Determination of Plasma Procalcitonin and Its Application in Sepsis Infection

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Abstract: Infectious diseases are a common disease in clinic. The incidence rate is high every year, and the infected patients will suffer from septic shock. Sepsis is a common cause of death. Generally speaking, sepsis is a systemic inflammatory response of the host to infection, with certain characteristics such as questioning and heart rate. If patients with sepsis are diagnosed and treated in the early stage, the mortality of such diseases can be greatly reduced. PCT is the precursor of calcitonin. PCT is specific for bacterial infection and can help to judge viral infection and bacterial infection. Based on this, this article through the investigation and research to understand the determination method of plasma procalcitonin, establish the reaction mode based on immune chromatography technology in time-resolved technology, and combine it with sepsis infection. By analyzing the plasma procalcitonin level of patients with positive blood culture, the optimal critical value of PCT was 1.34 μg/ml. Then the PCT level of patients with sepsis and severe sepsis was analyzed, and the optimal critical value of PCT for diagnosis of sepsis and severe sepsis was 1.09 μg/ml. Through this experiment, we determined the characteristics of procalcitonin in patients with sepsis and provided a reference method for the application of procalcitonin in sepsis infection.

1. Introduction

Procalcitonin, the English name of PCT, is the precursor of human calcitonin discovered in 1992. It is a hormone free active protein, which is generally a precursor protein of calcitonin produced by thyroid C cells [1]. The protein contains 116 amino acids and the relative molecular weight is about 13x103kd. Generally speaking, this substance cannot enter the blood. It's very low in the plasma of healthy people. The content of PCT in healthy human plasma is less than 0.1ng/ml [2]. PCT was found to be the expression product of calcitonin-i gene. Calcitonin originated from the single copy gene of chromosome 11 in human body. After transcription, it is specially cut in human body and
then translated into PCT precursor. Then it is hydrolyzed in Golgi complex to produce procalcitonin. In human body, the expression of CALC-I gene in tissues other than thyroid gland is prohibited. When the human body is infected by pathogenic microorganisms, the expression of calc-i gene in human body is increased. Therefore, human peripheral blood leukocytes, parenchymal tissues other than thyroid gland and vascular endothelial cells produce and release procalcitonin, thus increasing the content of procalcitonin in human circulation mechanism.

Procalcitonin is a specific indicator of bacterial infection. When the human body suffers from severe systemic infection, the level of procalcitonin in the human body will rise. When the human body is infected locally, the level of procalcitonin in plasma will be increased to a certain extent, but the overall level price is low. So far, a large number of studies have found that in addition to human infection, there are many non-infectious factors that can also make the level of human procalcitonin to a certain extent. Such as surgery, stroke, acute respiratory distress syndrome, trauma, burns, organ transplantation, cardiopulmonary resuscitation, autoimmune diseases, alcoholic cirrhosis, cardiac and respiratory arrest, myocardial infarction, cardiogenic shock, etc. In addition, in recent years, there are many related studies on the relationship between liver and kidney function and procalcitonin metabolism. Studies have confirmed that liver and kidney function have little effect on plasma procalcitonin level, and the main clearance path of procalcitonin is not metabolism of renal organs. Procalcitonin [3] in the human body has serious renal function and does not need all dialysis treatment and other conditions will have a slow increase. In general, the production, distribution and metabolic factors of procalcitonin in plasma will affect the content of procalcitonin in plasma.

Sepsis [4] is a life-threatening organ dysfunction, which is caused by the imbalance of infection response. Septic shock is one of the most serious sepsis. After fluid resuscitation, blood pressure still needs to be maintained by vasoactive substances. At this time, the human body also has a relatively high serum lactic acid content. Although many studies have been done in septic shock, there has been some progress in its pathogenesis and treatment. However, the incidence rate of this disease is still increasing. Sepsis is now one of the leading causes of death in ICU patients. Septic shock is a process of continuous development and occurrence. It is a very serious inflammatory reaction in human body. It can make a lot of inflammatory mediators enter the blood circulation and cause functional damage of organs and tissues. The course of septic shock is very rapid, and the mortality rate is always high. In the process of treating patients with sepsis, it needs a lot of money, but the success rate of treatment is not high. Therefore, the key to the treatment of septic shock is to identify, predict and effectively prevent the occurrence and development of septic shock. Some studies have shown that the level of procalcitonin in patients with sepsis after total thyroidectomy will be significantly increased, and the content of procalcitonin in patients with sepsis will increase, and the content of calcitonin will remain basically unchanged. It can be proved that other tissues and cells other than C cells can synthesize and secrete procalcitonin. In the case of inflammation, procalcitonin production process and calcitonin production process are independent of each other. In addition, it has been suggested that the sites producing procalcitonin may also be neuroendocrine cells and lungs. Studies abroad have shown that the concentration of procalcitonin and procalcitonin in normal human blood is very low. Under certain conditions, the concentration of procalcitonin and the protein composed of procalcitonin will increase obviously. Animal experiments showed that procalcitonin may be a secondary inflammatory factor. Procalcitonin itself cannot cause sepsis, but it can aggravate and enlarge the pathological process of sepsis. Previous studies by morgenthal et al showed that PCT can inhibit the synthesis and release of nitric oxide by inhibiting the inducible nitric oxide synthase (NOS) and inducible (indirect object) proinflammatory factors, thus
preventing infection in the occurrence of hypotension, and playing a protective role in infection [5]. One study found that the performance of plasma PCT was very stable. The PCT concentration decreased by 12% at room temperature and 6% after sampling at 4 °C for 24 hours. PCT was degraded by specific protease with a half-life of 25-30 hours.

The accuracy of procalcitonin is higher than that of leukocytes and high-sensitivity CRP [6]. Among the common pathogens of pulmonary infection, viral infection is much lower than procalcitonin on average in bacterial infection. Relevant studies have pointed out that in the process of human being infected by virus, human - gamma interferon in human body will reduce the up-regulation effect of procalcitonin and cut off the production of procalcitonin, so the content of procalcitonin in human body is low. The level of procalcitonin in viral and mycoplasma pneumonia was obviously lower than that in bacterial pneumonia, but there was no significant difference between viral and mycoplasma pneumonia. There are also research results show that viral pneumonia patients are lower than bacterial pneumonia patients, tuberculosis induced pneumonia and atypical pathogen pneumonia are also lower than bacterial pneumonia patients. The level of procalcitonin in patients with bacterial meningitis is significantly increased, but the level of procalcitonin in patients with viral meningitis is low. The specificity and sensitivity of procalcitonin in the auxiliary diagnosis of bacterial meningitis is obvious. Procalcitonin can accurately distinguish bacterial bacteremia and fungal bacteremia. Low concentration of procalcitonin is an independent predictor of fungal bacteremia. However, some researchers have pointed out that the level of procalcitonin in human body will be slightly or moderately increased when the human body is infected with critical invasive fungi, and the general range is about 1 ug / L. The level of plasma procalcitonin in patients with infective endocarditis caused by Staphylococcus aureus [7] is relatively high, while that of patients infected with Escherichia coli, Enterococcus, Streptococcus, Pseudomonas aeruginosa, Proteus mirabilis and other bacteria is lower. The American Academy of infectious diseases and the society for critical care jointly recommended procalcitonin as an auxiliary diagnostic marker for the differential diagnosis of sepsis and pulmonary infectious systemic pneumonia.

It can be found that the dynamic monitoring of procalcitonin and early clinical diagnosis is of great significance, so it is necessary to establish a simple, rapid and low error rate method to measure procalcitonin. In this study, time resolution combined with immunochromatography was used to establish a rapid quantitative method for monitoring procalcitonin, which was applied to the early diagnosis of sepsis and the continuous monitoring of the course of sepsis.

2. Determination of Plasma Procalcitonin

2.1 Immediate Detection (POCT)

Today, in addition to improving the process of inspection, diagnosis and quality in the past, it is also necessary to increase the early detection and prevention of diseases, so as to prevent the occurrence of diseases from the source. Whether it is small and medium-sized hospitals, pharmacies, clinics, or small and medium-sized hospitals and families, the number of detection and diagnosis is rapidly increasing. In the current rapid and efficient social environment, the development of science and technology is also faster and faster. Patients are eager to find and treat diseases as soon as possible. Early detection and treatment can strive for more rescue time for patients, treatment success rate will be greatly improved, also can save doctors and patients a lot of time and economic burden. With the rapid development of inspection technology, quarantine work has become
convenient and fast, and inspection workers get results more quickly, which greatly improves the efficiency and quality of quarantine work. In addition, such high-efficiency work for critically ill patients to fight for more rescue and treatment time. Clinical examination originated from bedside examination in the 19th century. With the rapid development of emergency medicine, rapid detection and testing has become more and more important in the rescue of critically ill patients, so that patients can get the most accurate diagnosis and treatment at the earliest time [8]. This factor makes science and technology progress, so bedside diagnosis appears. With the continuous research and development of testing equipment and reagents, this kind of test has been widely used in food inspection, environmental protection and forensic medicine due to its fast and convenient characteristics. Therefore, POCT (point of care testing) came into being.

Bedside detection can also be called instant detection. Bedside detection has the advantages of portability, convenient operation, multiple applications, accurate and timely reliable results. Bedside detection can make today's people know their health status, quickly and accurately let doctors know the diagnosis results and treatment needs of patients. Therefore, bedside detection is an important development direction of modern medical examination. At the same time, bedside detection is also one of the fastest developing fields in quarantine medicine [10]. Due to the change of medical model, the function of hospital has changed from simple treatment to multi-functional treatment place, including prevention, treatment, health care and rehabilitation. Today's hospital integration provides a full range of excellent services to people and society. The emergence of bedside detection is the product of rapid and efficient work and life rhythm in today's society, which can make patients get diagnosis and treatment as soon as possible. Bedside testing can be performed in most situations, such as patients' bedside, operating room, intensive care unit, home, open field, clinic and doctor's office. Bedside detection not only has the advantages of simple and fast operation and easy to carry, but also saves many complex operation steps for many of the money laundering and analysis stages of the system, and greatly shortens the detection cycle of patients. In addition, bedside testing also allows patients to self-test their condition. Bedside detection has the following characteristics: the operation tool is compact and convenient to carry; the detection object does not need to be preprocessed; the operation method is simple and can be used quickly. Bedside detection can complete the test in the shortest time and get the test results report, which is greatly beneficial to the rapid diagnosis of emergency patients, especially the critical emergency patients. Bedside detection can be used for early diagnosis of emergency and severe patients and dynamic monitoring of patients' condition changes. It can also be used in the evaluation of treatment effect of critical emergency patients.

2.2 Immunochromatography

In order to make the steps of immunoassay simple and improve the detection efficiency, scholars have studied a new simple detection technology in China immune chromatography (ICA) [11]. The development of immunochromatography in the early 1980s is a unique solid-phase membrane immunoassay method based on chromatography and antigen-antibody specific immune reaction, which is a combination of very edge sealing, rapid immunological detection technology and
immunochromatography technology. The structure of immunochromatography mainly includes binding pad, sample pad, absorbent pad, PVC base plate, nitrocellulose membrane (NC membrane), quality control line (C line) and detection line (T line). The main principle of immunochromatography is to use porous microporous filter membrane as the solid phase carrier for detection. Through the capillary action of fiber membrane, the antigen sample or antibody sample to be tested flows forward on the surface of the membrane made of strip fiber. When the antigen sample or antibody sample to be tested flows to the area to be detected, the antigen sample or antibody sample to be tested and the matching specific immune reaction of the monitoring area label number form a complex, and then the antigen sample or antibody sample to be tested is fixed in this area, and the combined reactant and liquid-solid interface are formed by this method Separation of the complex. The results can be obtained in a very short period of time by using enzymatic reaction or direct use of colored markers, using human eyes to observe or using instrument matching detection. Because this technology does not need to separate the binding marker and the free marker, it saves more complex steps of sample adding and washing, which makes the operation of this technology simple and the detection speed is faster. Operators can operate independently without long training. Immunochromatography is not only highly sensitive and specific, but also can be completed without specific equipment. Therefore, immunochromatography is very suitable for on-site detection, self-examination of chronic patients and self-care of ordinary people.

There are two dominant methods of immunochromatography, namely, non-competitive method and competitive method. Non-competitive method can also be called direct action. The non-competitive method is double antibody sandwich method [12]. The detection reagent used in this method is a specific ligand of antigen to be tested coupled with colloidal metal or latex particles. The specific ligand precipitates and accumulates on the binding pad. After the sample to be tested is added to the sample pad, the sample to be tested flows forward along the membrane strip due to capillary action, and finally fuses with the detection reagent on the binding pad. Finally, they continue to flow along the membrane. Immunochromatography is a complex immunochromatographic technique with the fastest development rate so far. After years of research, Immunochromatography has been successfully transformed into a very mature in vitro diagnostic technology and has been widely used in many fields such as medicine, biology, veterinary medicine and so on.

2.3 Time Resolved Fluoroimmunochromatography

Kaplan and cons used antibodies to bind fluorescein to locate antigens in tissues, and then proposed the concept of fluorescence immunoassay [13] in 1941. In the field of analysis, organic molecules such as fluorescein are usually used as markers. Under the irradiation of specific excitation light, these fluorescent markers are particularly easy to be excited to fluorescence. In addition, the fluorescence labeling can be repeated several times over a period of time. The main principle of fluorescence immunoassay is to combine the measurability and sensitivity of fluorescence with the high specificity of antigen antibody reaction and use fluorescein as a marker. Under the action of specific excitation light, fluorescein can absorb light energy, and then change into excitation state, and then release the absorbed light energy through electromagnetic radiation, thus producing a very strong fluorescence signal. The emission of fluorescence signals can be quickly and accurately concluded by fluorescence analyzer, of course, can also be observed by fluorescence microscope. This technology is widely used in medicine, forensic medicine, food hygiene, biology and other fields. Fluorescence immunoassay is the most sensitive detection
technology in the world. Fluorescence immunoassay has the characteristics of long storage period, high sensitivity, no radioactivity and few instruments. However, the traditional fluorescence labeling method usually has small measuring range and unstable detection, so it cannot meet the needs of rapid quantitative detection. In this case, it is urgent to develop a more perfect immunolabelling method. In 1983, k-ray’s and soini’s et al. Developed a new non-radioactive micro immunoassay (TRFIA) using lanthanide as a fluorescent marker.

TRFIA is a new immunofluorescence analysis technology after immunofluorescence analysis [14]. It is different from the traditional fluorescein labeling, which is characterized by the use of special lanthanide as a marker. After the biological reaction, the instrument matching with the analytical technology is used to detect the fluorescence signal value after the specific binding between the sample and the labeled fluorescent element, and then the user needs to calculate the concentration of the sample to be tested. At present, there are five kinds of special fluorescent elements that can be used in time-resolved fluorescence technology, which are terbium, samarium, europium and dysprosium. Among them, Eu3+ is commonly used in antigen antibody. In the free state, the fluorescence signal of lanthanide is very weak, and energy can only be transferred through resonance between molecules, so the probability of non-radiative transition is very small. However, the chelates of lanthanide metals can emit strong fluorescence under the irradiation of ultraviolet light source. Compared with the traditional fluorescent markers, the excitation spectra of lanthanide metals are wider, but the emission spectra are narrower, there is a longer time of fluorescence decay, and the stocks shift in emission wavelength and excitation wavelength is larger. With the help of time resolution technology and spectral resolution technology, the interference of external fluorescence, excitation light and non-specific fluorescence can be greatly reduced, so the sensitivity of the detection instrument can be enhanced.

3. Application of Plasma Procalcitonin in Sepsis Infection

3.1 Principle

Procalcitonin is an effective index for the diagnosis of sepsis. The high level of procalcitonin has high specificity for sepsis caused by bacterial infection. In severe bacterial infections and sepsis, procalcitonin levels increased significantly. The level of procalcitonin did not change significantly in mild infection and limited bacterial infection. The level of procalcitonin can directly reflect the severity of human infection and systemic inflammation. The systemic inflammatory response caused by human bacterial infection is related to the concentration of procalcitonin in serum. After human infection, procalcitonin changes with the severity of human infection, such as local infection, concentration shock, sepsis and severe sepsis. The concentration of procalcitonin in serum increased with the severity of human infection. Because the level of serum procalcitonin can be reduced with the reduction of human inflammation, procalcitonin can be used to detect the changes of human condition for treatment detection. At the same time, procalcitonin is also an excellent prognostic marker of disease, because many inflammatory markers other than calcitonin do not have the characteristics similar to calcitonin, so they cannot be used in the detection of diseases. In people with sepsis, elevated levels of calcitonin in the body indicate poor prognosis. In patients with multiple dysfunction syndrome, elevated levels of calcitonin may also indicate poor prognosis. The absolute level of calcitonin will not directly affect the prognosis of human disease, but the change of procalcitonin concentration can show whether the human body is sick or not. In the treatment of patients, continuous detection of procalcitonin can more accurately judge the prognosis of patients. It has been found that high or high levels of procalcitonin can be used as one of the independent
predictors of mortality. Procalcitonin can help doctors predict the mortality rate of sepsis patients in which syndrome is reported. At present, the critical value of procalcitonin in the diagnosis of sepsis is 0.5 ng.ml. In other words, if the level of procalcitonin in patients is lower than 0.5ng/ml, the possibility of high-risk infection is very small, and the probability of bloodstream infection is very small.

3.2 Application

After the introduction of immune response and molecular biology technology, bedside detection has become more widely used. Bedside detection can be applied in many fields of medicine, such as immunology, endocrinology and hematology. Bedside detection was first used in diabetes mellitus, blood glucose and urine glucose detection, which greatly improved the level of self-monitoring and self-monitoring of patients. With the development of bedside detection technology, bedside detection is also used in the detection of infectious diseases. So far, a large number of bedside detection methods for detection of pathogens have been developed. There are HIV infection screening, syphilis, C-reactive protein and hepatitis screening. Through the rapid accuracy of bedside detection in microbial diagnosis, doctors in primary hospitals and community hospitals can quickly and accurately get the diagnosis conclusion, help doctors better determine the patient's condition, so as to develop a more appropriate and effective treatment plan for patients.

Bedside detection can be divided into three stages [15]. The first stage is the use of test strips and strips, also known as qualitative testing. This stage of detection does not need supporting instruments and does not rely on the supporting system required by supporting instruments. The results are judged by naked eye observation of dry pieces or adhesive strips of paper for chemical reaction. The second stage is semi quantitative and semi quantitative examination. In this stage, the detection relies on the bedside detection system of the instrument, which uses micro-electronic computer and micro motor and sensor to detect the whole inspection process in real time. The digital information is input into LIS by using barcode and microchip. The third stage is bedside detection in the future. In the future, bedside detection technology will pay more attention to less trauma or even non-invasive technology. In this way, the pain of patients can be reduced as much as possible, and the detection error can be reduced from the source. This kind of detection may be more automatic and intelligent. With the development of detection technology, researchers have developed a more rapid and convenient new quantitative detection technology, that is, time-resolved fluorescence immunochromatography, which is based on the combination of time-resolved fluorescence immunochromatography. With the help of fluorescent markers, fluorescent markers can generate fluorescence when ultraviolet light is far away. Then the operator uses the recommended equipment to measure the fluorescence intensity, and then calculates the concentration of the measured sample, so as to achieve quantitative. This technique uses lanthanide rare earth elements as markers. Lanthanide rare earth elements have strong specificity, high sensitivity, simple and fast operation steps, good stability of tracers, wide range of dynamic monitoring, no radioactive pollution, small storage conditions of markers and no interference of natural fluorescence of samples. With these advantages, lanthanide rare earth elements replace radioisotope and enzyme immunoassay as a micro or excess quantitative immunoassay. The highlight of fluorescence analysis technology lies in the unique fluorescence of lanthanide elements and the wavelength resolution method and time delay technology used in the detection. After combining the advantages of time-resolved fluorescence analysis and immunochromatography, samples can be measured accurately and quickly. This study uses time-resolved fluoroimmunoassay,
which can measure procalcitonin rapidly and accurately.

4. Results and Discussion

4.1 Time of Septic Shock and Antibiotic Treatment is Also a Risk of Death

Shown as Figure 1, the risk of death increases with the time of septic shock receiving antibiotic treatment. If sepsis patients are not correctly diagnosed, they cannot receive effective antibiotic treatment, and the risk of death will gradually increase. Every 1 hour, the risk of death will increase by 7.9%.

4.2 PCT Concentration in Serum

Figure 1 The time of septic shock receiving antibiotic treatment was also related to the risk of death
Shown as Figure 2, serum PCT concentration is positively correlated with the course of disease. In other words, the more severe the course of disease, the higher the level of procalcitonin in patients.

### 4.3 PCT Level in Patients with Positive Blood Culture

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<th>Table 1 PCT levels in each group</th>
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Shown as Table 1, the median PCT values of positive blood culture group and all culture negative groups were 4.55ng/ml and 0.51ng/ml. The area under AUC curve of PCT original value to positive blood culture was 0.715, and the optimal critical value was 1.39ng/ml. This study specially analyzed the bacteria cultured in Bazhong blood, including Escherichia coli, Staphylococcus aureus, Enterococcus faecium, Enterococcus faecalis, Acinetobacter baumannii, Klebsiella pneumoniae, Enterobacter cloacae and Pseudomonas aeruginosa. Among them, the median PCT value of patients infected with Enterobacter cloacae was the highest, followed by that of patients infected with Klebsiella pneumoniae.

4.4 Investigation of Patients in Department

![Pie chart of departments in sepsis patients with procalcitonin more than 100g/L within 24 hours.](image)

**Figure 3 Distribution of departments in sepsis patients with procalcitonin more than 100g/L within 24 hours.**

Shown as Figure 3, among the sepsis patients with procalcitonin greater than 100 μg/ml within 24 hours, the proportion of patients in ICU ward is the highest, and about one third of patients are admitted to beryllium copper surgical wards. The mortality rate of ICU ward was the highest.

4.5 Composition of Main Diagnosis
Figure 4 The main diagnosis of sepsis patients with procalcitonin more than 100 μg/ml within 24 hours after admission

Shown as Figure 4, the vast majority of sepsis patients have mods, trauma, burns, CPR and other diagnoses after major thoraco peritoneal surgery. The mortality of patients with MODS, burns, trauma and CPR were much higher than that of the survival group.

5. Conclusion

Sepsis is a systemic inflammatory response syndrome caused by infection. Sepsis and severe sepsis, sepsis shock caused by the death rate of organ failure is still high. Sepsis is a critical disease, its mortality is high, and the cost of treatment is not low. According to traditional ideas, the essence of sepsis is the uncontrolled reaction of systemic inflammation. The body produces inflammatory response under the attack of therapeutic elements, and then a large number of inflammatory mediators are released by inflammatory cells, which is the "cascade reaction" of inflammation. In recent years, researchers have found that PCT can be used as a new index in the early diagnosis of severe systemic bacterial infection. In this paper, the probability of septic shock is proportional to the time of antibiotic treatment, and the serum PCT concentration is positively correlated with the course of disease. In the investigation of PCT level in blood culture positive patients, the median
PCT value of patients infected with Enterobacter cloacae was the highest, and that of patients infected with Klebsiella pneumoniae was the second. The proportion of patients in ICU is the highest, and the mortality rate is the highest. About one third of the patients are admitted to the surgical ward of beryllium copper. The mortality of patients with MODS, burns, trauma and CPR higher. This study found that blood purification can partially remove PCT in the blood. In this experiment, the combination of timely diagnosis immunochromatography technology and time-resolved immunofluorescence quantitative detection technology can detect some diseases quickly and accurately, which is very suitable for bedside detection in intensive care unit and emergency department. With the trend of aging population in China, the social burden of sepsis is becoming more and more heavy. Early diagnosis and treatment of sepsis can effectively alleviate this problem. The detection of plasma procalcitonin can help to diagnose sepsis, which will be widely used in the future diagnosis and treatment.

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