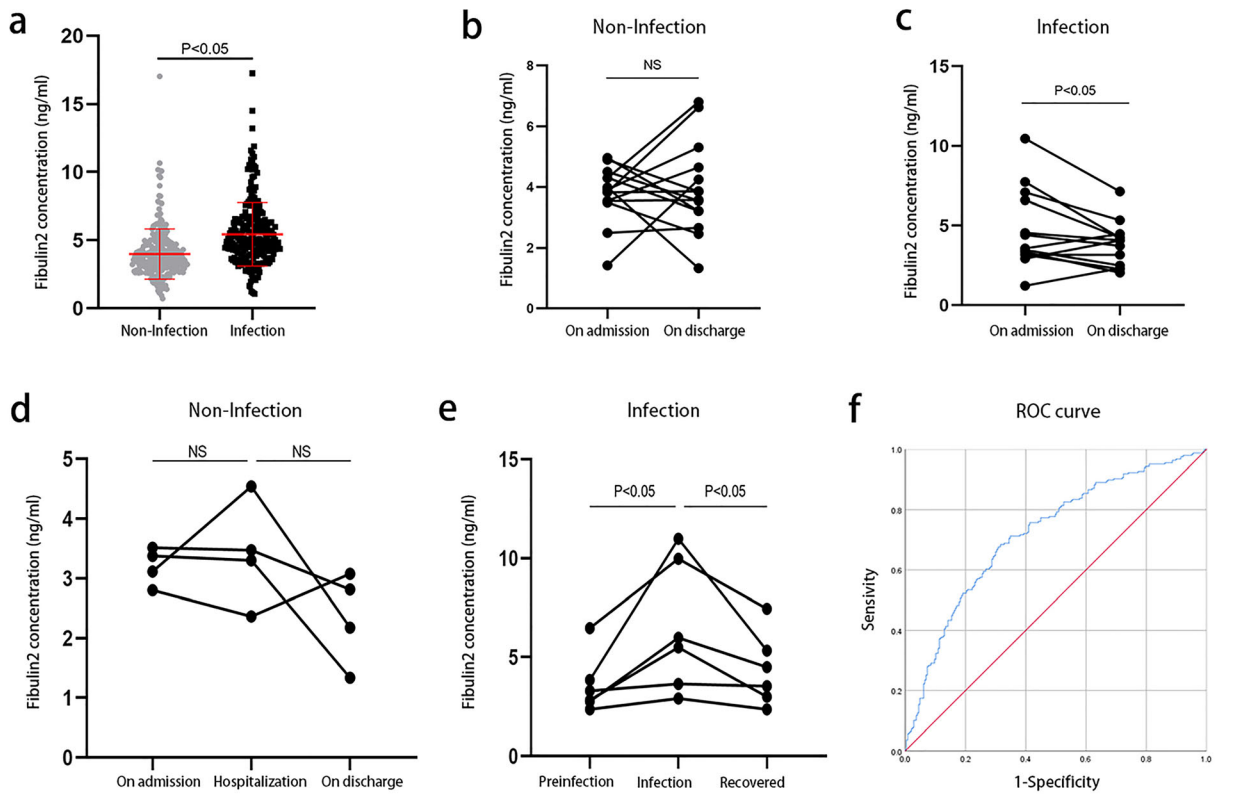

Fig. 2 Performance of fibulin-2 for infection detection. **a** Comparison of fibulin-2 between the infection group and the non-infection group (red horizontal bars are the mean ± SD). **b**, **d** Dynamic change in fibulin-2 in the non-infection groups. **c**, **e** Dynamic change in fibulin-2 in the infection group. **f** Receiver operating characteristic (ROC) curve of fibulin-2 for the diagnosis of infection after data matching. Area under the ROC curve 0.721 (95% confidence interval 0.676– 0.766), $P = 0.000$

95% CI 0.672–0.751), CRP (AUC 0.667, $P = 0.000$, 95% CI 0.622–0.712), PCT (AUC 0.632, $P = 0.001$, 95% CI 0.559–0.704), WBC (AUC 0.620, $P = 0.001$, 95% CI 0.573–0.666), NEU% (AUC 0.619, $P = 0.000$, 95% CI 0.573–0.664), IL-6 (AUC 0.561, $P = 0.153$, 95% CI 0.479–0.643) and D-dimer (AUC 0.630, $P = 0.000$, 95% CI 0.579–0.681) are shown. By comparing the value of AUC, we found that the diagnostic ability of fibulin-2 performed better than WBC ($Z = 2.966$, $P = 0.003$), NEU% ($Z = 3.025$, $P = 0.003$), IL-6 ($Z = 3.251$, $P = 0.001$) and D-dimer ($Z = 2.482$, $P = 0.013$). There was no significant difference between fibulin-2 and CRP ($Z = 1.480$, $P = 0.139$) and PCT ($Z = 1.901$, $P = 0.057$).

The cut-off values of these biomarkers when each of them obtained the highest odds ratio value are presented in Supplementary Table 1.

Next, the value of sensitivity, specificity, Youden index, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), negative likelihood ratio (NLR) and odds ratio are also shown. We also applied logistical regression to assess the association between different biomarkers and infection and the results are shown in Supplementary Table 2.

Performance of Fibulin-2 in the Diagnosis of Infection in Patients with Fever and Comparison Between Different Biomarkers

To validate the role of fibulin-2 in predicting infection, we screened the patients with fever from all patients and explored the performance

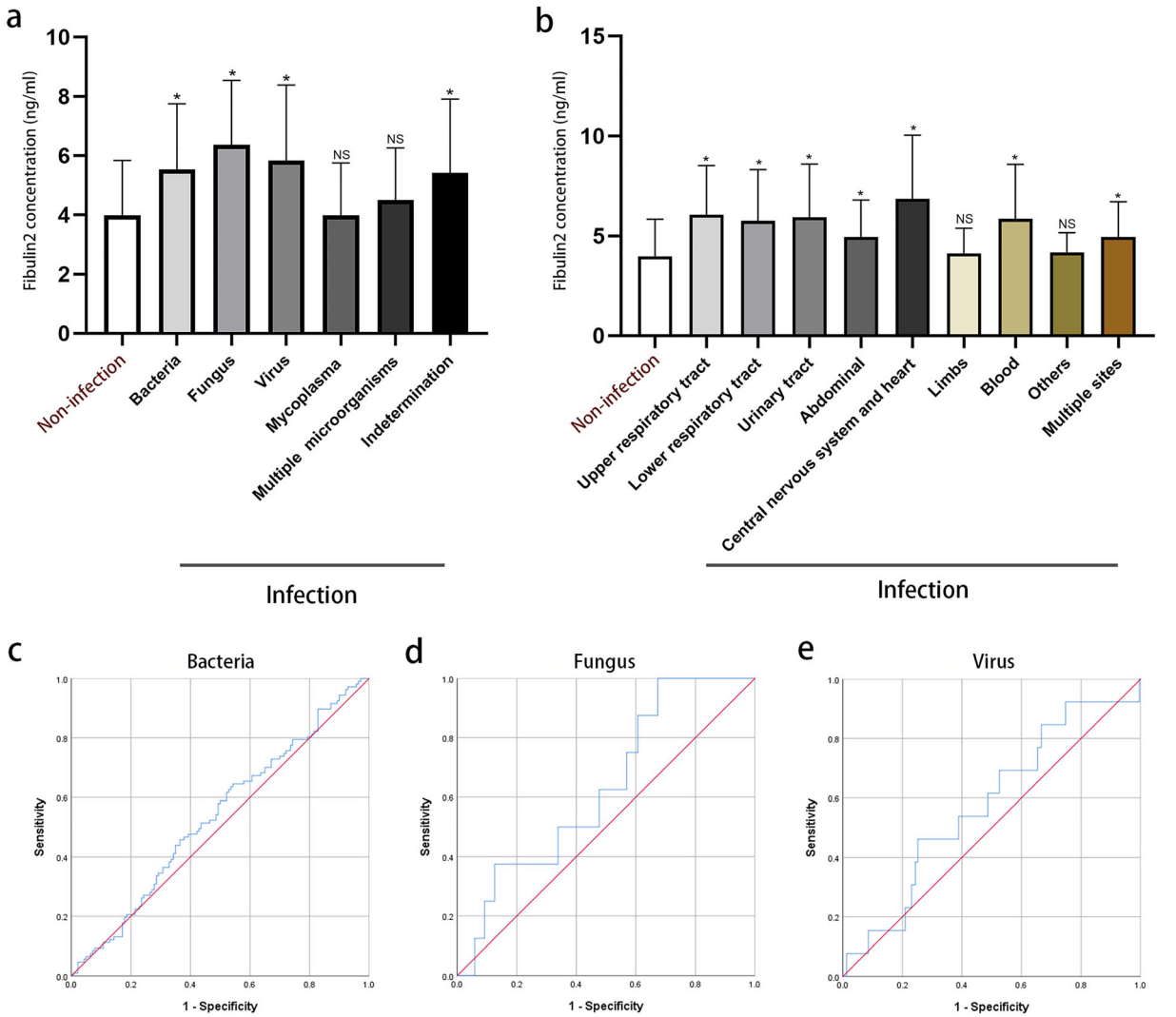

Fig. 3 Levels of fibulin-2 in patients with different infection types and performance of fibulin-2 in different diagnosis of infection. **a** Concentration of fibulin-2 between non-infection controls and patients infected with different microorganisms. **b** Concentration of fibulin-2 between non-infection controls and patients with infection at different sites. **c–e** Receiver operating characteristic (ROC) curves of fibulin-2 for the differential diagnosis of bacterial infection (AUC 0.535, $P = 0.346$, 95% CI 0.463–0.607), fungal infection (AUC 0.632, $P = 0.203$, 95% CI 0.465–0.799) and viral infection (AUC 0.577, $P = 0.351$, 95% CI 0.422–0.732) from all the patients with infection. $^*P < 0.05$, *NS* no significance

of fibulin-2 in the diagnosis of infection. In patients with fever, the level of fibulin-2 in the infection group (5.106 ± 2.063 ng/mL) was significantly higher than that in the non-infection group (3.842 ± 1.356 ng/mL) ($t = 2.463$, $P = 0.017$) (Fig. 5a). ROC curves of fibulin-2, CRP, PCT, WBC, NEU%, IL-6 and D-dimer for the diagnosis of infection in patients with fever are shown in Fig. 5b, c, d, e, f, g, h, respectively. Overall, only fibulin-2 (AUC 0.728, $P = 0.005$, 95% CI 0.589–0.868) and PCT (AUC 0.798, $P = 0.018$, 95% CI 0.612–0.983) had diagnostic ability for infection in these patients. There was no significant difference between fibulin-2 and PCT ($Z = 0.391$, $P = 0.796$). Moreover, CRP (AUC 0.666, $P = 0.059$, 95% CI 0.514–0.818), WBC (AUC 0.486, $P = 0.872$, 95% CI 0.324–0.648), NEU% (AUC 0.634, $P = 0.120$,

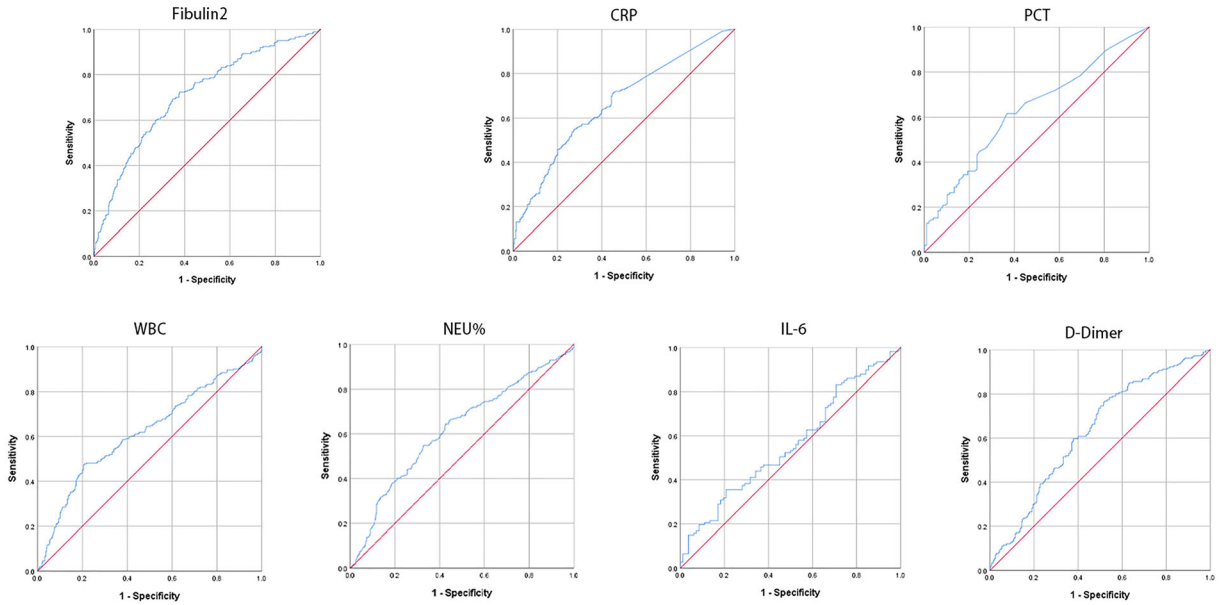

Fig. 4 Receiver characteristic curves of different biomarkers for the diagnosis of infection in all involved patients. Fibulin-2 (AUC 0.712, $P = 0.000$, 95% CI 0.672–0.751), CRP (AUC 0.667, $P = 0.000$, 95% CI 0.622–0.712), PCT (AUC 0.632, $P = 0.001$, 95% CI 0.559–0.704), WBC (AUC 0.620, $P = 0.001$, 95% CI 0.573–0.666), NEU% (AUC 0.619, $P = 0.000$, 95% CI 0.573–0.664), IL-6 (AUC 0.561, $P = 0.153$, 95% CI 0.479–0.643) and D-dimer (AUC: 0.630, $P = 0.000$, 95% CI 0.579–0.681)

95% Cl 0.468–0.799), IL-6 (AUC 0.555, $P = 0.708$, 95% Cl 0.350–0.759) or D-dimer (AUC 0.609, $P = 0.240$, 95% Cl 0.414–0.805) had no predictive ability in the diagnosis of infection in patients with fever.

The cut-off value of 4.300 ng/mL for fibulin-2 provided optimum diagnostic power by balancing the ability to detect infection (sensitivity 64.90%) and case control (specificity 80.00%) and had the highest Youden index (0.449). The cut-off value of 0.250 ng/mL for PCT provided optimum diagnostic power by balancing the ability to detect infection (sensitivity 70.80%) and case control (specificity 85.70%) and had the highest Youden index (0.565).

Performance of Fibulin-2 in the Diagnosis of Infection in Patients with Trauma and Comparison Between Different Biomarkers

In patients with trauma, the level of fibulin-2 in the infection group (5.305 ± 1.528 ng/mL) was significantly higher than that in the non-infection group (3.539 ± 1.182 ng/mL) ($t = 5.394$, $P = 0.000$) (Fig. 6a). ROC curves of fibulin-2, CRP, PCT WBC, NEU%, IL-6 and D-dimer for the diagnosis of infection in patients with trauma were shown in Fig. 6b, c, d, e, f, g, h, respectively. Overall, only fibulin-2 (AUC 0.844, $P = 0.000$, 95% Cl 0.757–0.931) had diagnostic ability for infection in patients with trauma. Conversely, CRP (AUC 0.640, $P = 0.061$, 95% Cl 0.478–0.801), PCT (AUC 0.620, $P = 0.203$, 95% Cl 0.424–0.816), WBC (AUC 0.491, $P = 0.900$, 95% Cl 0.334–0.648), NEU% (AUC 0.378, $P = 0.100$, 95% Cl 0.234–0.521), IL-6 (AUC 0.587, $P = 0.364$, 95% Cl 0.388–0.786) and D-dimer (AUC 0.445, $P = 0.487$, 95% Cl 0.282–0.609) had no predictive ability in the diagnosis of infection in patients with trauma.

The cut-off value of 4.312 ng/mL for fibulin-2 provided optimum diagnostic power by balancing the ability to detect infection (sensitivity 81.00%) and case controls (specificity 82.50%) and had the highest Youden index (0.634).

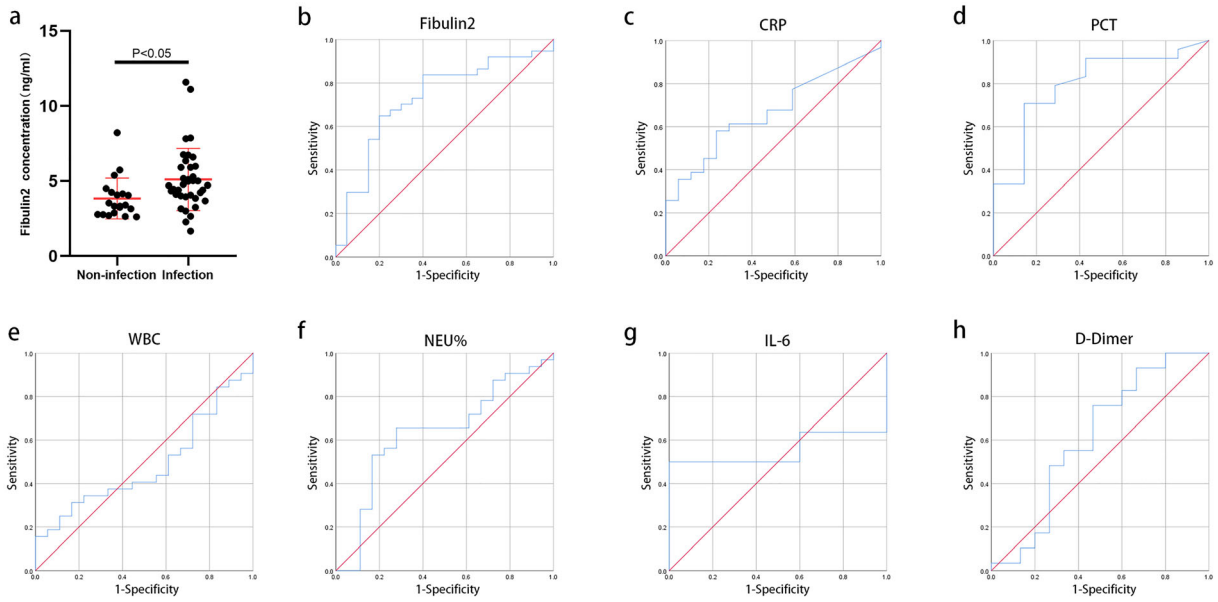

Fig. 5 Performance of fibulin-2 in the diagnosis of infection in patients with fever and comparison between different biomarkers. **a** Levels of fibulin-2 in plasma from patients with fever and with or without infection. **b–h** Receiver operating characteristic (ROC) curves of fibulin-2, CRP, PCT, WBC, NEU%, IL-6 and D-dimer for the diagnosis of infection in patients with fever. Fibulin-2 (AUC 0.728, $P = 0.005$, 95% Cl 0.589–0.868), CRP (AUC 0.666, $P = 0.059$, 95% Cl 0.514–0.818), PCT (AUC 0.798, $P = 0.018$, 95% Cl 0.612–0.983), WBC (AUC 0.486, $P = 0.872$, 95% Cl 0.324–0.648), NEU% (AUC 0.634, $P = 0.120$, 95% Cl 0.468–0.799), IL-6 (AUC 0.555, $P = 0.708$, 95% Cl 0.350–0.759) and D-dimer (AUC 0.609, $P = 0.240$, 95% Cl 0.414–0.805)

DISCUSSION

Despite the improvement in medical care, the case fatality rate for patients with infection has still ranged from 20% to 30% in recent decades [25]. In general, a timely and accurate diagnosis helps to improve patient outcomes, and early warning biomarkers contribute to diagnosis [26]. This study is the first to report that fibulin-2 is a potential early biomarker for infection. Initially, on the basis of proteomics studies we found that fibulin-2 is upregulated in patients with infectious osteomyelitis (data not published). In our ensuing research involving a small-sample clinical study, we observed that fibulin-2 was also upregulated in the plasma of patients with different types of infections. Therefore, to further demonstrate the feasibility of fibulin-2 as a good biomarker for infection, we performed this prospective study in our hospital. As EDs are increasingly recognized as not only acute diagnostic centres but also as important centres of infectious disease surveillance, prevention and control [27], we chose the ED as the point of volunteer recruitment to carry out this clinical study. Most of the study population in the experimental group had early infection, thereby helping to evaluate the effectiveness of different biomarkers in early prediction performance.

A total of 992 individuals were recruited for our study, and 722 were eligible for inclusion. The mean age of 261 patients with infection was 59.586 ± 21.554, and that the mean age of 461 patients without infection was 52.258 ± 19.436; thus, the age of the infection group was higher than that of the non-infection group ($P < 0.05$). This discrepancy might be attributable to the fact that older individuals are more susceptible to various infections due to immunological changes that occur during the ageing process [28]. To increase comparability between the groups and to maintain consistent baseline values, we matched patients by age and

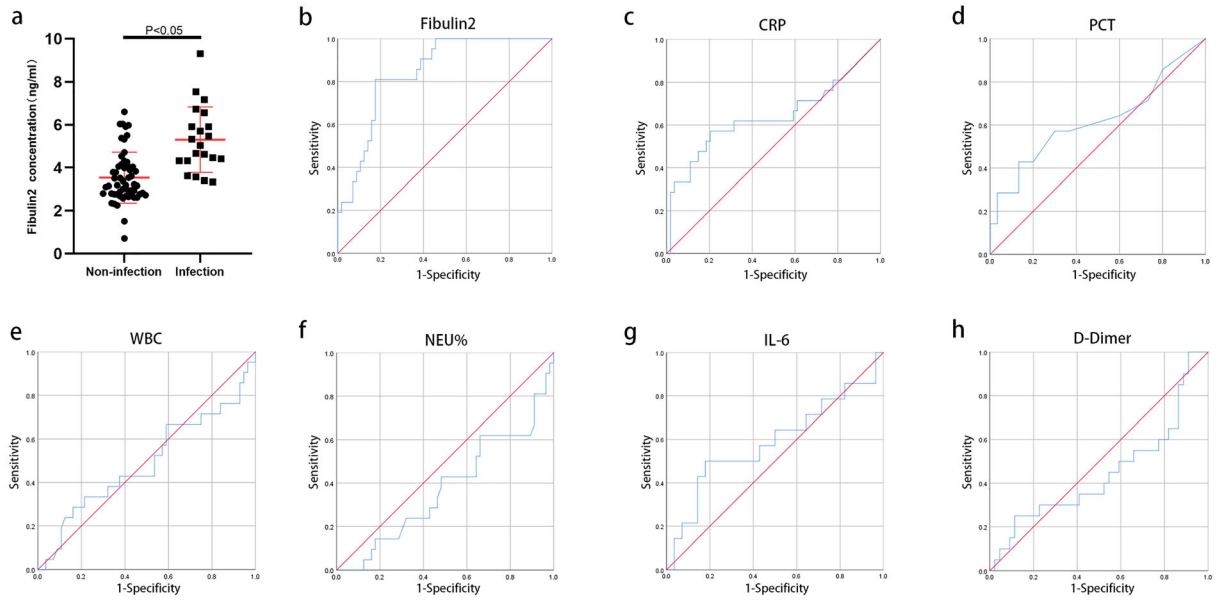

Fig. 6 Performance of fibulin-2 in the diagnosis of infection in trauma patients and comparison between different biomarkers. **a** Levels of fibulin-2 in plasma from traumatic patients with or without infection. **b–h** Receiver operating characteristic (ROC) curves of fibulin-2, CRP, PCT, WBC, NEU%, IL-6 and D-dimer for the diagnosis of infection in patients with trauma. Fibulin-2 (AUC 0.844, $P = 0.000$, 95% CI 0.757–0.931), CRP (AUC 0.640, $P = 0.061$, 95% CI 0.478–0.801), PCT (AUC 0.620, $P = 0.203$, 95% CI 0.424–0.816), WBC (AUC 0.491, $P = 0.900$, 95% CI 0.334–0.648), NEU% (AUC 0.378, $P = 0.100$, 95% CI 0.234–0.521), IL-6 (AUC 0.587, $P = 0.364$, 95% CI 0.388–0.786) and D-dimer (AUC 0.445, $P = 0.487$, 95% CI 0.282–0.609)

sex in a ratio of 1:1, as in previous studies [29, 30]. Finally, 247 patients remained in each group, with no significant difference at baseline in age, sex, weight, race, medical history and comorbidities. In the infection groups, the lower respiratory tract was the most common site of infection, and bacteria were the most common pathogenic microorganism, consistent with previous studies [25, 31].

By comparing fibulin-2 levels in the infection and non-infection groups, we found it to be significantly higher in the former. To further observe the dynamic change of fibulin-2 in the progression of infection, we continuously detected its plasma level in different stages of infection. The results showed that fibulin-2 was elevated in the stage of infection but returned to normal in the convalescent stage. Conversely, fibulin-2 remained at the same level in the different stages of non-infectious diseases. These results confirm that fibulin-2 is closely associated with infection. Nevertheless, the number of cases measured continuously was small, and more large-sample studies are needed to confirm our findings.

We then used ROC curve analysis to demonstrate the role of fibulin-2 in the clinical prediction of infection. The AUC of fibulin-2 was 0.721, which was better than chance (AUC 0.5) [32]. Therefore, fibulin-2 might be a potential biomarker for early infection. To estimate the application coverage of fibulin-2 in predicting infection, we specified the source of microorganisms and the site of infection and compared fibulin-2 levels. Fibulin-2 was upregulated in bacterial, fungal and viral infections but not in mycoplasma or multiple microorganism infections, demonstrating that fibulin-2 may act as a biomarker in predicting not only bacterial infection but also viral and fungal infections. However, there was no significant difference in predicting mycoplasma infection, possibly because mycoplasmas do not commonly cause infection and the sample size was

small. It is also possible that mycoplasma infection may not lead to a change in fibulin-2 level. The fibulin-2 level was elevated in the respiratory tract, urinary tract, abdominal, central nervous and heart, blood and multiple infection sites, though it remained the same in the infection of the limbs and other sites. This finding may be attributed to the fact that limb infection and infection at other sites are considered local infection, and that most are mild infections insufficient to affect fibulin-2 expression. More basic research and stronger evidence are needed to confirm this hypothesis.

To clarify the feasibility of fibulin-2 as an excellent biomarker for infection, we compared the AUCs of fibulin-2, CRP, PCT, WBC, NEU%, IL-6 and D-dimer by ROC curve analysis. For all ED patients examined, ROC analysis showed that the AUC of fibulin-2 was higher than that of CRP, PCT, WBC, NEU%, IL-6 and D-dimer, which have been explored in previous studies [33–36]. Among these biomarkers, CRP and PCT are widely used in the clinic to predict infection. In this study, the performance of PCT was lower than that reported in a previous study, with AUCs ranging from 0.64 to 0.79 [37, 38]. The first reason for the discrepancy is that most of our study population was at an early stage of infection, as opposed to patients with sepsis. In addition, the patients were enrolled, and blood samples were obtained at the time of ED admission, when the concentration of PCT had not increased or reached a maximum [25]. Finally, our study involved some patients infected with fungi, viruses and other microorganisms, and PCT is a good biomarker for predicting bacterial infection but not for fungi or viruses [39, 40]. Moreover, the AUC of CRP was consistent with previous studies in which the AUC ranged from 0.57 to 0.79 [37, 41]. Fibulin-2 may be a good predictor that is not worse than PCT and CRP for the following reasons. PCT is produced by C cells of the thyroid gland or neuroendocrine cells in the lung or intestine, and very few PCT molecules are released into the circulation [42]. CRP is synthesized by the liver in response to IL-6 stimulation [43]. Fibulin-2, an ECM protein, is expressed in epithelia and many other cells throughout the body [44]. As early infection may only result in local tissue destruction and not systemic impairment [45, 46], upregulation of CRP and PCT may not occur. In particular, if the infection does not stimulate the thyroid gland or liver, the level of CRP or PCT may not change.

Last, fever and trauma are the most common patient complaints in the ED; however, patients with trauma or fever have various degrees of stress that elicit an inflammatory response, leading to an upregulation of CRP, PCT, WBC, NEU%, IL-6 and D-dimer even in the absence of infection [47, 48]. Therefore, these biomarkers may perform poorly in the diagnosis of infection in patients with fever or trauma. Accordingly, we screened patients with fever or trauma among all patients and explored the performance of fibulin-2 in the diagnosis of infection. In patients with fever, fibulin-2 was not better than PCT but better than others in the diagnosis of infection. Above all, in patients with trauma, fibulin-2 performed better than CRP, PCT, WBC, NEU%, IL-6 and D-dimer. As a result, with a wider range of applications, fibulin-2 may serve as a novel promising biomarker for the early differential diagnosis of infectious and non-infectious systemic inflammatory responses, even in patients with fever or trauma.

This study had several limitations. First, it was a single-centre study, and the sample size needs to be further expanded. Second, the measuring error should be noted, as the detection of fibulin-2 in plasma was not finished at once. Third, because the fibulin-2 level was elevated in patients with tumours, we excluded them from this study. Some patients with unclear diagnoses of infection and with incomplete medical records were also not included in the analysis. As a result, many individuals were eliminated from the study. Finally, we did not explore the predictive capacities of fibulin-2 for prognosis secondary to infection.

CONCLUSIONS

With good predictive value, fibulin-2 is a promising novel biomarker for early diagnosis of infection, even in patients with fever or trauma.

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Authorship. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Author Contributions. SDL, YZ, XX and JF conceived and developed the study. DLZ and DQT collected the blood samples. HJ, SDL, SCW, CYM, JJZ, and PL maintained the biological repository and processed the biological samples. SDL maintained the database for analysis, directly participated in the analyses, and wrote the manuscript. XX, LL, WX and YZ aided in conceiving of and developing the study, the patient classification and the manuscript revision. All authors read and approved the final manuscript.

Disclosures. Shidan Li, Hao Jiang, Wei Xing, Shaochuan Wang, Yao Zhang, Youbin Li, Chengyi Mao, Delian Zeng, Ping Lan, Dongqin Tang, Jijie Zhan, Lei Li, Xiang Xu and Jun Fei have nothing to disclose.

Compliance with Ethics Guidelines. The study followed the law and was approved by the Clinical Ethics Committee of Daping Hospital (approval number: Medical research review (2021) NO 07) and conducted in compliance with the principles outlined in the Declaration of Helsinki of 1964 and its later amendments. All patients involved in our study provided informed consent.

Data Availability. The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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