ORIGINAL RESEARCH

Utility of Human Neutrophil Lipocalin as a Diagnosing Biomarker of Prosthetic Joint Infection: A Clinical Pilot Study

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Purpose: The discrimination of prosthetic joint infection (PJI) from aseptic failure is regarded as a major clinical challenge. The key function of human neutrophil lipocalin (HNL) in regulating bacterial infection rationalizes its potential as a biomarker to diagnose PJI. This work evaluated the accuracy of serum human neutrophil lipocalin as a biomarker to diagnose PJI.

Methods: This prospective cohort study enrolled altogether 58 patients suffering from miserable knee or hip arthroplasty and receiving revision surgery from 2018 to 2020. Related laboratory and clinical information of these patients were retrieved. Following the Musculoskeletal Infection Society (MSIS) criteria, the diagnosis of PJI was conducted. Collecting preoperative blood samples, we measured HNL by the standard assay. Thereafter, plotting the receiver-operating characteristic curve (ROC), the area under the curve (AUC) values were calculated to analyze the diagnosis accuracy.

Results: According to the MSIS criteria, 38 cases (65.5%) were classified into the PJI group, while 20 (34.5%) into the aseptic loosening group, with age ranging from 38 to 87 (median, 66.9) years. The median serum HNL level of the PJI patients was 199.01 (range, 85.34–357.79) ng/mL, significantly higher as compared with that of 64.81 (range, 20.73–157.89) ng/mL of the aseptic loosening group. Using the Youden index, the optimal threshold value was 105.1ng/mL, while the specificity, sensitivity, and AUC were 85.0%, 81.6%, and 0.919, respectively.

Conclusion: Serum HNL is the creditable test that can be employed as the laboratory biomarker to screen PJI. The threshold HNL level is 105.1 ng/mL, which may distinguish PJI from aseptic failure.

Keywords: prosthetic joint infection, human neutrophil lipocalin, biomarker, arthroplasty

Introduction

The severe complication secondary to total joint arthroplasty (TJA) is periprosthetic joint infection (PJI) which may lead to catastrophic results, like limb loss, revision surgery, and even death.[1–3] Nonetheless, it is still not easy to diagnose PJI and often gets delayed, in particular in the presence of low-degree or chronic infections, because of the absence of any "gold standard" examinations available and the existing diagnostic approaches fail to achieve high accuracy.[4–6]

Standard tests, including erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), are unable to achieve satisfactory diagnostic value.[7,8] Therefore, many efforts are made to elucidate novel approaches to effectively detect PJI, such as circulating cytokine analysis, molecular approaches, and implant sonication.[9–11] Nonetheless, such novel diagnostic approaches are expensive, time-consuming, and need special equipment, restricting their clinical application. This necessitates the development of highly sensitive, specific, cost-effective markers or marker panels for diagnosis so as to rapidly diagnose and differentiate disease under varied clinical conditions.

Human neutrophil lipocalin (HNL) can also be referred to as neutrophil gelatinase-associated lipocalin (NGAL) and lipocalin 2.[12,13] Neutrophil granulocytes reflect the major HNL source, while the main site for protein generation in some conditions is epithelial cells.[14–16] HNL was frequently associated with tumor size, stage, and invasiveness. These characteristics thus make it a potential biomarker for malignancy.[17] Carlson et al reported HNL served as a unique marker of neutrophil inflammation in ulcerative colitis and proctitis.[18] Immunoassays estimating HNL/NGAL in different body fluids suggested escalation of its concentration in urine and blood among cases suffering from acute kidney injury (AKI) and those having bacterial infections, respectively.[19,20] Thus, serum HNL level is presumed to distinguish viral from bacterial infections, which can achieve high likelihood, and negative (NPV) and positive (PPV) predictive values (NPV) in the N90% distinction.[21] As verified by other articles, the potent discrimination of serum HNL content on adults and children indicates that measuring serum HNL level can be the potential biomarker to differentiate viral from acute bacterial infections.[22–24] The joint fluid HNL has also proved the existence of closed correlations between PJI and aseptic loosening.[25–27] Deirmeingian et al were the first to predicted HNL in synovial fluid with 100% sensitivity and specificity,[26] and Vergara et al reported 86% sensitivity and 77% specificity applying MSIS definition.[25] Thirdly, Dijkman reported synovial fluid HNL 92% sensitivity and 83% specificity when applying the MSIS criteria.[27] Based on the above findings, the HNL level has been recognized as the potential diagnostic factor to diagnose postoperative prosthesis infection. Nonetheless, there is no available article demonstrating HNL's function in blood as the candidate biomarker to diagnose PJI. Thus, this single-center study mainly focused on the significance of serum HNL in distinguishing between aseptic failure and PJI.

Methods
Study Design, Patient Inclusion, and Exclusion Criteria
This single-centered prospective cohort study was conducted in one medical university hospital. The Board Review Committee of Zhengzhou Orthopaedics Hospital approved our study protocols. Each partaker provided informed consent for participation. This work included cases who underwent knee or hip arthroplasty revision between 2018 and 2020 in our institution. Patients conforming to the following criteria were enrolled: those undergoing total knee or hip arthroplasty to treat osteoarthritis and those who were about to receive revision due to aseptic loosening and suspected infection. The exclusion criteria involved cases who took antibiotics for a long time (>7 days) in 2 weeks prior to admission, cases who had other sites of infections (including urinary or respiratory tract infection), acute or chronic renal function injury patients, chronic renal insufficiency, and patients with active malignant disease. Moreover, cases with heavy smoking (≥20 cigarettes per day) or those with obesity (body mass index (BMI) >35 kg/m^2) were also eliminated from this study.

PJI diagnosis was defined according to the MSIS definition.[28] We determined the need for revision in patients due to aseptic causes (like wear, aseptic loosening, malalignment, instability) or PJI. Preoperative blood was sampled to determine ESR, CRP, D-dimer, PCT (Procalcitonin), and HNL.

Bacterial Culture
Synovial fluids after collection in the operating room were cultured in BD BACTEC aerobic/anaerobic/fungal and acid-fast bacilli vials for 14 days (35 °C). Intraoperative periprosthetic tissue samples were homogenized for 1min using BHI broth (3mL). Thereafter, the as-obtained homogenate was further cultured on Columbia blood agar containing selective medium, chocolate agar and 5% sheep blood for 7 days at 35 °C both anaerobically and aerobically. The same amount of homogenate (1mL) was inoculated into BD BACTEC aerobic/anaerobic/fungal and acid-fast bacilli bottles for 14 days.

Measurement of Serum HNL Levels
HNL levels in heparinized blood or EDTA-plasma were determined using the double monoclonal ELISA kit (Diagnostics Development, Uppsala, Sweden) following the specific protocols. In the ELISA assay, the <6% CV imprecision was allowed. HNL levels within whole blood samples were analyzed based on the previous description.

Statistical Analysis

Results were expressed in the form of medians. The data obtained from diagnostic tests were compared using Fisher's exact test and independent-sample *t*-test. Meanwhile, this study plotted receiver-operating characteristic (ROCs) curves to present HNL-index within the blood, CRP, ESR, PCT, and D-dimer levels, respectively, which were subsequently adopted for establishing the best thresholds to diagnose PJI based on Youden's J statistic. The specificity, sensitivity, as well as area under the curve (AUC) values were evaluated with the help of the MedCalc 13.2.2 Software (MedCalc, Ostend, Belgium). Further, this study also adopted SPSS24.0 (SPSS, Chicago, Illinois, USA) for statistical analysis. P<0.05 represented statistical significance.

Results

General Characteristics and Comorbidities of Patients

This study enrolled a total of 58 patients for revision knee or hip arthroplasty from 2018 to 2020. The MSIS criteria on PJI categorized 38 patients into the PJI group, while 20 were into the aseptic group (Figure 1). The study population comprised 35 (57.4%) males and 26 (42.6%) females, yielding the ratio of male to female of 1.3. The mean age was 66.4 ±6.7 years and 67.3 ±8.2 years for the PJI and non-PJI groups, respectively. Preoperative blood was obtained from the hip in 7 (17.2%) and knee in 51 (82.8%) cases. Patient baseline features and underlying diseases are detailed in Table 1.

The Diagnostic Accuracy and ROC Curve of PJI Diagnosis by Using Serum HNL

Relative to the aseptic group, the PJI group manifested significantly high serum PCT, ESR, and HNL levels (Figure 2). However, blood CRP and D-dimer levels failed to demonstrate significant differences between both groups, respectively (p = 0.204, p = 0.414). The median serum HNL level was 199.01 ng/mL (ranging from 85.34 to 357.79) among patients with PJI compared to 64.81 ng/mL (20.73 to 157.89) among those with no PJI (p < 0.001). Meanwhile, the PJI group revealed the median level of PCT of 0.52 mg/L (0.22–1.34) relative to that of 0.27 mg/L (0.04–0.45) for the non-PJI group (p = 0.000). Moreover, the median CRP level was 22.27 mg/L (1.03–89.26) in the PJI group, whereas 15.99 mg/L (3.01–50.40) in the non-PJI group (p = 0.204). As evident from Table 2, the median ESR and median D-dimer of the PJI group were 41.63 mm/H (9.00–80.00) and 1.58 mg/L (0.19–3.38), respectively, while those in the non-PJI group were 13.70 mm/H (3.00–25.00) and 1.39 mg/L (1.87–2.05), respectively (p = 0.000, p = 0.414).
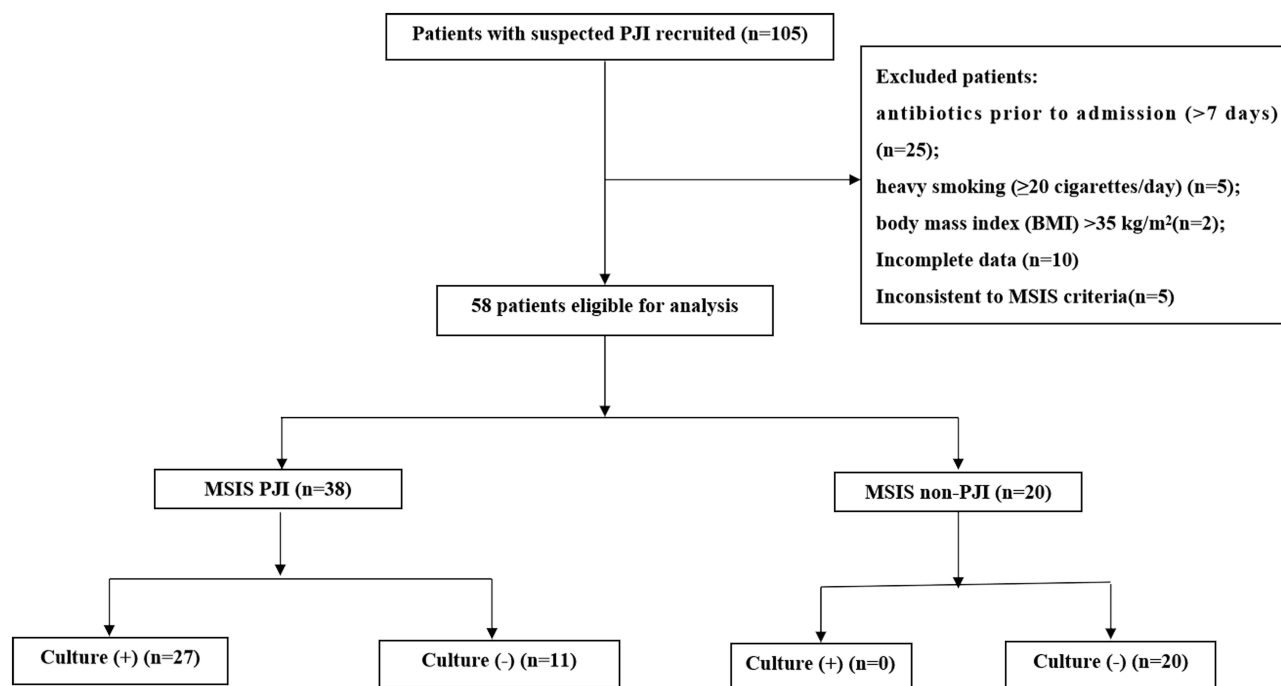

Figure 1 Flowchart of patient inclusion.

Table I Demographic Data for the Study Population

Characteristics	PJI (n=38)	Non-PJI (n=20)	p value
Age, years (mean ± SD)	66.4±6.7	67.3±8.2	0.43
Sex, n			0.00*
Male	24	11	
Female	18	8	
Body mass index, kg/m^2	26.9±3.6	27.9±2.8	0.56
Comorbidities, n (%)			
Hypertension	16	9	0.00*
Diabetes	12	3	0.00*
Smoking	15	10	0.83
Alcoholism	12	4	0.01*
Joints, n			0.00*
Knee	33	18	
Hip	5	2	
Sinus tract, n	5	0	0.003*
Laboratory findings			
ESR (mm/H, mean ± SD)	41.63±19.26	14.20±7.12	0.000*
CRP (mg/L, mean ± SD)	22.27±19.6	15.99±13.1	0.204
D-dimer (mg/L, mean ± SD)	1.59±0.9	1.41±0.5	0.414
PCT (ng/mL, mean ± SD)	0.39±0.1	0.20±0.1	0.000*
HNL (ng/mL, mean ± SD)	199.01±94.3	64.81±42.4	0.000*

Notes: *P<0.05.
Abbreviations: ESR, erythrocyte sedimentation rate; CRP, C reactive protein; PCT, procalcitonin; HNL, human neutrophil lipocalin.

To analyze the discriminating ability of these inflammatory parameters of PJI versus non-PJI groups, ROC curves regarding the serum parameters were plotted (Figure 3). Results revealed the AUC value for serum CRP was 0.531 (95% CI 0.429–0.719), while that for D-dimer was 0.579 (95% CI 0.384–0.678). The PJI and non-PJI groups could be well discriminated by the serum HNL, ESR, and PCT, and their AUC values were 0.919 (95% CI 0.851–0.987), 0.878 (95% CI 0.773–0.982), and 0.856 (95% CI 0.678–0.913), respectively. Table III narrates the comprehensive features of serum CRP, ESR, PCT, D-dimer, and HNL in diagnosis. To distinguish aseptic failure from PJI, the best threshold for serum HNL was 105.15 ng/mL, and the maximal specificity and sensitivity were 85.00% (95% CI 74.6–96.9%) and 81.6% (95% CI 71.0–93.0%), respectively. To detect PJI, PCT at level of 0.32 mg/L reflected the specificity and sensitivity of 90.0% (95% CI 85.4–99.1%) and 78.9% (95% CI 65.7–85.8%), separately; those for CRP level (19.2 mg/L) were 80.0% (95% CI 71.1–89.7%) and 57.9% (95% CI 43.5–67.1%), separately. ESR demonstrated a specificity of 76.3% (95% CI 62.3–82.2%) and a sensitivity of 93.5% (95% CI 88.8–100.0%) at the 24.5 mm/H threshold; D-dimer at level of 2.13 mg/L highlighted the specificity and sensitivity of 100.0% (95% CI 95.0–100.0%) and 31.6% (95% CI 21.8–40.7%), separately.

Microbiological Findings

All of these patients had ≥2 positive culture specimens with the same organism. Totally, 27 (27/38, 71.1%) cultures were positive in diagnosed PJI patients. *S. epidermidis* (12/27, 42.9%) and *S. aureus* (5/27, 21.4%) were the most commonly cultured bacteria, followed by fungal (3/27), *Mycobacterium tuberculosis* (2/27), *Pseudomonas aeruginosa* (2/27), *Escherichia coli* (2/27) and *Brucella* (1/27). There were no polymicrobial culture results. Five out of 11 patients with PJI are negative cultures.

Discussion

Despite recent advancements in exploring the approaches to diagnose PJI, it is still not easy to definitely detect PJI prior to surgical treatment. More efforts are warranted to develop novel strategies to timely and correctly diagnose PJI.
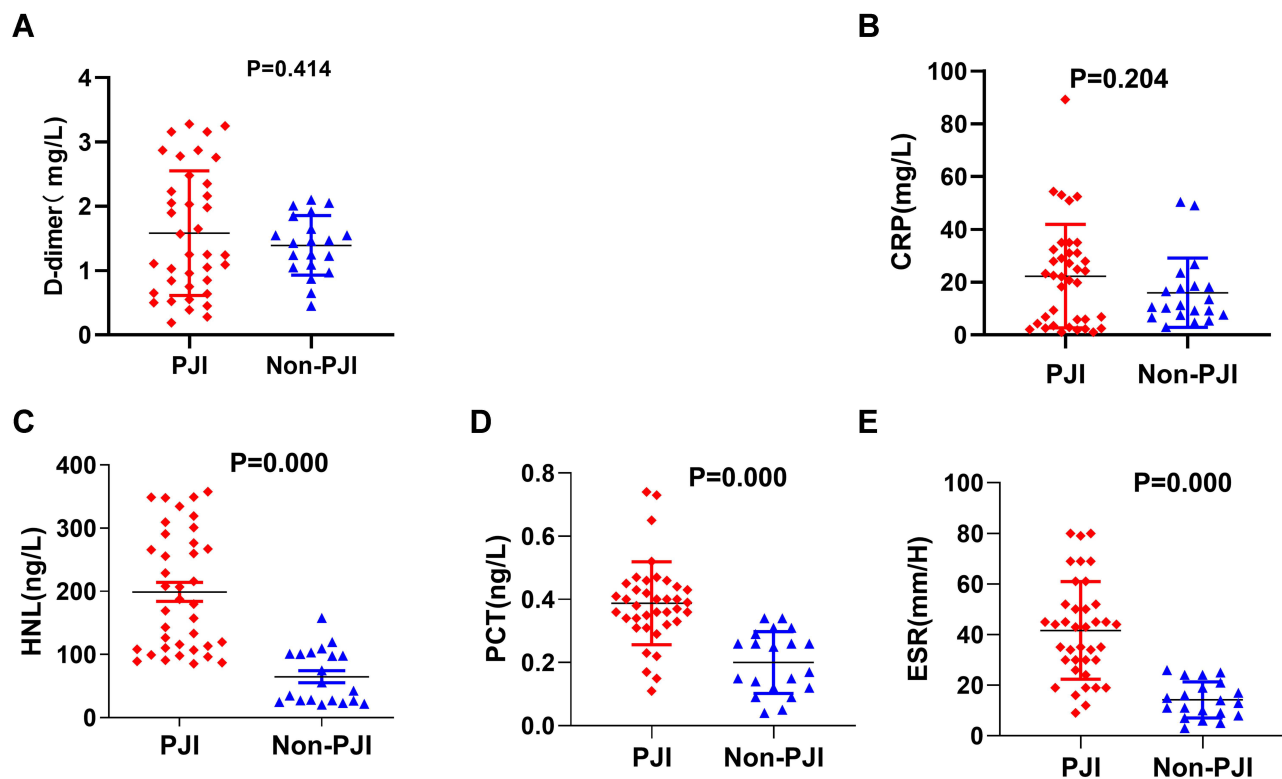
Figure 2 Comparison of different serum biomarkers levels between PJI group and non-PJI group. (**A**). There was not significantly different of D-dimer value between PJI group and non-PJI group (P = 0.414) (**B**). There was not significantly different of CRP value between PJI group and non-PJI group (P = 0.204) (**C**). There was significantly different of HNL level between PJI group and non-PJI group (P = 0.000) (**D**). The PCT value of the PJI group was significantly higher than that of the non-PCT group (P = 0.000) (**E**). There was significantly different of ESR level between PJI group and non-PJI group (P = 0.000).

A degranulation product of neutrophils, HNL, is found to be expressed at a low level in the kidney, lung, stomach, colon, and prostate under normal physiological conditions. HNL expression escalates when inflammation stimulates neutrophil activation or epithelial cell injury.[29] Research findings substantiate the close association of HNL to inflammation, signal transduction, immune response, and the occurrence and development of tumors. The HNL begins to rise at 6–8 h following infection and reaches the peak at 24–48 h. Recent results predict a more rapid release time of HNL. Thus, alterations in serum HNL levels are beneficial in detecting acute infection.

The results of this study emphasized that differences in PCT, HNL, and ESR were of statistical significance between the PJI and non-PJI groups. Meanwhile, serum D-dimer and CRP levels did not reveal any differences between both groups.

Table 2 Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value, and Accuracy of Inflammatory Markers

	Sensitivity	Specificity	PPV	NPV	PLR	NLR	AUC (95% CI)	Cutoff value	P value
HNL	81.6% (71.0–93.0%)	85.0% (74.6–96.9%)	91.2% (88.1–98.7%)	70.8% (66.5–80.2%)	3.438 (1.895–4.606)	0.216 (0.109–0.330)	0.919 (0.851–0.987)	105.1 ng/mL	0.000
CRP	57.9% (43.5–67.1%)	80.0% (71.1–89.7%)	84.6% (76.1–92.3%)	50.0% (41.7–60.2%)	2.894 (1.156–4.246)	0.526 (0.353–0.783)	0.531 (0.429–0.719)	19.2 mg/L	0.204
ESR	76.3% (62.3–82.2%)	93.5% (88.5–100%)	93.5% (84.3–98.9%)	66.7% (54.3–77.7%)	3.632 (2.025–4.759)	0.263 (0.147–0.471)	0.878 (0.773–0.982)	24.5 mm/H	0.001
PCT	78.9% (65.7–85.8%)	90.0% (85.4–99.1%)	93.8% (83.9–99.7%)	69.2% (60.2–79.7%)	5.894 (4.098–9.701)	0.233 (0.125–0.437)	0.856 (0.678–0.913)	0.32 mg/L	0.001
D-dimer	31.6% (21.8–40.7%)	100% (95.0–100%)	100.0% (95.0–100%)	43.5% (31.1–50.5%)	+∞	0.284 (0.151–0.384)	0.579 (0.384–0.678)	2.13 mg/L	0.417

Abbreviations: ESR, erythrocyte sedimentation rate; CRP, C reactive protein; PCT, procalcitonin; HNL, human neutrophil lipocalin; AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio.
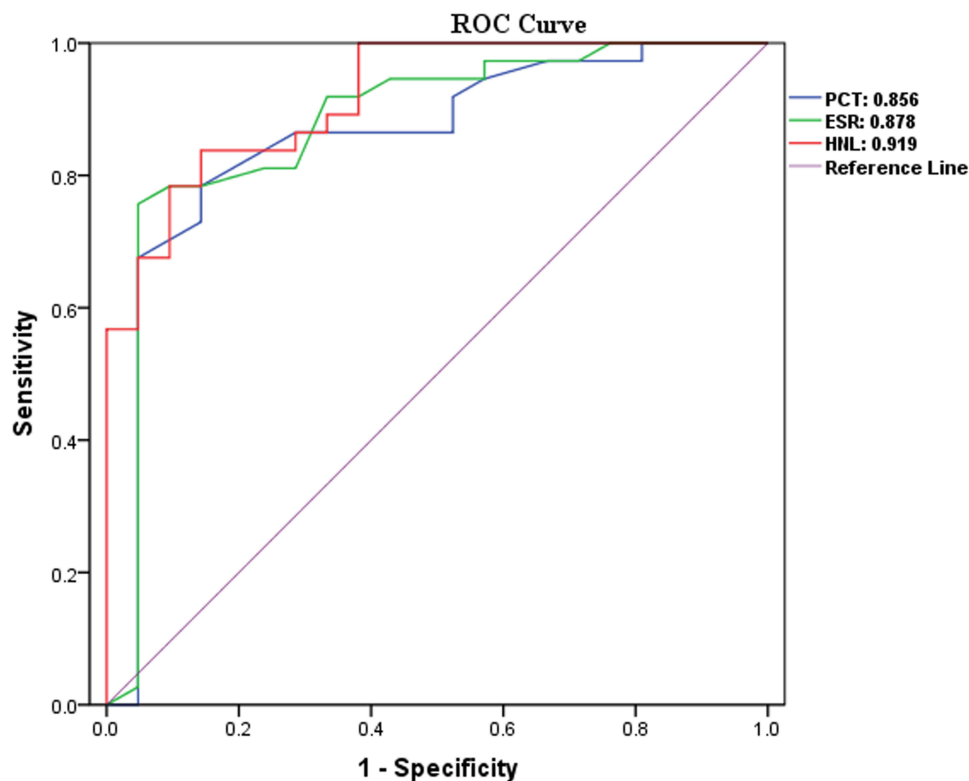
ROC Curve

Figure 3 ROC curves for diagnostic indicators with an AUC >0.7, which included PCT, ESR, HNL.

ROC curve is the most commonly employed method to estimate the diagnostic value. It takes the 1-specificity (misdiagnosis rate or false-positive rate) corresponding to different cutoff points as the abscissa and the sensitivity (true-positive rate) as the ordinate to connect the obtained operating points into a curve. The diagnostic value of the diagnostic test is determined by the ROC AUC, typically 0.5≤AUC≤1. A higher AUC value reflects a higher diagnostic value. Analyzing the ROC curves of the five indicators, the AUC of HNL and ESR were all higher than 0.90 with no significant difference, but HNL exhibited the highest diagnostic value. In the diagnosis of bacterial infectious diseases, the cutoff values of HNL, ESR, and PCT were 105.1 ng/mL, 24.5 mm/h, and 0.32 ng/mL, respectively. The cutoff values of ESR and PCT in the diagnosis of bacterial infectious diseases differed from those in other studies, which may be attributed to the differences in disease patterns, regions, populations, reagents, instruments, etc. Further investigation will greater sample size will help to identify the most accurate cutoff value to provide relevant data for clinical diagnosis.

In conclusion, HNL, ESR, PCT, CRP, and D-dimer are all increasing at PJI, but the sensitivity, specificity, and accuracy of HNL, ESR, CRP, D-dimer, and PCT in the diagnosis of PJI varied. The sensitivity of HNL was the highest, with 85.1% sensitivity. To our knowledge, this is the first study evaluating serum HNL as diagnostic biomarker for PJI. Deirmeingian et al were the pioneer to detect synovial fluid HNL, demonstrating 100% for both sensitivity and specificity based on the MSIS criteria.[27] Subsequently, the sensitivity and specificity of synovial fluid HNL detected by Vergara et al demonstrated 86% sensitivity and 77% specificity, respectively.[25] Dijkman then reported 92% sensitivity and 83% specificity when applying the MSIS criteria, and sensitivity 95% and specificity 95% when according to the pro-Implant criteria.[26] Notably, their cut-off values (2.2 μg/mL, 0.152 μg/mL and 0.7355 μg/mL, respectively) were distinguished from the values our analysis revealed (199.01 ng/mL=0.199μg/mL, MSIS criteria), which could potentially result from using of different analyzers and type of sample. HNL was detected by an Enzyme-linked immunosorbent assay in our study. At present, Shahi et al described the benefits of D-dimer in the diagnosis of PJI, with a sensitivity of 89% and a specificity of 93%.[30] In our study, D-dimer was presented the highest specificity (100%), but less sensitivity (31.6%) to distinct between PJI and non-PJI. Serum PCT is also not a good adjunct in diagnosing PJI in other study.[31] The reason may be a different detection methods and reagents used and different cutoff for infection in the present study.[32] Fjaertoft et al[24] also believed that the process of bacterial infection could be better reflected by HNL than CRP

and PCT tests. Hemodynamic studies of HNL and CRP revealed that CRP still maintained a high level after the elimination of bacterial infection, while HNL was consistent with the degree of disease recovery.[24] These findings justified the application of HNL as a detection indicator to guide use more effectively and avoid the irrational of antibiotics. To offer the foundation of rapid diagnosis of PJI in the clinic, the research group will further explore the sensitivity, specificity, and accuracy of the combined diagnosis of multiple detection indicators.

Several limitations of this study included: Firstly, although MSIS criteria are well-accepted at present, the gold-standard test to detect PJI is lacking for the time being. In this regard, the criteria selected in the present work might misclassify the PJI or non-PJI patients. Secondly, the small sample size of this study might affect the NPV/PPV of the indicators. Furthermore, underlying diseases in certain cases might affect the HNL level. Moreover, the present single-center study only enrolled Asian patients. Thus, to eliminate biases of race and researcher, more multicenter studies are required.

Conclusion

This work confirmed the remarkably higher serum HNL levels in PJI cases with high specificity and sensitivity in diagnosis. Serum HNL was identified as the candidate diagnostic biomarker for PJI, which might be incorporated in the diagnostic algorithm of MSIS criteria.

Abbreviations

PJI, periprosthetic joint infection; HNL, human neutrophil lipocalin; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; BMI, body mass index; PCT, procalcitonin.

Ethics Approval and Consent to Participate

The study protocol was approved by the Institutional Review Board of Zhengzhou Orthopaedics Hospital (202203). This study was conducted in accordance with the declaration of Helsinki and patient data were kept confidential.

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Disclosure

The authors declare that there are no conflicts of interest in this work.

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