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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Significance of procalcitonin in bacterial infections among acute leukemia patients with post-chemotherapy agranulocytosis

Zhigang Qu, Bingmu Fang, Guangli Ma, Jinhong Jiang, Xiaoli Wang, Junnv Wang, Shuping Liu, Xiaoqiu Wang, Yonghua Liu, Qiaolei Zhang

People's Hospital of Lishui City, Department of Hematology, Lishui, Zhejiang, China; Wenzhou Medical University, Sixth Affiliated Hospital, Wenzhou, Zhejiang, China

SUMMARY

Introduction/Objective Bacterial infection caused by the lack of granulocytes that results from the chemotherapy of acute leukemia is the leading cause of death. At present, there are few sensitive markers to reflect the bacterial infection, and there is no obvious specificity for the diagnosis of infection. Procalcitonin (PCT) is a precursor of calcitonin, and it has been found that PCT is a rapid and accurate marker of infectious diseases in various studies, but its clinical value remained unclear.

This study aimed to explore the clinical significance of PCT levels in patients with acute leukemia who have acquired bacterial infections during the agranulocytosis period post-chemotherapy.

Methods Serum PCT levels were analyzed from samples collected from 92 patients with acute leukemia who had acquired bacterial infections during the agranulocytosis period post-chemotherapy.

Results Serum PCT levels in patients with positive blood cultures were significantly higher than those in patients with negative blood cultures ($p < 0.05$). Gram-negative bacterial infection group was significantly more frequent cause of infection than the Gram-positive group ($p < 0.05$). Furthermore, for patients with positive blood cultures, serum PCT levels were significantly higher in patients who subsequently died than in those who survived ($p < 0.05$).

Conclusion In the period of agranulocytosis combined with bacterial infection that occurred after the chemotherapy of acute leukemia, PCT can show the status of bacterial infection, infected bacterial types and severities.

Keywords: procalcitonin; acute leukemia; bacterial Infections; agranulocytosis

INTRODUCTION

Acute leukemia is a malignant, life-threatening, clonal disease of the hematopoietic tissue. The preferred treatment for acute leukemia is chemotherapy [1]. Due to the characteristics of the disease, and the side effects of chemotherapy drugs, chemotherapy can often result in severe bone marrow suppression, leading to agranulocytosis, thrombocytopenia, and anemia. This can consequently result in increased risk of infection, bleeding, or, in some severe cases, death. With developments in medical technology, administration of hemostatics and transfusion of red blood cells and platelets, the incidence of anemia and death has been greatly reduced.

However, chemotherapy-induced agranulocytosis is still very serious, and can cause sepsis, leading to fever, chills, necrosis, organ failure, or even death [2]. Bacterial infections still contribute to the high mortality rate seen in patients with acute leukemia, in spite of the application of broad-spectrum antibiotics. During the agranulocytosis period, patients with acute leukemia are susceptible to infection with both Gram-positive and Gram-negative bacteria [3].

Culture of pathogens is still the gold standard for their identification in a variety of specimen types. However, there are inevitable limitations to this method, such as the delay in obtaining results [4]. For patients with acute leukemia in the agranulocytosis period post-chemotherapy, time waiting for pathogen culture results is limited, as the onset of infection is acute and severe. To date, there are no specific and sensitive evaluation indexes to monitor the onset of infection and its severity. Thus, the development of methods that enable early diagnosis of bacterial infections, and evaluation of the prognosis of disease, has become a popular research focus.

Many researchers are focused on identifying accurate and quickly measured markers for monitoring of infectious diseases. A recently identified infection-related biomarker, procalcitonin (PCT) has been measured in a clinical setting, and this strategy has been effective in diagnosing infection at an early stage, in grading severity of infection, and in enabling prognostic assessment [5–8]. Some researchers believe that PCT can be used as an early indicator of infection in patients with acute leukemia during the agranulocytosis period [9], while others have argued that the significance of PCT remains unclear at this stage [10]. Therefore, we believe it is necessary to further investigate the clinical

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Correspondence to:
Bingmu FANG
Department of Hematology
People's Hospital of Lishui City and the Sixth Affiliated Hospital of Wenzhou Medical University,
No. 15 Dazong Street Liandu District,
Lishui 323000, Zhejiang, China
bingmufang@126.com

significance of PCT in providing a basis for subsequent anti-infective therapy and in assessing patients' clinical condition, in patients with acute leukemia who acquire bacterial infections during the agranulocytosis period post-chemotherapy. This study was conducted in accordance with the declaration of Helsinki, with approval from the Ethics Committee of the People's Hospital of Lishui City and the Sixth Affiliated Hospital of Wenzhou Medical University. Written informed consent was obtained from all participants.

The aim of the study was to investigate the clinical value of PCT in granulocyte deficiency with bacterial infection of patients with acute leukemia after chemotherapy, which provides evidence for clinical anti-infection treatment and disease assessment.

METHODS

Subjects

A total of 92 patients with acute leukemia (48 males and 44 females; 12–65 years old, with median age of 38.5 years) who were admitted to the Department of Hematology of the People's Hospital of Lishui City and the Sixth Affiliated Hospital of Wenzhou Medical University from July 2009 to December 2013 were enrolled in this study.

The inclusion criteria were the following: the age of the patients' was 12–65 years; the fever occurred in pro-chemotherapy agranulocytosis period; the first blood culture was bacteria-positive or negative, but clinical symptoms indicated infection; cytomegalovirus, Epstein–Barr virus, herpes simplex virus, varicella zoster virus and glucan negative tests were negative.

The exclusion criteria were as follows: fungal infection indicated by the pathogen culture and chest CT; virus infection indicated by the serological examination; transfusion associated fever; persistent fever without infection due to use of high-dose cytarabine. According to the hematopoietic and lymphoid tissue tumor classification standard from WHO, there were 68 patients with acute myeloid leukemia (AML) and 24 patients with acute lymphoblastic leukemia (ALL). Sixty-eight AML patients consisted of eight t(8;21) cases, 12 inv(16) cases, seven t(16;16) cases, eight t(15;17) cases, and 33 cases without specific classification (M1, 3; M2, 12; M4, 13; M5, 5). The 24 ALL patients included 19 cases of B-ALL and five cases of T-ALL. All the patients were cases with complete bone marrow remission after chemotherapy. The intensive treatment scheme for AML patients included HDAra-C, IDA, ID-Ara-C+Mit, ID-Ara-C+VP16, etc., and that for ALL patients included HDAra-C+HD-MTX, HD-MTX+VP, CDOLP, etc.

All the patients were at the agranulocytosis period post-chemotherapy and had acquired bacterial infections. The diagnosis of neutropenic fever, infection and severe sepsis was as follows: (1) neutropenic fever (neutropenia was defined as neutrophil count of $< 0.5 \times 10^9$/L, or count of $< 1 \times 10^9$/L with a predicted decrease to $< 0.5 \times 10^9$/L; the fever was defined as a single oral temperature of ≥ 38.5°C or a temperature of ≥ 38°C for ≥ 1 hour) [11]; (2) bacterial infection (the single oral temperature was ≥ 38.5°C or the body temperature was ≥ 38°C for ≥ 1 hour; the blood culture suggested bacterial infection, with or without clinical infection sign, defined as bacteremia; for blood culture negative patients, there were clinical infection signs such as cough, diarrhea, abdominal pain, urine or anal pain; the patients had an unexplained drop in blood pressure or blood oxygen saturation. Computed tomography, X-ray, and abdominal B ultrasound indicated a new infection focus; antibiotic treatment was effective; fungus and virus infections were excluded); (3) severe sepsis (the sepsis was associated with organ dysfunction, hypoperfusion or hypotension; perfusion abnormalities included, but were not limited to, lactic acidosis, oliguria, or acute alteration in mental status [12]).

Based on blood culture results, specimens were divided into a positive group (n = 30), and a negative group (n = 62). Positive blood culture referred to patients with at least one positive blood culture result; negative blood culture referred to patients who had negative blood culture results, but had the signs and symptoms of bacterial infection, as well as positive imaging findings for bacterial infection, and were sensitive to antibiotics. Bacteriological examination was performed using the VITEKAMS60 automatic bacterial analyzer (BioMerieux, Lyon, France). Among the positive blood culture group, specimens were further divided into a Gram-positive bacteria subgroup (n = 14), and a Gram-negative bacteria subgroup (n = 16).

Before chemotherapy, all the patients were given tests in routine blood, routine urine, liver and kidney function, C-reactive protein, PCT, cardiac ultrasound, hepatobiliary and urinary B ultrasound, chest computed tomography, hepatitis B virus, cytomegalovirus, Epstein–Barr virus, herpes simplex virus, and varicella zoster virus. If there was no abnormality, intensive therapy was performed. After the emergence of agranulocytosis, respiration, pulse, blood pressure, and peripheral oxygen saturation were daily monitored for revealing the potential infection focus. For the occurrence of fever, blood culture was conducted, as were other cultures, such as urine and throat swab culture. The routine tests were performed again. Finally, broad-spectrum antibiotic treatment was administered. The evaluation standard of curative effect was as follows: after using antibiotics for three to five days, the patient's body temperature gradually returned to normal, and vital signs gradually grew stable. The disappearance of clinical symptoms suggested that the patient was sensitive to antibiotics. After the white blood cells recovered to normal, the computed tomography, X-ray, and abdominal B ultrasound were performed to re-examine the new infection focus. The obvious remission or disappearance of infection focus presented the cure.

PCT measurement

Venous blood (2 ml) from patients with neutrophil counts $\leq 0.5 \times 10^9$/L and a single oral temperature of ≥ 38.5°C or axillary temperature of ≥ 38°C for ≥ 1 hour were collected before using antibiotics (D0) for serum isolation. Serum PCT levels were determined within four hours by using colloidal gold technology and detection with the LIAISON

automated fluorescence immunoassay analyzer (Sorin, Modena, Italy). Values of ≥ 0.5 ng/ml were regarded as cut-off.

Blood culture

Venous blood (16–20 ml) from patients with neutrophil counts of ≤ 0.5×10^9 /L and single oral temperature of ≥ 38.5°C or axillary temperature of ≥ 38°C for ≥ 1 hour were collected at D0 for two sets of blood culture (the interval between them was within 5 minutes). Each set of blood culture included one bottle of anaerobic bacteria and one bottle of aerobic bacteria.

Statistical analysis

Statistical analysis was performed using SPSS 12.0 statistical software (SPSS Inc., Chicago, IL, USA). Comparisons of serum PCT between the groups were carried out using the χ^2 test.

RESULTS

Treatment results

All the patients were given conventional treatment with broad-spectrum antibiotics. For some patients the antibiotics were adjusted according to drug sensitive test results. Five patients died due to severe sepsis during the treatment. Other patients were cured according to the efficacy evaluation standard.

Relationship between blood culture results and serum PCT

The median blood neutrophil absolute value of 92 patients was $0.03 \pm 0.16 \times 10^9$ /L. In 92 specimens tested by blood culture, 30 specimens obtained from 30 patients were bacteria-positive (32.6%, 30/92), and 62 specimens obtained from 62 patients were bacteria-negative (67.4%, 62/92). The bacteria of the blood culture are shown in Table 1. On D0, 68 cases exhibited PCT ≥ 0.5 ng/ml, accounting for 73.9% of total patients. PCT ≥ 0.5 ng/ml was found in 87% of the bacteria-positive specimens, with 67.7% of bacteria-negative specimens ($p < 0.05$). The D0 serum PCT was negative in 13% of bacteria-positive specimens, with 32.3% of bacteria-negative specimens ($p < 0.05$). The expression intensity for serum PCT ≥ 0.5 ng/ml in the bacteria-positive group was 12.4 ng/ml, while it was 5.6 ng/ml in the bacteria-negative group. PCT in the bacteria-positive group was significantly higher than that in the bacteria-negative group ($p < 0.05$, Table 2).

Serum PCT levels in patients with different bacterial infections

Of the 30 specimens obtained from 30 patients that were bacteria-positive, 14 specimens showed Gram-positive bacterial infection (46.7%, 14/30), and 16 specimens showed Gram-negative bacterial infection (53.3%, 16/30). In serum PCT positive patients, the average serum PCT levels in the Gram-negative group was 14 ng/ml, Gram-positive group was 7.5 ng/ml, and the serum PCT levels in the Gram-negative group were significantly higher than those in the Gram-positive group ($p < 0.05$, Table 3).

Table 1. Bacterial species in 30 patients with positive blood culture

Bacterial species	Number of strains
Gram+ bacteria	14
Staphylococcus epidermidis	3
Staphylococcus aureus	4
Streptococcus viridans	2
Hemolytic streptococcus	2
Enterococcus faecalis	1
Rothia mucilaginosa	1
Enterococcus faecium	1
Gram- bacteria	16
Escherichia coli	3
Enterobacter cloacae	2
Pseudomonas maltophilia	2
Pseudomonas aeruginosa	2
Klebsiella pneumoniae	4
Fusobacterium	2
Morganella morganii	1

Table 2. Serum PCT levels in 92 specimens grouped by the blood culture results

Groups	cases (n)	PCT subgroup, No. and mean (ng/ml)	
		< 0.5	≥ 0.5
Bacteria-positive	30	4 (13%) 0.1	26 (87%) 12.4
Bacteria-negative	62	20 (32.3%) 0.11	42 (67.7%) 5.6
Total	92	24	68

Table 3. Serum PCT levels in the Gram-positive (G+) and Gram-negative (G-) infection groups

Groups	Cases (n)	PCT, No. and mean (ng/ml)	
		< 0.5	≥ 0.5
G-	16 (53.3%)	1 0.11	15 14
G+	14 (46.7%)	3 0.09	11 7.5
Total	30	4	26

The prognostic significance of serum PCT

To explore the prognostic significance of serum PCT levels for the patients with acute leukemia during the agranulocytosis period post-chemotherapy who had acquired bacterial infections we further analyzed data on serum PCT levels from patient specimens that were bacteria-positive. In this study, five patients died of infections, the blood culture of four patients was Gram-negative bacteria, and one case was Gram-positive bacteria.

The comparison of the remaining 25 patients with bacteria-positive specimens is as follows: in five cases of death, the average PCT value was 28.2 ng/ml; the patients who survived had the average PCT value of 12 ng/ml; serum

PCT levels in patients who died were significantly higher than those found in patients who survived ($p < 0.05$).

DISCUSSION

PCT constitutes 116 amino acids and has a molecular weight of 13 kDa [13]. PCT is cleaved in vivo into PCT and binding calcitonin, with binding calcitonin being further converted enzymatically into calcitonin, which plays a physiological role in vivo. In the event of infection, PCT levels increase hundred-fold. Thus, serum PCT measurement has been increasingly applied to the monitoring of infectious disease in a clinical setting [14, 15].

During the agranulocytosis period, post-chemotherapy patients with acute leukemia are at an increased risk of bacterial infections, the leading cause of death in these circumstances. Thus, early diagnosis and prompt treatment of infection is very important in patients' prognosis. Studies have shown that, with the cut-off value for PCT level set to 0.5 ng/ml, diagnostic sensitivity to bacterial infection was 65%, and specificity 96%. Additionally, at serum PCT levels higher than 1.2 ng/ml, sensitivity reaches 100% [16]. In many studies, 0.5 ng/ml is often used as the cut-off value of PCT [17]. We conducted this study according to the above reported methods and found that, patients with etiologically confirmed bacterial infections had significantly higher serum PCT, as did patients that were diagnosed clinically, but had negative blood culture results, especially for PCT-positive cases. This is consistent with other studies mentioned above. Our study found that, on the exact day of fever, 73.9% of the patients had PCT over the threshold, the PCT-positive-rate of the positive blood culture group (≥ 0.5 ng/ml) was higher than the negative blood culture group (87% vs. 67.7%, $p < 0.05$), indicating that the PCT value of the positive blood culture group was higher than the negative blood culture group. Our study also shows that patients infected with Gram-negative bacteria had significantly higher serum PCT levels than patients infected with Gram-positive bacteria ($p < 0.05$). In serum PCT-positive patients, the average serum PCT level in the Gram-negative group was 14 ng/ml, while the level in the Gram-positive group was 7.5 ng/ml. Furthermore, serum PCT levels in patients that died were also significantly higher than in those that survived; this indicates that measurement of serum PCT can be applied to the early diagnosis of infection and to the classification of the disease severity. These results are consistent with the findings of Jeddi et al. [18], Neofytos et al. [19], and Hatzistilianou et al. [20]. Our study has confirmed this conclusion. However, this is a single-center study. Due to a smaller sample size it needs to be further verified.

For patients with agranulocytosis who do not show obvious symptoms of infection, measurement of serum PCT at the early stages of infection will not only help to assess the infection severity and increase the efficacy of anti-infective therapy but also help to distinguish between Gram-negative and Gram-positive bacterial infection to provide a reasonable basis for the clinical administration of antibiotics [21, 22, 23]. For patients with acute leukemia acquiring bacterial infection during the agranulocytosis period post-chemotherapy, our findings are consistent with most other studies [24, 25]. We found that the PCT level in Gram-negative infected patients was higher than in Gram-positive infected patients. In five cases of death, the average PCT reached 28.2 ng/ml, and the average PCT level of these patients was higher than other positive blood culture patients. Therefore, we initially thought that the increasing level of PCT could reflect the severity of bacterial infection, especially in severely infected patients, in whom PCT was obviously increased. Our results initially considered that in the period of agranulocytosis combined with bacterial infection that occurred after the chemotherapy of acute leukemia, procalcitonin had a clinical significance in predicting bacterial infection, infected bacterial types, and severities. Combined with the previous studies, it could be used as a confirmed clinical examination index to guide the clinical judgment.

CONCLUSION

Our results showed that PCT of bacterial infection confirmed by etiology was significantly increased, while positive results of patients who were considered te be suffering from a bacterial infection with blood-free culture also increased. This indicates that PCT can be used not only in early diagnosis of diseases, but also in the classification of the severity of a disease. For patients with granulocyte deficiency who have no obvious symptoms and whose condition changes rapidly, the detection of serum PCT levels in early infection can not only make a judgment on the severity of infection and anti-infection effect, but can distinguish Gram-negative from Gram-positive bacteria, providing the basis for the reasonable application of antibiotics. For patients in granulocyte deficiency with bacterial infection with acute leukemia after chemotherapy, serum PCT levels have an important value for reflecting the extent of bacterial infection and the anti-infection treatment, which is a reliable index for monitoring and has important clinical value.

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Прокалцитонин као показатељ бактеријске инфекције у постхемиотерапијској агранулоцитози код акутне леукемије

Џиганг Ћу, Бингму Фенг, Гуангли Ма, Ћинхунг Ћанг, Сјаоли Венг, Ћунв Венг, Шупинг Љу, Сјаоћу Венг, Јунгхуе Љу, Ћаолеј Џанг

Народна болница Лишуја, Одељење за хематологију, Лишуј, Џеђанг, Кина;
Медицински универзитет у Венџоуу, Шеста универзитетска болница, Венџоу, Џеђанг, Кина

САЖЕТАК

Увод/Циљ Бактеријска инфекција због мањка гранулоцита је последица хемиотерапије акутне леукемије и водећи је узрок смрти. Тренутно постоји мали број довољно осетљивих показатеља који би указивали на бактеријску инфекцију и који немају јасну одређеност за постављање дијагнозе инфекције. У више студија прокалцитонин (ПКТ), прекурсор калцитонина, показао се као брз и сигуран показатељ инфекције, али је његов клинички значај нејасан.
Циљ овог рада је био да се одреди клинички значај нивоа ПКТ-а код болесника са акутном леукемијом и инфекцијом услед постхемиотерапијске агранулоцитозе.

Методе Ниво ПКТ-а у серуму је анализиран код 92 болесника са акутном леукемијом и инфекцијом услед постхемиотерапијске агранулоцитозе.

Резултати Ниво ПКТ-а у серуму болесника са позитивном хемокултуром је значајно виши него код болесника са негативном хемокултуром ($p < 0.05$). Грам-негативне бактерије су биле значајно чешћи узрочник инфекције од грам-позитивних ($p < 0.05$). Осим тога, болесници са позитивном хемокултуром и повишеним ПКТ-ом су значајно чешће умирали него преживљавали ($p < 0.05$).

Закључак У периоду агранулоцитозе са бактеријском инфекцијом услед хемиотерапије акутне леукемије ПКТ може указати на бактеријску инфекцију, врсту и тежину инфекције

Кључне речи: прокалцитони; акутна леукемија; бактеријска инфекција; агранулоцитоза