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ORIGINAL ARTICLE

Enhanced Understanding of Procalcitonin Utilization as an Infection Biomarker in Non-Hodgkin's Lymphoma

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SUMMARY

Background: **This investigation aimed to assess the diagnostic relevance of procalcitonin (PCT) in non-Hodgkin's lymphoma (NHL) and determine factors impacting its diagnostic precision. Additionally, the study endeavored to delineate its judicious application in clinical settings.**
Methods: **By employing a retrospective analysis of clinical records, serum PCT levels were gauged utilizing an automated immunoassay, followed by the generation of a receiver operating characteristic (ROC) curve.**
Results: **The established threshold for serum PCT in diagnosing infection was identified as 0.120 ng/mL. Serum PCT levels were markedly elevated in patients with bloodstream infections compared to those with localized infections. Infections due to Gram-negative bacilli manifested higher PCT levels relative to those caused by Gram-positive cocci. Within the bloodstream infection cohort, patients with coagulase-negative staphylococci infections exhibited increased PCT levels compared to those in the contamination cohort. The interplay of various elements induces the onset of tumor metastasis and progression as notable risk factors that augment serum PCT levels in NHL patients.**
Conclusions: **Serum PCT levels are heightened in NHL patients, influenced by tumor stage and evolution. The diagnostic application of a solitary PCT test is limited for infection detection. It is recommended that NHL patients be hospitalized to establish a baseline serum PCT level, facilitating its use as a benchmark in infection diagnostics.**
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INTRODUCTION

NHL refers to one of the common malignancies in the clinic. Given the latest global cancer statistics in 2018, there are 509,590 new cases with NHL and 248,724 deaths, taking up 2.8% and 2.6% of all cancer cases, respectively [1]. In China, the incidence rate increased significantly, with a population incidence rate of nearly 7/100,000 [2]. The co-infection acts as one of the common complications and causes of death in NHL patients, as impacted by the nature of the disease and the decrease of autoimmune system activity attributed to radiotherapy and chemotherapy [3,4]. Early detection of tumors complicated with infection is critical to an appropriate anti-infection treatment and prognosis improvement.

PCT, an acute time-limited reaction protein, is important in diagnosing infectious diseases [5-7]. Some studies have shown that patients exhibit signs of infection, but the pathogen or infection site is unknown. The increase in PCT level can be an important basis for the initial use of antibiotics [8,9]. Numerous studies reported that PCT is highly significant for diagnosing infection in tumor patients [10-12]. The change in PCT level can act as a laboratory test index for early predicting postoperative infection of colorectal cancer [13]. PCT is highly accurate in diagnosing lung cancer patients' infections and cancerous fever and can act as a clinical diagnosis method [14]. However, although PCT is closely associated with inflammation, it may not be completely specific for infection; studies have suggested that the serum PCT level of not infected malignant tumor patients can be higher than that of normal people [15-17], but few reports exist on serum PCT and its role in diagnosing NHL with infection.

Serum PCT is used as a diagnostic marker for NHL with infection, as it is more suitable for clinical use. In this paper, the clinical data of NHL patients were collected, the ROC curve was drawn by comparing the infected group with the not infected group, and the cutoff value was calculated in order to analyze the changes in serum PCT level in the same patient before infection, during infection, and after anti-infection treatment, to analyze its diagnostic value in the infection site and pathogenic bacteria species differences in the infection group, to further analyze the influencing factors of serum PCT detection in NHL patients, and to explore its rational application in NHL patients.

MATERIALS AND METHODS

Research data

The research objects were 736 NHL inpatients at the Clinical Oncology School of Fujian Medical University, Fujian Cancer Hospital, from January 1, 2018, through December 31, 2021, i.e. 412 males and 324 females, aged 58 years on average (10 - 84 years). A total of 1,106 PCT cases were collected, with 260 cases (306 times) in the infection group and 476 cases (800 times) in the non-infection group. Among them, 326 (578 cases) were in the non-infected state on the first day of admission. Moreover, there were 80 cases with complete data of serum PCT detection before, during, and after the effective anti-infection treatment. All cases were diagnosed as NHL by pathology. The pathological diagnosis and classification complied with 2016 WHO Classification Standard for Lymphocyte Tumors. Furthermore, the clinical staging abided by the Ann Arbor Staging Standard modified by Lugano, and the prognosis score followed the NCCI International Prognostic Index (IPI). Upon admission, the patients signed an informed consent form and were informed that their relevant clinical data (anonymized) could be used for related scientific research.

Criteria for inclusion and exclusion

Pathology diagnosed all cases as NHL (referencing the WHO Classification Standard for Lymphocyte Tumors in 2016 for diagnosis and classification [18]). Besides, the PCT test was performed routinely or during hospitalization, and the infection diagnosis standard was as follows: patients were diagnosed as being infected by clinical manifestations, physical examination, imaging examination, microbiological examination, and others, and the anti-infection treatment was effective.

The exclusion criteria included the existence of factors affecting serum PCT level (e.g. autoimmune diseases, trauma, severe acute pancreatitis, cardiogenic shock, poisoning, acute renal insufficiency), antibiotics used one week before admission, and missing case data.

Object group

According to whether the patients were infected, they were assigned to the infection group or the non-infection group. Given the IPI score (0 - 4 points), the patients were divided either into the 0 - 2 points group or the 3 - 4 points group. According to the pathological types, the patients were divided into the T cell, the B cell, or the NK cell groups. By complying with the Ann Arbor Staging Standard modified by Lugano, the patients were split into stage I, stage II, stage III, and stage IV. Moreover, according to tumor progression, the patients were assigned to the cured/partially cured group and the progressive group. In line with the number of neutrophils, the patients were separated into two groups, i.e. neutropenia and non-neutropenia group. Based on the infection site, the patients were divided into the bloodstream infection group and the local infection

group. Furthermore, based on the types of pathogens, they were split into Gram-positive and Gram-negative bacteria groups.

Data collection and detection methods

The present study conducted a retrospective analysis, and the general clinical data of NHL patients were collected (e.g. genders, ages, serum PCT levels, granulocyte counts, pathological tumor types, tumor stages, IPI scores, tumor progression, infection sites, and microbial species). All specimens were collected on the first day of admission, within 24 hours of fever, or from the fasting venous blood collection in the early morning of the hospitalized patients. Furthermore, the serum was taken for PCT detection after 3,000 rpm/min centrifugation. All study protocols were approved by the Clinical Research Ethics Committee of Fujian Cancer Hospital. In addition, all experiments were performed in accordance with relevant guidelines and regulations.

Instruments and reagents

The PCT was detected by the electrochemiluminescence automatic immunoassay analyzer (Cobas E411) of Roche Diagnostics GmbH. The concentration of serum PCT ranged from 0.03 to 100 ng/mL. For the microbial detection, Americal BD Bactec™ FX and BD Phoniex™ 100 were used, and all reagents acted as the instrument. PCT quality control products were purchased from Roche Diagnostics Co., Ltd., in Germany, and microbial identification quality control products originated from Staphylococcus aureus ATCC29213, ATCC25 923, Escherichia coli ATCC25922, and Pseudomonas aeruginosa ATCC27853 by National Center for Clinical Laboratories.

Statistical analysis

The data were analyzed and processed with SPSS 26.0. As indicated by the Kolmogorov-Smirnov test, the sample data of this study displayed a skewed distribution, and the median and quartile M (25%, 75%) was used to describe the distribution of quantitative data. The non-parametric Mann-Whitney U and chi-squared tests were performed to draw the inter-group comparison, and logistic regression analysis was used to explore independent risk factors. GraphPad Prism (version 5.0) was used to draw ROC to evaluate the sensitivity, specificity, positive predictive value, and negative predictive value of serum PCT for diagnosing infection. $p < 0.05$ was considered a statistically significant difference.

RESULTS

The cutoff value of serum PCT in diagnosing the infection reached 0.120 ng/mL in NHL

By comparing 800 cases in the non-infection group with 306 cases in the infection group, the optimal critical value for serum PCT diagnosis of infection determined from the ROC curve was 0.120 ng/mL, AUC was 0.7885, and the sensitivity and the specificity took up 54.9% and 89%, respectively. The positive predictive value was 65.6%, and the negative predictive value was 83.8% (Figure 1A - B).

Changes in serum PCT level in the same patient before and after infection

Serum PCT values of 80 NHL patients were continuously monitored. As indicated from the results, the serum PCT of the before infection was 0.065 ng/mL (0.040, 0.100), the during infection was 0.175 ng/mL (0.085, 0.653), and the effective anti-infection treatment was 0.060 ng/mL (0.040, 0.095). All three showed a statistically significant difference (Table 1 and Figure 2).

Comparison of serum PCT in different infection sites and different flora in NHL patients with infection

Among 301 cases in the infection group, 63 (20.93%) were positive for blood culture. The serum PCT level of patients with bloodstream infection was significantly higher than that of local infection ($p < 0.001$). Among 136 NHL patients infected by pathogenic bacteria, the serum PCT level of G^- was significantly higher than that of G^+ ($p = 0.012$) (Tables 2 and 3).

Diagnostic significance of the serum PCT level in coagulase-negative staphylococci infection in blood culture of NHL patients

Serum PCT level in the coagulase-negative staphylococci infection group was significantly higher than that in the contaminated group ($p = 0.007$). ROC curve displaying the PCT optimal cutoff value diagnosed as coagulase-negative staphylococci infection reached 0.165 ng/mL, AUC was 0.7143, and the corresponding sensitivity, specificity, positive predictive value, and negative predictive value reached 60%, 85.71%, 75%, and 60%, respectively (Figure 3).

Analysis of influencing factors of serum PCT detection in NHL patients

This study showed there were significant differences in gender, histopathological type, and IPI score (0 - 2 points vs. 3 - 4 points) ($p < 0.05$); the serum PCT level of stage IV was 0.070 ng/mL (95% CI: 0.040 - 0.140) higher than that of stages I - III ($p < 0.05$); serum PCT levels were significantly higher in the tumor progression group than in the partial cured groups ($p < 0.001$). Among NHL patients with neutropenia, serum PCT levels were significantly higher in those with concurrent infection compared to those without infection ($p = 0.003$). Tumor stage IV and progression were independent risk factors for increased PCT levels (Table 4, 5).

Table 1. Comparison of serum PCT levels in tumor patients before and after infection (ng/mL).

Group	PCT value M (P_{25}, P_{75})	Z value	p-value
Before treatment group	0.065 (0.040, 0.100)	-5.096	0.000 *
Infection status	0.175 (0.085, 0.653)		
Treatment group	0.060 (0.040, 0.095)	-4.626	0.000 *

* Compared with the infection group, $p < 0.001$.

Table 2. Comparison of serum PCT levels in different infection sites.

Infection site	Number of cases (%)	PCT level M (P_{25}, P_{75})	p-value
Bloodstream infection	58 (19.0)	0.660 (0.158, 11.067)	0.000 *
Local infection	238 (79.07)	0.098 (0.070, 0.238)	
Lung	156 (51.83)	0.080 (0.060, 0.180)	
Soft tissue	26 (8.64)	0.110 (0.080, 0.320)	
Upper respiratory tract	16 (5.32)	0.100 (0.060, 0.413)	
Others (including urinary tract, intestinal tract, etc.)	40 (13.2)	0.140 (0.070, 0.308)	

* Comparison of serum PCT levels between bloodstream infection and local infection.

Table 3. Comparison of serum PCT levels among different pathogens.

Group	Number of cases (%)	PCT level M (P_{25}, P_{75})		p-value
		bloodstream infection	local infection	
G^+ group	62 (45.6)	0.546 (0.203, 7.574)	0.080 (0.070, 0.225)	0.003
G^- group	74 (54.4)	3.885 (0.224, 36.463)	0.240 (0.065, 0.450)	0.008
p-value		0.012	0.137	

The G^+ group was infected by gram-positive bacteria, and the G^- group was infected by gram-negative bacteria.

DISCUSSION

NHL is a general term for a group of independent diseases with strong heterogeneity attributed to heterogeneous B and T lymphocytes or natural killer (NK) cells [19]. NHL acts as a common tumor in China. Researchers generally consider NHL related to patients' autoimmune dysfunction, chemical stimulation, and virus infection [20]. The infection is a highly common complication and cause of death in NHL patients [21]. PCT is an inactive propeptide substance of calcitonin, with an extremely low content in healthy people ($p < 0.05$ ng/mL) [22]. However, under pathological conditions, almost all tissues and organs can secrete PCT, and its production is regulated by numerous factors (e.g. bacterial toxins and inflammatory cytokines). Serum PCT levels increase in severe bacterial infection, sepsis, and multiple organ failures, whereas the values are normal or only slightly increased in aseptic inflammation and viral infection [23].

Serum PCT, a biomarker of infectious diseases, exhibits high sensitivity and great specificity. In immunocompetent patients with infection, elevated serum PCT levels typically correlate with infection severity and can guide antibiotic therapy decisions. However, the diagnostic significance of PCT in cancer patients remains controversial, as tumor cells may either directly secrete PCT or stimulate its production, leading to non-infectious PCT elevation and consequently limiting its utility as an infection marker in this population [14,24]. Nevertheless, one study reported that serum PCT levels in pediatric cancer patients effectively excluded bloodstream in-

Table 4. Correlation analysis of serum PCT levels in 326 not infected NHL patients.

Item	Grouping	Number of cases (%)	PCT level ng/mL (P_{25}, P_{75})	Z value	X^2 value	p-value
Gender	male	184 (56.4)	0.070 (0.040, 0.110)	-	5.044	0.025 $^{\Delta}$
	female	142 (43.6)	0.060 (0.030, 0.070)			
Age (years)	< 65	218 (66.9)	0.060 (0.037, 0.073)	-1.272	-	0.496
	≥ 65	108 (33.1)	0.060 (0.040, 0.100)			
IPI score	0 - 2	128 (39.3)	0.052 (0.020, 0.170)	-2.898	-	0.004 $^{\Delta}$
	3 - 4	198 (60.7)	0.125 (0.020, 0.390)			
Pathologic staging	B lymphoma	248 (76.1)	0.065 (0.020, 0.360)	-	4.114	0.043 $^{\Delta}$
	T lymphoma	52 (21.0)	0.107 (0.020, 0.260)			
Tumor staging	Stage I	32 (9.8)	0.040 (0.030, 0.070)	-3.734	-	0.000 $^{*,\Delta}$
	Stage II	60 (18.4)	0.040(0.030, 0.080)	-2.844		0.004 $^{*,\Delta}$
	Stage III	76 (23.3)	0.060 (0.030, 0.080)	-2.551		0.025 $^{*,\Delta}$
	Stage IV	158 (48.5)	0.070 (0.040, 0.140)			
Treatment situation	progress	242 (74.2)	0.070 (0.040, 0.100)	-	64.015	0.000 $^{\Delta}$
	partial cure	84 (25.8)	0.050 (0.030, 0.070)			
Neutrophil count	neutropenia	18 (5.5)	0.080 (0.040, 0.173)	-2.932	-	0.003 $^{a,\Delta}$
				-1.877		0.066 ^{b}
				0.533		0.521 ^{c}
	non-neutropenia	308 (94.5)	0.060 (0.040, 0.080)			

*** Comparison with stage IV, p < 0.01, ^{a} comparison between neutropenia and infection group and non-neutropenia and non-infection group, ^{b} comparison between non-neutropenia and non-infection group and neutropenia and non-infection group, ^{c} comparison between neutropenia and infection group and non-neutropenia and infection group, $^{\Delta}$ difference is statistically significant.**

Table 5. Logistic regression analysis of serum PCT levels in 326 not infected NHL patients.

Factors	β value	SE value	Wald value	Exp (β)	95% CI	p-value
Gender	0.245	0.330	0.553	1.278	0.670 - 2.437	0.457
Tumor type	0.622	0.419	2.198	1.862	0.819 - 4.234	0.138
IPI score	0.042	0.324	0.017	1.043	0.553 - 1.967	0.896
Whether progress has occurred	0.871	0.357	5.947	2.390	1.187 - 4.812	0.015
Whether there is tumor stage IV	0.914	0.325	7.911	2.495	1.319 - 4.718	0.005
Constant	-2.271	0.499	20.683	0.103		0.000

fections [25]. For immunocompetent infected patients, PCT monitoring has been widely implemented in clinical practice, particularly for respiratory infections and sepsis cases. It serves to complement clinical judgment, guide antibiotic therapy, improve patient outcomes, support antimicrobial stewardship programs, and potentially reduce antibiotic resistance [9,26]. In this study, 80 NHL patients were analyzed before infection, during infection stage, and after the effective anti-infection treatment. The serum PCT level was continuously monitored to be 0.065 ng/mL vs. 0.175 ng/mL vs. 0.060 ng/mL, respectively, demonstrating that the serum PCT level could act as an early diagnosis index of NHL patients with infection and monitoring the effect of anti-infection treatment. Notably, our study observed that NHL patients may not achieve the same magnitude of PCT reduction as immunocompetent patients following effective anti-infective therapy, likely attributed to persistently elevated PCT level associated with underlying malignancy. Consistent with prior literature [27], serial PCT monitoring may serve as a valuable tool for antibiotic de-escalation and stewardship in cancer patients

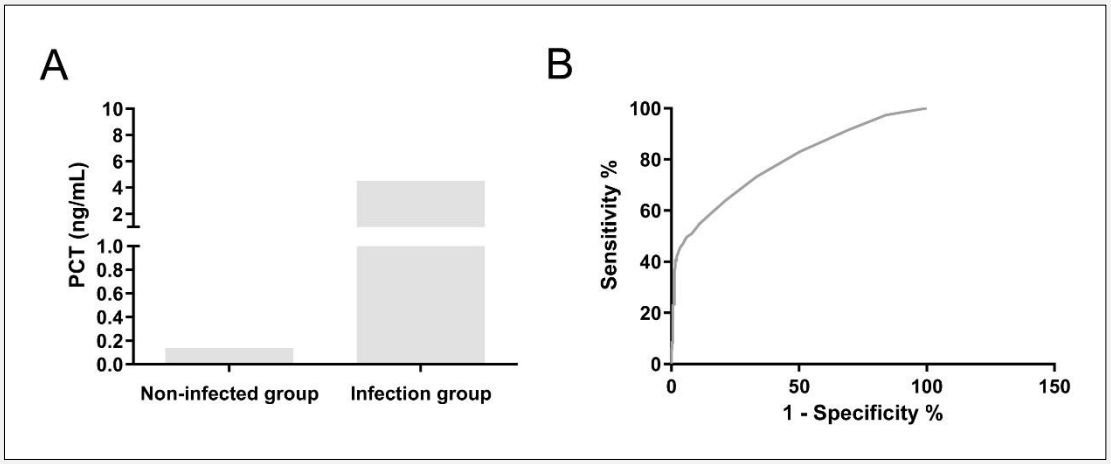

Figure 1. Comparison of serum PCT levels in NHL patients with and without infection.

A Serum PCT levels of not infected and infected NHL patients. B ROC curve of serum PCT levels in the diagnosis of NHL patients with infection. The optimal critical value for serum PCT diagnosis of infection determined from the ROC curve was 0.120 ng/mL, the AUC was 0.7885, and the sensitivity and specificity took up 54.9% and 89%, respectively. The positive predictive value was 65.6%, and the negative predictive value was 83.8%.

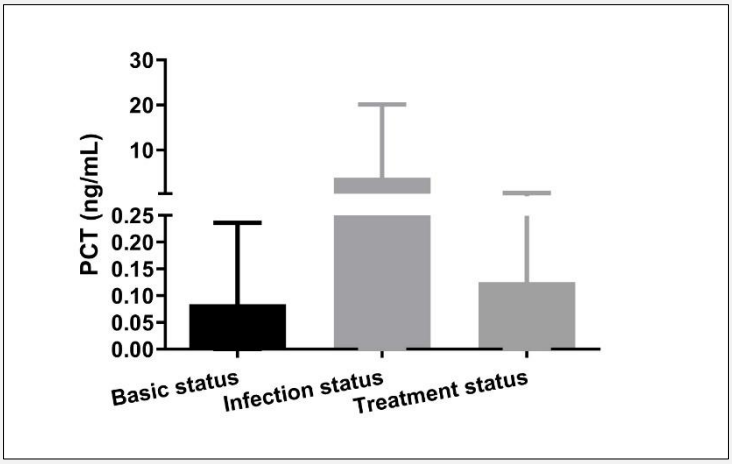

Figure 2. Serum PCT levels before and after appropriate treatment in NHL patients with infection.

with concurrent infections, with studies demonstrating a median 30% reduction in PCT level following successful treatment of infection. Some studies reported that the optimal cutoff value of PCT in diagnosing infection in patients with and without tumor infection was 0.17 ng/m [28]. In this study, the optimal critical value for serum PCT to diagnose infection was 0.120 ng/mL, and the negative predictive value was 83.8%, suggesting that 83.8% of NHL patients with a PCT level higher than 0.120 ng/mL at the hospital studied may develop the infection.

As suggested from the comparison of the difference in serum PCT level in different infection sites in the infection group, the serum PCT level in patients with blood-

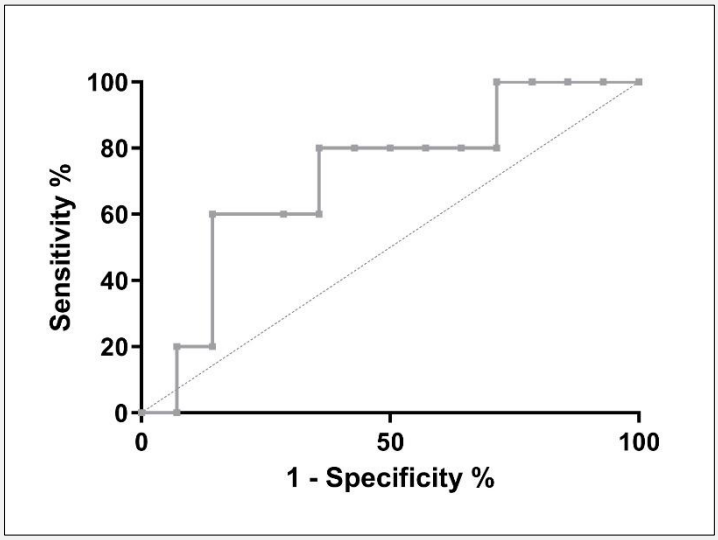

Figure 3. ROC curve for identification of coagulase-negative staphylococci caused by bloodstream infection and contamination by serum PCT level in NHL.

The PCT optimal cutoff value diagnosed as coagulase-negative staphylococci infection reached 0.165 ng/mL, the AUC was 0.7143, and the corresponding sensitivity, specificity, positive predictive value, and negative predictive value reached 60%, 85.71%, 75%, and 60%, respectively.

stream infection was significantly higher than that in those with local infection, complying with the literature report [29]. Thus, it was demonstrated that the significant increase in the PCT level could commonly indicate adverse results (e.g. bloodstream infection and septic shock). The serum PCT level of Gram-negative bacteria infection was significantly higher than that of Gram-positive cocci (3.885 ng/mL vs. 0.546 ng/mL), demonstrating that serum PCT level was different, as impacted by various infectious pathogens. On that basis, the diagnosis of clinical infectious pathogens can be expedited. As reported in existing studies, serum PCT levels can distinguish Gram-negative bacterial sepsis from Gram-positive bacterial and fungal sepsis, with a cutoff value of 2.44 ng/mL [30,31]. Besides, this study reported a difference in the PCT level between the bloodstream infection and the contamination attributed to coagulase-negative staphylococci. The optimal cutoff value of the ROC curve was 0.165 ng/mL, and the AUC was 0.7143. As revealed in the abovementioned results, the PCT level can help identify the contamination and the coagulase-negative staphylococcus bloodstream infection, as well as provide a reference for the clinical and laboratory determination of the blood culture contamination. The literature reports indicate that serum PCT levels of patients with tumor non-complicated infection are higher than that of healthy humans [32-34]. In this study, the serum baseline PCT level in NHL patients was higher than that in healthy humans, which might be associated with the particular state of NHL patients, similar to inflammation. According to previous studies [35,36], tumor cells can stimulate the body's immune system to produce a sustained inflammatory state, thereby facilitating the proliferation and differentiation of tumor cells and further inhibiting apoptosis. This study analyzed the factors in depth. For IPI score, group 0 - 2 was significantly higher than group 3 - 4, which demonstrated that the higher the IPI score of NHL patients, the worse the prognosis and the higher the PCT level would be, consistent with a previous study [10]. Tumor stage IV and tumor progression acted as independent factors of higher serum PCT level in non-infectious NHL patients. Cytokines (e.g. IL-6, IL-2, and TNF) could mediate the production of PCT during tumor metastasis [32]. The value of PCT in patients with liver metastasis of lung cancer significantly increased, allowing PCT to predict tumor metastasis and the advanced stage. The results also indicated that the serum PCT level of NHL patients with tumor progression was higher than that of partially cured or cured patients (0.070 ng/mL vs. 0.050 ng/mL). Next, a previous study suggested that serum PCT was positively correlated with the disease progression of small cell lung cancer [37]. According to our in-depth results, the serum PCT level in the infection group of NHL patients with neutrophil count deficiency was significantly higher than that in the non-infection group, which indicated that PCT level could act as an infection diagnostic marker for patients with neutrophil

count deficiency, consistent with a previous study [38]. Several important limitations in our study should be acknowledged. The relatively small cohort size and incomplete ancillary biomarker data precluded meaningful comparison between PCT levels and established progression markers in NHL patients, as well as assessment of potential synergistic effects when combining PCT with conventional biomarkers. The critical question of whether PCT possesses incremental prognostic value for monitoring disease progression and treatment response in NHL remains unresolved in our analysis, highlighting an important area for future research.

CONCLUSION

In brief, serum PCT is a good diagnostic marker for NHL patients with infection and anti-infection monitoring. The cutoff value is 0.12 ng/mL. However, due to the influence of multiple factors, a single PCT measurement test cannot establish the diagnosis of infection. It is recommended that patients with NHL be admitted to the hospital to detect serum PCT baseline level as a control for the diagnosis of infection.

Declaration of Interest:
The authors declare that there are no conflicts of interest regarding the contents of this article.

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