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Serum human neutrophil lipocalin: An effective biomarker for diagnosing bacterial infections

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ABSTRACT

Background: Human neutrophil lipocalin (HNL) is used as a novel biomarker for infections. However, only a few studies have focused on the usefulness of HNL. The purpose of this study was to evaluate the diagnostic efficiency of HNL for identifying bacterial infections and to compare HNL with procalcitonin (PCT) and C-reactive protein (CRP).

Methods: Hospital patients with acute infections of bacterial origin (n = 439), viral origin (n = 71), and healthy volunteers (n = 67) were included in the study. The infection status of each patient was verified using microbiological, serological, and PCR testing. Additionally, CRP, HNL, and PCT levels were measured by established methods.

Results: In distinguishing bacterial and viral infections, area under the curve (AUC) analysis showed that, with a value of 0.81 (95% CI, 0.76–0.86), HNL was superior to CRP at 0.73 (0.68–0.79) and PCT at 0.64 (0.58–0.70). Interestingly, the combination of HNL, PCT, and CRP improved the diagnostic potential significantly with an AUC of 0.86 (0.82–0.90, $P < 0.05$). Furthermore, when comparing different infection site subgroups with healthy patients, HNL levels were higher in all bacterial groups, albeit to widely varying degrees ($P < 0.0001$), and HNL reached a higher level in bloodstream and abdominal infections. CRP levels showed the same trend as HNL levels. PCT levels were significantly increased in bloodstream infections, abdominal infections, and in bacterial pneumonia ($P < 0.0001$), while no significant differences were found in soft tissue ($P = 0.4378$) or urinary tract infections ($P = 0.423$). There was no difference in HNL and CRP levels between patients with Gram-negative bacterial (GNB) or Gram-positive bacterial infections. However, compared with controls, PCT was only increased in GNB-infected patients.

Conclusion: HNL detection can help diagnose patients with infectious diseases, and the diagnostic efficacy of HNL is not affected by the infected site or by pathogenic bacterial species. The combination of HNL, PCT, and CRP has a superior performance at identifying bacterial infections compared with traditional biomarkers.

1. Introduction

Infectious diseases are a serious threat to global public health, and infections are also a common and costly complication in patients with established diseases [1–3]. For bacterial infections, antibiotic therapy is an effective strategy, and earlier treatment is optimal. Therefore, it is important to determine the microbiological sources of infections in a timely manner, which is difficult even for experienced physicians. Microbiological culturing and serological markers, such as C-reactive protein (CRP) and procalcitonin (PCT), which are extensively used in the clinic, can improve the diagnosis of infectious diseases. Nevertheless, current markers are not sufficiently accurate and rapid. Microbiological culture has long been regarded as the gold standard for pathogen identification. However, infection diagnoses using culture-based methods are often delayed, because culturing and identifying pathogens takes at least 48 h. Research has shown that the serum PCT test does not help to diagnosis patients with soft tissue infections. Moreover, the sensitivity of PCT for Gram-positive bacteria (GPB)-infected patients was very low. CRP is criticized for its low specificity, and its levels can be elevated by a variety of pathologies such as rheumatic disease and surgical trauma [4–10]. Those deficiencies lead to a number of infection misdiagnoses. Misdiagnoses have a significant bearing on the use and misuse of antibiotics [11,12]. Consequently, there is an urgent need for rapid and accurate biomarkers with high sensitivity and specificity in clinical diagnosis.

Human neutrophil lipocalin (HNL) has been purified from the

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secondary granules of human neutrophils, with a weight of 45 kDa (unreduced). Two similar subunits constitute the glycoprotein molecule. HNL is readily mobilized in neutrophils upon stimulation [13,14]. Previous studies have reported that HNL has enormous diagnostic performance in discriminating viral and bacterial infections, suggesting that HNL is a potential marker for clinical diagnosis [15–17]. However, HNL has not been evaluated for infections at different sites or caused by different pathogenic bacterial species.

Therefore, in the present study, we evaluated HNL, CRP, and PCT performance at identifying the presence of bacterial infections in patients. Additionally, we grouped patients based on their infection sites and bacteria, and then compared the levels of HNL, PCT, and CRP. Besides, we further explore the potential for a more accurate method of diagnosing bacterial infection.

2. Materials and methods

2.1. Patients

The study cohort included 574 participants from September 2017 to April 2018. Patients, 327 males and 180 females, were hospitalized in AnHui Provincial Hospital, China. The 67 healthy controls consisted of 43 males and 24 females. All patients (n = 436 with bacterial infections and n = 71 with viral infections) had microbiological, serological, and/or PCR testing confirming the etiology of their infections. Diagnoses were made by three experienced physician internists based on the information available: symptoms and signs, course, temperature, cultures, X-ray or other imaging examinations, and blood analyses including white blood cell counts, erythrocyte sedimentation rate. CRP and PCT levels were not available to reviewers. There are 506 parents in whom the etiology could not be confirmed was excluded from this research. The inclusion criteria for patients in this study included:

(1) The signs and symptoms of acute infection: to be specific, patients with pneumonia has cough, sputum, fever (> 37 °C), and the support of a positive chest-X-ray [18]; patients with the classic symptoms of urinary tract infections with varying degrees of frequency, and with pain and urgency to urinate [20]; patients with soft tissue infections from an obvious injury, skin redness, and swelling including acute cellulitis, erysipelas, and soft-tissue abscess [21]. Abdominal infections with peritoneal irritation, abdominal tenderness and tension, fever (> 37 °C), including abdominal abscess, bacterial peritonitis, and purulent appendicitis [19]. The diagnostic criteria for bloodstream infections included fever (> 38 °C), chills, inflammatory variables, hemodynamic variables, organ dysfunction variables, and tissue perfusion variables [22].
(2) The diagnosis of bacterial infection was supported by positive cultures from blood, wound abscesses, urine, percutaneous peritoneal drainage, sputum, or bronchoalveolar lavage fluid. The diagnosis of infection with viruses such as influenza A/B, Epstein-Barr, and others was supported by serological and/or PCR testing of samples from the respiratory tract or blood.

The exclusion criteria included: (1) patients who had received antibiotic therapy at the start of the study. (2) Multi-infection cases and patients with known chronic viral infections, such as hepatitis B virus. (3) Patients with immune-related diseases (rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus, vasculitis, or multiple sclerosis), which may influence biomarker levels. This study was approved by the Ethics Committee of the Anhui Provincial Hospital. Patient characteristics are shown in Table 1.

2.2. Methods

All specimens were drawn before the start of antibiotic treatment when patients had the clinical signs and symptoms of infection. Serum

Table 1
Characteristics of patients in the study.

Groups	Number of patients with confirmed etiology	Age (median and IQR)	Sex ratio(% male)	Etiological results (%)
Healthy	67	55(43–70)	64.2%	
Viral infection	71	14(5–31)	64.7%	Cytomegalovirus (22.5%), Epstein-Barr virus (22.5%), Herpes simplex virus (16.9%), Hepatitis virus (14%), Influenza virus (12.6%)
Bacterial infection	436	59(42–74)	64.4%	
Bloodstream infection	69	60(39–68)	68.1%	*Escherichia coli* (31.8%), *Klebsiella pneumoniae* (17.4%), *Staphylococcus aureus* (13.0%), Others (37.8%)
Bacterial pneumonia	199	61(39–78)	71.3%	*Acinetobacter baumannii* (23.1%), *Pseudomonas aeruginosa* (17.1%), *Klebsiella pneumoniae* (14.6%), *Staphylococcus aureus* (13.6%), Others (31.6%)
Abdominal infection	80	56(45.25–74)	50%	*Escherichia coli* (41.3%), *Enterococcus* spp. (13.8%), *Klebsiella pneumoniae* (10%), Others (34.9%)
Soft tissue infection	50	49(29–65.25)	76%	*Staphylococcus aureus* (40%), *Escherichia coli* (18%), *Pseudomonas aeruginosa* (8%), Others (34%)
Urinary tract infection	38	63(49.75–70.75)	36.8%	*Escherichia coli* (60.5%), *Enterococcus* spp. (10.5%), *Klebsiella pneumoniae* (10.5%), Others (18.5%)

Table 2
Concentrations of studied bio-markers.

Groups	Medians (inter-quartile ranges) for:		
	HNL(μg/L)	CRP(mg/L)	PCT(μg/L)
Bacterial infection	170.9(110.4–263.1)[a] N = 436	39.4(12.32–100.7)[a] N = 344	0.64(0.20–2.47)[a] N = 300
Viral infection	77.5(55.6–119.0)[b] N = 71	11.1(3.65–23.09)[a] N = 71	0.31(0.18–0.64)[a] N = 71
Healthy	67.27(49.64–88.96) N = 67	4.81(3.14–6.39) N = 41	0.18(0.10–0.32) N = 41

[a] $P < 0.0001$,
[b] $P < 0.05$, compared to healthy.

was obtained by allowing whole blood to clot for 2 h at room temperature, after which the serum was recovered after centrifugation of the blood at 450 × *g* for 10 min, followed by storage at −80 °C until analysis. Subgroups of bacterial infections were based on culture results and clinical diagnoses.

2.3. Assays

Serum CRP levels were measured by a turbidimetric inhibition immunoassay using an automatic analyzer (SIEMENS BNII system). Plasma PCT levels were measured using the Roche Cobas e601 Automatic Electrochemiluminescence Immunoassay System. The above tests were performed in the routine clinical laboratory at Anhui Provincial Hospital, China. Serum HNL levels were measured with an ELISA kit provided by Changchun Brother Biotech Co. Ltd (the product manual indicates an accuracy rate above 85% and an intra-assay coefficient of variation less than 10%). The assays were performed in accordance with manufacturer guidelines.

2.4. Statistics

Quantitative data were expressed as medians and inter-quartile ranges. Group comparisons were performed by the non-parametric Mann–Whitney test or the non-parametric Kruskal–Wallis test for independent groups, where appropriate. Receiver operating characteristic (ROC) analysis was performed to estimate the clinical performances of the different biomarkers. A combining analysis including HNL, CRP, and PCT was generated. We calculated the predicted probabilities of the combination of several biomarkers through logistic regression analysis, drawing ROC curves based on predicted probabilities. Comparisons of data for the area under the curve (AUC) were analyzed by c-statistics. Youden's index was used to estimate the optimal discriminatory concentration of a biomarker. Sensitivities, specificities, and positive (LR +) and negative likelihood ratios (LR-) were calculated based on this index. A P-value of < 0.05 was regarded as statistically significant. The statistical program SPSS18.0 was used for all calculations. *P < 0.05, **P < 0.01, ***P < 0.001, ns = not significant.

3. Results

3.1. Biomarker diagnosis performance for bacterial infection

We compared the levels of each biomarker in the infection group with those of the healthy controls. The results are summarized in Table 2. Compared with the healthy group, the levels of all the biomarkers were higher in the groups with bacterial infections. Fig. 1 shows the ROC curves for HNL, PCT, and CRP. The diagnostic performances of these markers were judged by ROC curve analysis, the results of which are shown in Table 3. As shown in the table, the AUCs for HNL and CRP (P = 0.1492) were nearly 0.9, which is a significantly higher value than that for PCT (p < 0.0001) in terms of differentiating the patients with bacterial infections from healthy people. Upon further analysis of the ability of biomarkers to distinguish bacterial and viral infections, we found that the AUC of each biomarker dropped. The AUC for HNL fell to 0.81, but this value was still significantly higher than those for CRP (P = 0.0001) and PCT (P < 0.0001).

The results indicated a better performance of HNL in discriminating bacterial infections from viral infections. However, the sensitivity and specificity of a single biomarker for distinguishing bacterial infection from a viral infection or from a healthy status were not suitable for clinical usage. We sought to combine the HNL, PCT, and CRP markers to improve their diagnostic power. The constructed discriminant function consisted of a linear equation of original independent variables, shown as:

$$P(h) = 0.026 * C1 + 0.186 * C2 + 0.285 * C3 - 0.3079.$$

$$P(v) = 0.016 * C1 + 0.018 * C2 + 0.04 * C3 - 1.561$$

The *P(h)* is the predicted probabilities for combining to discriminate bacterial infections from healthy, while *P(v)* for distinguishing bacterial and viral infections. The *C1* are the value of HNL, and *C2* for CRP, *C3* for PCT.

The ROC of the combination of three biomarkers shows the best performance, which gave an AUC of 0.96 (0.95–0.98, P < 0.05) when compared with samples from healthy patients and 0.86 (0.82–0.90, P < 0.05) when compared with viral infections, both of which were

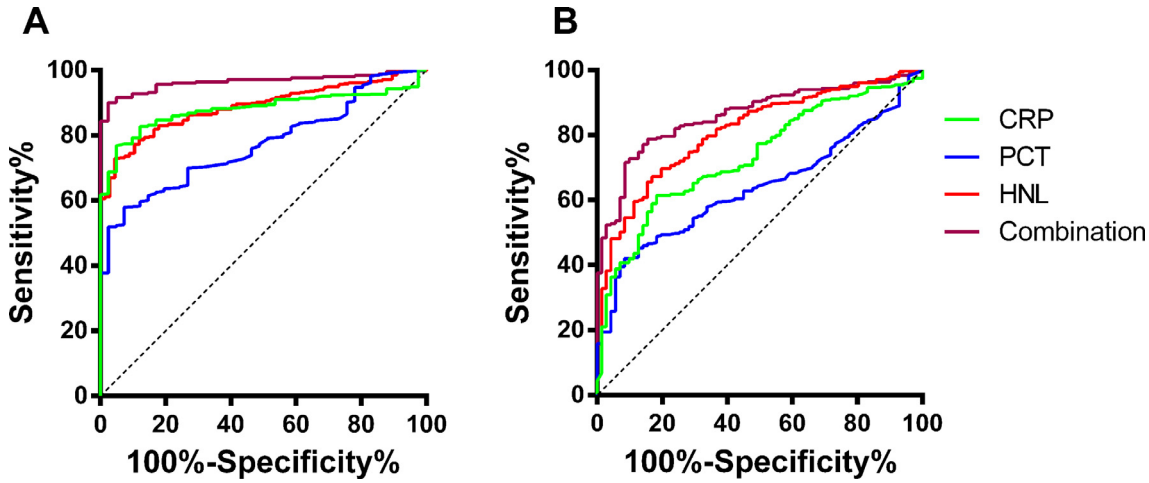

Fig. 1. A. ROC curves of HNL, CRP, PCT, and their combination in discrimination between healthy subjects and patients with bacterial infections. B. ROC curves of HNL, CRP, PCT, and their combination in discrimination between patients with bacterial infections and viral infection.

Table 3
AuROCs analysis of the distinction between bacterial infection and healthy control or viral infection and the biomarker performances.

	AuROC (95%CI)	*P* value of AuROC Vs. HNL	MAX Youden index	Cut-off	Sensitivity,% (95%CI)	Specificity,% (95%CI)	*OR* (95%CI)	Likelihood ratio (95%CI)	
								LR +	LR −
Distinguish between bacterial infection and healthy									
HNL	0.89(0.86–0.92)	–	0.68	116.3 μg/L	72.94(68.46–77.00)	95.55(86.53–98.83)	57.49(17.72–186.5)	3.53(3.0–4.15)	0.06(0.02–0.19)
CRP	0.88(0.84–0.92)	0.1492	0.71	8.38 mg/L	82.80(78.06–86.71)	90.24(75.94–96.83)	44.54(15.24–130.2)	8.49(3.34–21.56)	0.19(0.15–0.24)
PCT	0.77(0.71–0.83)	< 0.0001	0.51	0.43 μg/L	57.95(51.95–63.73)	92.68(78.99–98.09)	17.46(5.26–57.89)	7.92(2.65–23.65)	0.45(0.39–0.52)
Combination(HNL,PCT,CRP)	0.96(0.95–0.98)	< 0.0001	0.87	0.84	90(85.43–93.30)	97.62(85.91–99.88)	360(47.4–2732)	37.8(5.45–262.23)	0.10(0.07–0.15)
Distinguish between bacterial infection and viral infection									
HNL	0.81(0.76–0.86)	–	0.50	125.9 μg/L	67.20(62.54–71.55)	83.1(71.94–90.59)	10.07(5.25–19.34)	3.98(2.36–6.68)	0.39(0.34–0.45)
CRP	0.73(0.68–0.79)	0.0001	0.43	24.85 mg/L	61.47(55.81–66.83)	81.69(70.36–89.52)	7.12(3.74–13.54)	3.36(2.04–5.53)	0.47(0.41–0.55)
PCT	0.64(0.58–0.70)	< 0.0001	0.34	0.92 μg/L	42.05(36.27–48.05)	91.55(81.89–96.52)	7.86(3.30–18.74)	4.98(2.29–10.83)	0.63(0.57–0.70)
Combination(HNL,PCT,CRP)	0.86(0.82–0.90)	0.0039	0.38	0.71	78.4(72.86–82.22)	84.51(73.54–91.65)	19.78(9.73–40.27)	5.06(2.93–8.75)	0.26(0.20–0.33)

significantly higher than the AUC of HNL ($P < 0.05$).

The optimal concentration of each biomarker for distinguishing bacterial infections from healthy samples and viral infections was determined by the Youden index, the results of which showed that the optimal cut-off values for HNL, CRP, PCT, and their combination were 116.3 μg/L, 8.38 mg/L, 0.43 μg/L, and 0.84 when compared with healthy samples and 125.9 μg/L, 24.85 mg/L, 0.92 μg/L, and 0.71 when compared with viral infections, respectively. The combination shows superior performance in analysis indexes such as the positive likelihood ratio. The results are shown in Table 3.

3.2. HNL levels for different infection sites

It has been mentioned that the sensitivity for PCT was different between patients with soft tissue infection and sepsis [23–27]. To further investigate whether the biomarker level can be affected by infection sites, we grouped the bacterial infection patients based on their infection sites. As shown in Fig. 2, the levels of HNL and CRP were found to be significantly higher in each group with bacterial infections compared with healthy controls ($p < 0.0001$). A higher level was observed in patients with bloodstream infections and abdominal infections ($p < 0.0001$). As for PCT, there were significant increases for bloodstream infections, abdominal infections, and bacterial pneumonia groups ($p < 0.0001$). However, no significant elevated levels were found in the soft tissue-infected patients ($P = 0.4378$) or urinary tract-infected patients ($P = 0.423$).

3.3. HNL levels for infections caused by different bacterial species

Some studies have reported that PCT was more insensitive to GPB-infections. To investigate whether HNL has the same characteristic, we divided the bacterial-infected patients into GPB-infection and GNB-infection groups. There were no statistical differences observed in HNL between patients with GNB and GPB infections ($P = 0.566$). Similarly, no statistical differences were observed in CRP levels ($P = 0.1299$). Meanwhile, the level of PCT was higher in the GNB-infection group than the GPB-infection group ($P = 0.0258$) as shown in Fig. 3A–C, respectively. Finally, we grouped the bacterial-infected patients based on pathogenic bacterial species. For each markers, No statistical differences were found between pathogenic bacterial species subgroups. The results of each markers are shown in Fig. 3D–F, respectively.

4. Discussion

Symptoms related to infections are probably the most common reason for seeking health care worldwide. Early diagnosis of an infection is still challenging for clinicians. Therefore, this study focused on exploring the effectiveness of markers for diagnosing bacterial infections.

In general, the AUC values reveal that all the diagnostic marker have good performances for distinguishing bacterial infections, with HNL having the best efficiency. In the distinction between healthy controls and bacterial infections, Venge et al. showed that the AUC of HNL was 0.95 (95% CI 0.91–0.97), which is higher than our research [28,29]. The differences between studies may have been because we recruited patients with different infection sites and infection severities. We speculate that testing patients with less severe infections may have been the reason for the drop in the sensitivity and specificity of HNL, which caused the AUC of HNL to be lower than previously reported [28,29].

To further enhance the diagnostic value of markers, including combinations of each marker, we generated a more reliable index using a new algorithm. The combination of HNL, CRP, and PCT had the best AUC, and both the sensitivity of 97.62% and specificity of 90% are more attractive compared to single biomarkers. These findings indicate that the combination of HNL, CRP, and PCT was more effective in

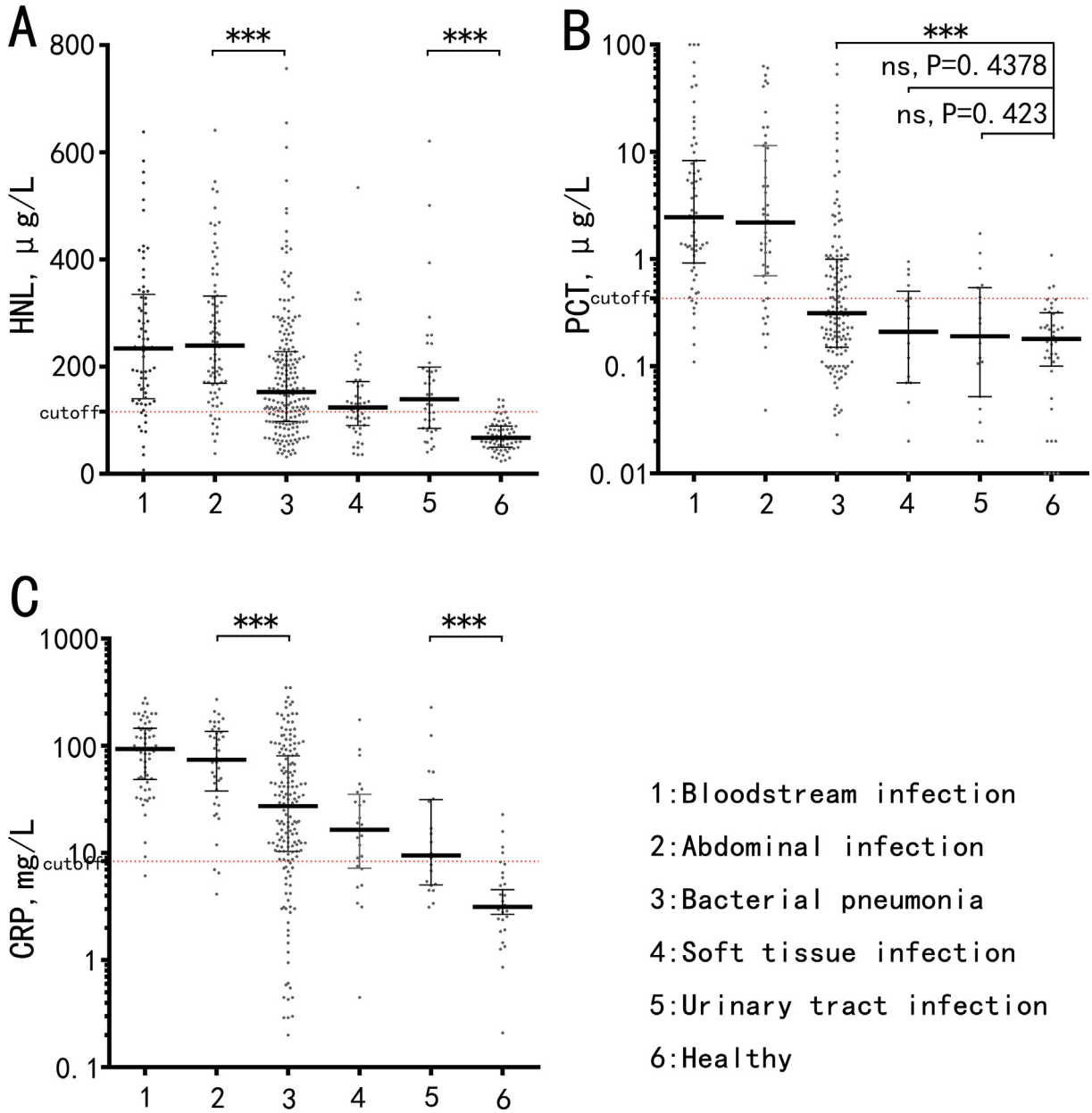

Fig. 2. A. HNL levels in patients with different infection sites from the bacterial infection group. B. PCT levels in patients with different infection sites from the bacterial infection group. C. CRP levels in patients with different infection sites from the bacterial infection group. *P < 0.05, **P < 0.01, ***P < 0.001, ns = not significant.

identifying bacterial infection. However, these results need to be confirmed in further prospective studies.

In addition to comparing diagnostic efficiencies, we further investigated the levels of each marker for infections in different sites. Previous research has shown that the plasma PCT level increases significantly in bloodstream infections, however the sensitivity of serum PCT has not been found to be of value in the diagnosis of soft tissue infections [21]. In the present study, patients with localized infection, such as soft tissue infections and urinary tract infections were recruited, and we found that PCT had a lower diagnostic performance in these individuals. With regard to HNL, we witnessed successful diagnoses, even in localized infections. Higher HNL levels were found in patients with abdominal or bloodstream infections than in those with soft tissue infections, urinary tract infections, or bacterial pneumonia. We speculate that the reason for these differences is that pathogens that enter the bloodstream or body fluids can stimulate granulocyte-macrophage colony-stimulating factor, which may directly or indirectly lead to HNL release. Our study revealed that elevated levels of HNL were detectable across all sites of bacterial infection in the different groups. Determining the HNL levels in patients may help to diagnose localized infections.

In accordance with prior research, serum PCT levels were found to be significantly higher in GNB-infected patients than in GPB-infected patients [23–27]. Interestingly, no difference was found in the HNL levels between GPB-infected patients and GNB-infected patients. Therefore, we predict that using HNL as a diagnostic marker would overcome this drawback and improve the accuracy and chances of the early diagnosis of GPB infection. It would also be helpful to clinicians early diagnoses GPB-infection when patients with high HNL levels and low PCT levels. We also found that the levels of HNL were high with all kinds of infection-causing bacterial species, meaning that HNL has a broad coverage for infections.

This study was subject to several limitations. Improving diagnosis accuracy was the key point in this study, so we combined objective

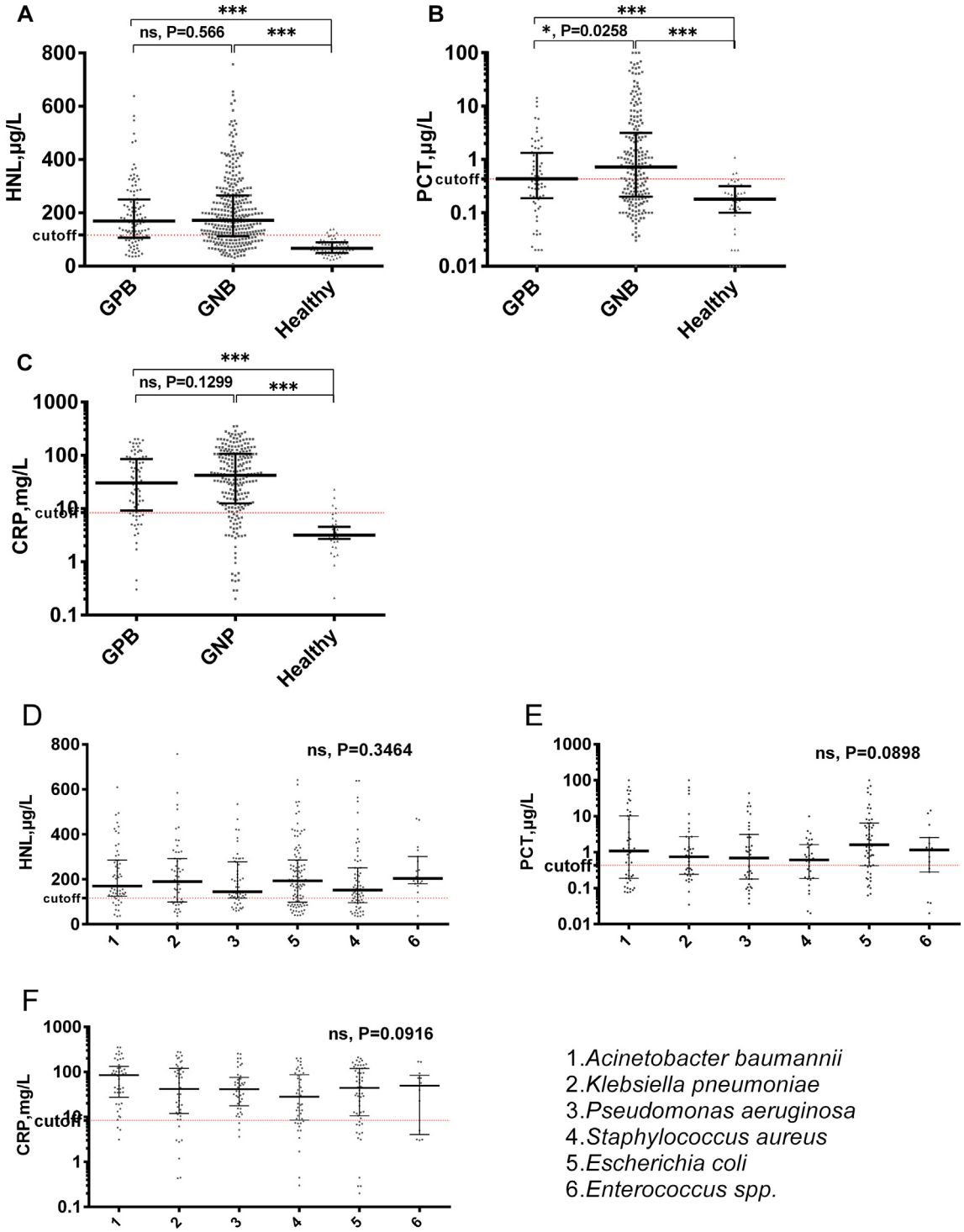

Fig. 3. The different levels of HNL grouped based on patients with infection caused by GNB or GPB was shown in Fig. 3A. And Fig. 3B and C is for PCT and CRP, respectively. Fig. 3D showed the different HNL levels corresponding to bacterial strains isolated from various cultures. And Fig. 3E and F is for PCT and CRP. GNB, Gram-negative bacteria; GPB, Gram-positive bacteria. *P < 0.05, **P < 0.01, ***P < 0.001. ns = not significant.

microbiological, serological, and PCR tests with clinical signs and symptoms of the patients, which greatly improved diagnostic accuracy. However, we cannot exclude the small possibility of misdiagnosis. Besides, in this study, patients with viral disease were younger than the other groups in this study. Previous studies have shown that HNL concentrations in healthy children were similar to the concentrations of healthy adults [14,15], which was also shown in a recent review[33]. As for PCT and CRP, the baseline levels in children are similar to those in adults [34]. Additionally, the proportion of males was moderately higher than females in this study. There were no statistical differences in the levels of each markers between males and females in our study (Fig. S1). It has been reported that HNL can help guide the use of empirical antibiotics [17], but further research in clinical settings is needed.

In previous studies, CRP was found to display higher sensitivity than other markers [30]. However, one remaining problem is the poor specificity of CRP. In this regard, there is evidence that HNL concentrations of patients with rheumatoid arthritis were normal despite active clinical

disease [31]. HNL concentrations are slightly elevated by major surgical trauma, but these changes are minor as compared with CRP [32]. Those previous studies indicated that non-infection inflammatory and other disease processes may not increase HNL levels, making HNL more specific in infection-driven disease.

In conclusion, we illuminated that serum HNL is a novel marker for diagnosing bacterial infections, especially for infections located in the bloodstream and abdomen. Meanwhile, HNL can diagnose almost any site of bacterial infection, because it also shows a credible performance in soft tissue and urinary tract infections. Detecting HNL levels in patients will help to diagnose GPB and GNB infections. HNL is useful for physicians to diagnose bacterial infection in an accurate and timely manner. The combination of HNL, CRP, and PCT will improve the diagnosis performance markedly.

Competing interests

All authors have completed disclosure form and declare that: (i) no support, financial or otherwise, has been received from any organization that may have an interest in the submitted work; and (ii) there are no other relationships or activities that could appear to have influenced the submitted work. (iii) Lining Sun provided technical support for experimental and didn't participate in the statistical analysis and writing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinbiochem.2019.10.003.

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