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Procalcitonin for differential diagnosis of graft rejection and infection in patients with heart and/or lung grafts

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Abstract *Objectives*: Investigation of the reliability of Procalcitonin (PCT) for differential diagnosis of acute rejections and non-viral infections in heart and lung transplanted patients.
Design: Retrospective study.
Setting: Transplant intensive care unit (ICU) at a university hospital.
Patients: 57 heart, 18 lung and 3 heart-lung transplant patients.
Measurements: PCT was measured in plasma samples of heart and lung transplanted patients using a commercial immuno-luminescence assay and was compared with values of C-reactive protein (CRP) and leukocytes (WBC).
Results: PCT was elevated in patients suffering from bacterial and fungal infections. The magnitude of values was clearly associated with the severity of the infection. Rejections and viral infections did not interfere with the PCT release.
Conclusion: PCT is a reliable predictor with discriminating power for non-viral systemic infections in patients after heart and/or lung transplantation. PCT allows an early differential diagnosis between rejection (AR) and bacterial/fungal infection (IF) and thus a rapid and focused therapeutic intervention. It avoids unnecessary antibiotic treatment which could be toxic for the graft itself in patients with rejection only. PCT provides vital information early to clinicians and allows them to improve the management of bacterial/fungal infections in immunocompromized transplant patients. PCT thus facilitates and improves the outcome of survival rate and the quality of life in the postoperative period of patients with heart and/or lung grafts.

Key words Procalcitonin · Acute rejection · Heart transplantation · Lung transplantation · Infection

Introduction

The history and management of an intensive care patient suffering from systemic infection or sepsis or who is in danger of becoming septic is totally different from that of a patient who will or has received a transplant. A patient who is registered on the waiting list for heart and/or lung transplantation has been chronically ill for months and even years. Patients waiting for a heart have circulatory and respiratory problems, multiple organ dysfunction and often show signs of immunodepression. Lung transplant patients usually have a long history of cystic fibrosis with all its known associated infections, or have suffered from chronic bronchitis, emphysema or idiopathic fibrosis, which result in hypoxia and hyperglobulinaemia. All such patients have been under close medical care and observation. Their underlying disease was known and was heavily and specifically treated.

All transplant patients have to undergo major surgery with a long period of anaesthesia and extended trauma. They often receive large numbers of blood

transfusions from foreign donors. After surgery the patient experiences aggressive and chronic immunosuppression. This treatment is strongest within the first seven days, but remains relatively high for another month. The steroids are usually tapered off as are other potent immunosuppressive drugs like Azathioprin, Cyclosporin or Tacrolimus.

A rejection episode, which occurs in more than 60 % of all transplant patients, needs an immediate boost of immunosuppression. Under this beneficial anti-rejection treatment patients have a significant risk for opportunistic infections. According to the European Transplant Registry more than 50 % of transplanted patients were found to have infections in the first post operative year, 40 % of which were of viral, 42 % of bacterial, 10 % of fungal and 8 % of protozoal origin. Their early and rapid detection is necessary for prevention measures and could improve the survival outcome of the transplant patient. It would facilitate post transplant monitoring and minimize unnecessary treatment of non-infected patients. This would be of vital advantage, because antibiotic treatment often has toxic side effects not only to the graft but also to the patient.

In contrast, in individuals who become septic after sudden and unexpected severe trauma or insult monitoring and managing of these serious microbial infections is therefore very different. Procalcitonin seems to be a new and excellent early marker for identifying non-viral infections in both groups of patients and also helps to differentiate non-viral infections from rejections in transplanted patients. Here PCT is much more specific than acute phase proteins and leukocyte populations.

Materials and methods

In a retrospective study 57 heart, 18 lung and 3 heart-lung transplant patients were monitored for inflammatory events. The most common indications for heart transplantation were dilatative cardiomyopathy (n = 35) and ischemic cardiomyopathy (n = 11). Of the 18 lung patients 11 received a unilateral and seven a bilateral graft. Seven patients were transplanted because of lung fibrosis, four suffered from emphysema and five from cystic fibrosis.

Biopsies under cyto-immunological monitoring were the standard for diagnosis of rejection [1, 2]. Rejection was classified according to the score of the International Society for Heart and Lung Transplantation (ISHLT) [3].

Infections were diagnosed by serology, smears or cultures. Local infections were defined as peripheral colonisation, mucous membrane infections or locally limited infections. If local infections spread to other organs within one week or were accompanied by multiple germs the events were classified as generalized or multiple infections. Sepsis was defined by positive blood-culture and simultaneous occurrence of typical clinical signs of sepsis.

For statistical evaluation and graphical representation the laboratory values on day of diagnosis of AR or IF were retrospectively used.

The transplant patients (TP) were split into five groups to differentiate between acute rejections and infections:

Group 1: TP with a negative biopsy result (degree 0–1A) and without infection (control group)
Group 2: TP with rejection (degree 1B-3)
Group 3: TP with an AR and simultaneous occurrence of infection
Group 4: TP with local infections
Group 5: TP with systemic infections including generalized/multiple infections or sepsis

and into four groups to ascertain the severity of infection:

Group A: TP on day of discharge regarded as healthy (control group)
Group B: TP with local infection
Group C: TP with generalized or multiple infections
Group D: TP with sepsis

Plasma PCT was measured by a specific ultrasensitive immunoluminometric assay. This test, which is species specific for the PCT molecule, requires 20 µl of plasma or serum and can be completed rapidly within three hours. It anticipates results of blood cultures by 24–48 h, avoiding unacceptable delays in the administration of the appropriate therapy. Inter- and intra-assay variations for both the low and high concentrations are less than 7 %. PCT is chemically stable at room temperature for more than 12 h [4].

The results were expressed as mean values with standard error of the mean. The non-parametric Mann-Whitney-U test was used to achieve robust results despite of small patient numbers and large variability and to assess the significance of differences. The Bonferroni-Holm procedure was applied to correct for multiple comparisons. P-values of less than 0.05 were accepted as significant.

Results

Levels of less than 0.5 ng/ml PCT (cut-off point) were regarded as non indicative of systemic bacterial, fungal or protozoal infection. These values were found in patients with acute rejection only (0.2 ± 0.2 ng/ml). On the day of discharge values levelled at 0.3 ± 0.5 ng/ml. A PCT of 0.6 and 7.3 ng/ml respectively was found during local and multiple non-viral infections. Mean values of 22.4 ± 17.8 ng/ml PCT were typical signs of sepsis, whether of bacterial, fungal or protozoal origin. This proves that the quantity of circulating PCT depends on the severity of the infection. PCT levels differed significantly between AR and local infection ($p < 0.01$) and between AR and systemic infection ($p < 0.001$) (Fig. 1, 4).

Even at discharge patients showed abnormal CRP values of a mean of 2.8 mg/dl. This value increased, in all three groups of patients with infections, to 6.8 mg/dl during local infection, to 6.4 mg/dl during multiple/generalized infections, and to 8.3 mg/dl during sepsis. In contrast to PCT, CRP levels increased during rejection episodes to a mean of 1.9 mg/dl (Fig. 2, 5).

The number of WBC ranged from 12 G/L during rejection to 13 G/L during local and 15 G/L during multi-

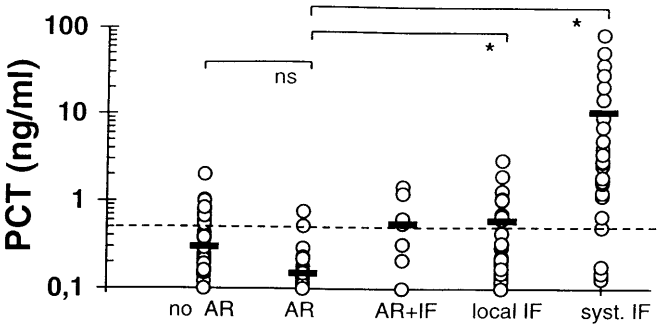

Fig. 1 PCT values in patients with and without rejections or infections; ns = not significant, * = significance $p < 0.01$. Normal values < 0.5 ng/ml (*line*)

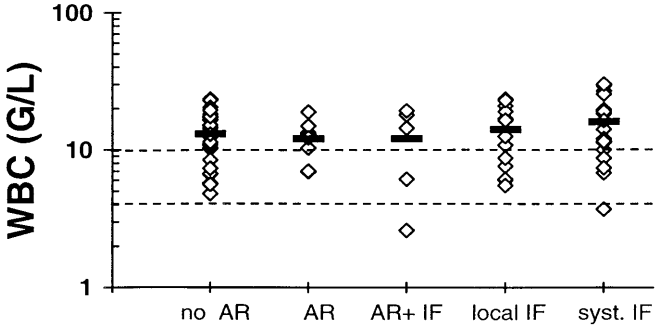

Fig. 3 WBC values in patients with and without rejections or infections. Normal values 4–10 G/L (*lines*)

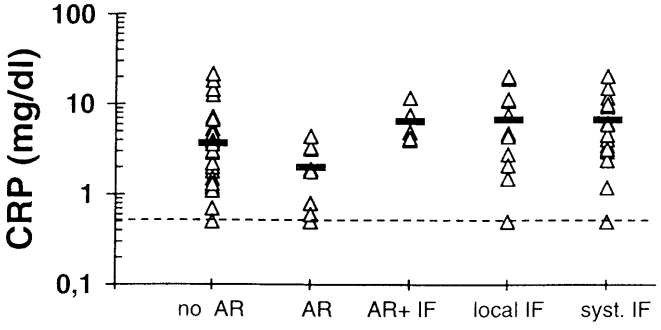

Fig. 2 CRP values in patients with and without rejections or infections. Normal values < 0.5 mg/dl (*line*)

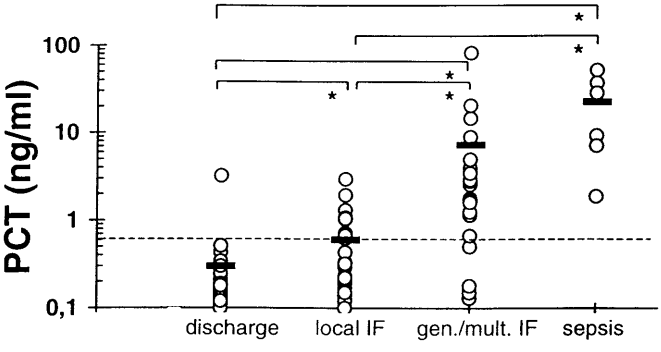

Fig. 4 Association of PCT values with severity of infections; * = significant $p < 0.01$. Normal values < 0.5 ng/ml (*line*)

ple infections. A count of 18 G/L was found in sepsis, thus allowing no reliable differential diagnosis. Only at discharge the levels dropped to almost normal counts of 9.5 G/L (Fig. 3, 6)

Discussion

PCT is a new and innovative indicator for the diagnosis of severe systemic bacterial and fungal infections in transplant patients. It is a reliable predictor with high discriminative power of inflammatory activities during sepsis.

PCT is the 116-aminoacid prohormone of calcitonin with a molecular weight of 13 kD but lacks hormonal activity [5]. Its biological function is unknown, so is the organ of origin [6]. Patients with near-complete eradication of the leukocyte population after chemotherapy still presented with very high levels of PCT. Injection of bacterial endotoxin or contaminated plasma expander lead within 24 h to PCT levels exceeding the baseline of < 0.5 ng/ml up to 300 fold [7, 8]. Since Aspergillum and Candida as fungi were found to induce extreme levels of PCT, other promoters than endotoxin must be responsible for PCT production [9]. The appearance of PCT in the circulation is slightly slower (2–4 h) than IL-6 (1 h) but faster than CRP (24 h) after a known stimulus. PCT has a half life of about 24 h in contrast to calcitonin with a half life of 10 min. PCT is cleaved while circulating in the periphery by specific proteases to calcitonin, katacalcin and a N-terminal residue [5].

The monitoring of PCT in transplant patients has led to a new and promising method for differentiating rejection from infection. PCT was not found in patients suffering from acute and chronic rejections [10]. It remained at normal levels in transplant patients with viral diseases and also in HIV patients, even those with terminal disease [11]. PCT was not detected in patients with autoimmune diseases [12] and was not influenced by conventional immunosuppression like Azathioprin, Cyclosporin and Tacrolimus [13]. Steroid bolus therapy for acute rejections had no impact on the levels of PCT while treatment with murine monoclonal anti-CD3-antibodies seemed to stimulate PCT production in a similar way to cytokine release (interleukin storm) [12].

PCT reflects the severity and dynamics of the non-viral infection and allows an estimation to be made of the effect of the antibiotic therapy. This is possible due to the half life of PCT of 24 h. A steady decrease indicates appropriate antibiotic treatment, while a steady in-

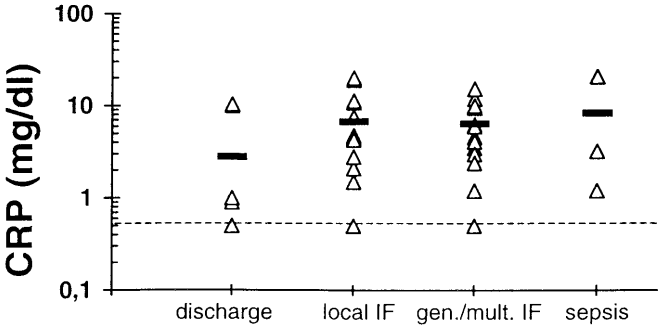

Fig. 5 Association of CRP values with severity of infections. Normal values < 0.5 mg/dl (*line*)

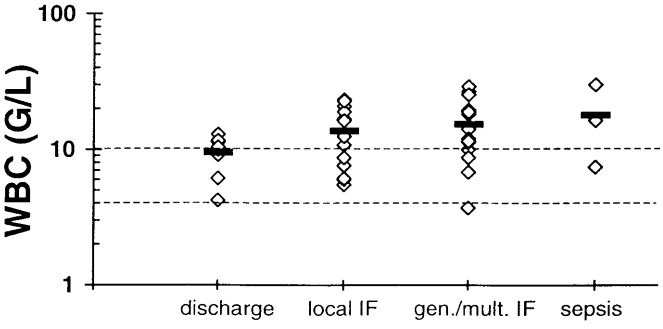

Fig. 6 Association of WBC values with severity of infections. Normal values 4–10 G/L (*lines*)

crease is a sign of persistence of pathogens, mainly found during aspergillum infection. These courses allow an early prognosis of the outcome [14].

For clinical purposes another advantage of PCT is that its evaluation is easy to establish and it provides an answer within 3 h. In contrast diagnosis of the different grades of rejection of heart and lung grafts using the standard techniques of endomyocardial biopsies and open lung biopsies in combination with histopathology are time consuming and inconvenient for the patient [1, 3]. Viral infections (Cytomegaly and Herpes type viruses) can be established by using the CD4/CD8 ratio [2], by determining the anti-bodies of the IgG and IgM class and immuno-histochemical stainings [15]. No valid method exists to safely diagnose non-viral infections.

Up-to-date blood cultures are the safest indicator for septicemias. But their low sensitivity is only at 25–42 % [16]. The detection of IgG and IgM antibodies has the disadvantage of usually being too late to adjust therapy to save life. Smears identify only superficial colonisations. Other humoral parameters such as CRP, Neopterin, leukocyte numbers and TNF react to all kinds of inflammatory events and not specifically to non-viral infections [17]. The marker coming closest to the characteristics of PCT is IL-6. This interleukin elevates during sepsis [18], correlates with the severity of infection and predicts the outcome of the patient [19]. It does not increase during rejections [20]. However, it increases in both, viral and bacterial infections [21] and may decrease despite persistency of bacteria in septic patients [18]. IL 6 is released in autoimmune disease [12] and increases regularly after major operations [17]. Because the latter methods are relatively unspecific they are of lower prognostic and therapeutic value than PCT.

Since PCT specifically responds to inflammatory events of bacterial and fungal origin, it also allows a distinction to be made between overlapping events such as AR and viral infection (no PCT increase), and AR and bacterial infection (increase according to the severity of infection). These characteristics also allow the differentiation between septic shock and cardiogenic shock [22], toxic and biliary pancreatitis [23], bacterial and viral meningitis [24] and infectious and non-infectious ARDS [25].

PCT was initially used to diagnose sepsis and septicaemia especially in new born infants [26]. It was especially helpful in bone marrow transplanted children with fever of unknown origin, where unnecessary myelotoxic antibiotic treatment could be avoided.

Summary

In our study we found PCT to be an elegant predictor in transplant patients not only of severe infections but also a reliable parameter to clearly distinguish infections from acute rejections.

Some questions remain open: What is the organ of origin? And what are the stimuli for PCT production? Its biological function is unclear. It is unknown which effect PCT has on the total organism.

One of the advantages is the fast detection of an infection within 3 h. PCT levels are correlated with activity and dynamic of the infection, as well as efficiency of therapy and prognostic outcome. Critical care patients, newborns and unconscious persons can be monitored reliable and rapid. Differentiation between infectious and non-infectious causes is possible and valuable.

On the other hand PCT can not replace tests defining the different types of germs involved, the sensitivity or the resistance to antibiotics. Thus PCT represents a new marker for infections which allows in combination with other parameters like for example CRP, leukocyte numbers and interleukins to improve and accelerate the appropriate treatment leading to a better clinical situation, quality of life and longer survival.

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