

PROCALCITONIN IN MULTIPLE MYELOMA

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Background: C-reactive protein (CRP) is an acute phase protein (APP) and has prognostic significance in multiple myeloma (MM). Procalcitonin (PCT) is also an APP and has been reported to be elevated in patients with severe systemic infection and sepsis. Aims: We investigated PCT level in patients with MM and its relationship with the stage and activity of the disease. The discriminatory value of PCT level in myeloma patients with and without infection was also assessed.

Patients and methods: PCT, CRP, interleukin-6 (IL-6) and IL-1 β levels were determined in 69 blood samples from 66 myeloma patients with various disease activity and 25 controls. Infections were verified by microbiological identification of an infectious specimen together with clinical presentation and radiological findings. Statistical analysis: The Mann-Whitney U test and Spearman's rank correlation test were performed. P values lower than 0.05 were considered significant.

Results: Patients with known infections and inflammatory diseases that might elevate PCT levels were excluded from the data in order to evaluate PCT merely in MM. None of the patients with MM had a PCT level higher than 0.5 ng/mL. Mean PCT level in myeloma patients with infection, without infection and controls were 1.42 \pm 0.36, 0.38 \pm 0.15 and 0.09 \pm 0.02, respectively. The difference between mean PCT levels in myeloma patients with and without infection was insignificant ($p > 0.05$). The sensitivity of PCT for the detection of infection was 64%, and the specificity 82%.

Conclusion: PCT level seems to be a good parameter to identify systemic bacterial infections and is a useful marker of the severity of infection in patients with MM if coexistent non-infectious conditions that may contribute to PCT elevation are excluded by appropriate clinical and laboratory investigations.

Key Words: Multiple Myeloma, Procalcitonin, Infection.

MULTİPL MYELOMDA PROKALSİTONİN

Dayanak: Bir akut faz reaktanı olan C-reaktif protein (CRP) multipl myelomda (MM) prognostik öneme sahiptir. Prokalsitonin de (PCT) akut faz reaktanıdır. PCT düzeyleri şiddetli sistemik enfeksiyonlar ve sepsiste yüksek bulunmuştur.

Amaç: Bu çalışmada MM'lu hastalarda PCT düzeylerinin hastalığın evresi ve aktivitesiyle ilişkisi araştırılmıştır. MM'lu olgularda enfeksiyonların ayırımında PCT'in öneminin değerlendirilmesi de çalışmanın diğer amacıdır.

Hastalar ve Metodlar: Altmışaltı MM'lu hastadan alınan 69 kan örneği ve 25 sağlıklı kontrolde PCT, CRP, interlökin-6 (IL-6), ve interlökin-1 β (İL-1 P) düzeyleri ölçüldü. Enfeksiyon tanısı hastanın kliniği, radyolojik bulgular ve enfeksiyon etkeninin mikrobiyolojik olarak izolasyonu kondu.

İstatiksel Analiz: İstatiksel değerlendirme Mann-Whitney-U ve Spearman korelasyon testleriyle yapıldı. 0.05'den küçük p değerleri İstatiksel olarak anlamlı kabul edildi.

Sonuçlar: MM'da PCT'in hastalığın aktivitesi ve evresiyle ilişkisini değerlendirmek için eşlik eden inflamatuvar veya enfeksiyöz hastalığı olanlar değerlendirmeye alınmadı. MM'lu hastaların tümünde PCT düzeyleri 0.5 ng/ml'den düşük bulundu. MM ve enfeksiyonu olan; sadece MM'lu olgular ve kontrol grubunda PCT düzeyleri sırasıyla 1.42 \pm 0.36, 0.38 \pm 0.15 and 0.09 \pm 0.02 olarak bulundu. Enfeksiyonu olan ve olmayan MM'lu olguların PCT düzeyleri arasındaki fark anlamlı bulunmadı. Enfeksiyonların tespitinde PCT'in duyarlılığı % 64, özgünlüğü % 82 olarak bulundu. Sonuç: MM'lu olgularda, PCT düzeyini yükseltebilecek enfeksiyon dışı nedenler klinik veya laboratuvar araştırmalarıyla dışlanabilirse, PCT düzeyleri sistemik bakteriyel enfeksiyonların tanısı ve enfeksiyonun şiddetini değerlendirmek için faydalı bir tanı yöntemi olarak görünmektedir.

Anahtar Sözcükler: Multipl Myelom, Prokalsitonin, Enfeksiyon

Multiple myeloma (MM) accounts for 10% of all malignant hematologic neoplasms. There is a significant variation in the survival of patients with myeloma. Prognostic factors are needed to identify groups of patients with widely different survival rates to assist in counseling and therapeutic strategies. Studies have shown that serum levels of interleukin-6 (IL-6) have prognostic value in MM. The serum C-reactive protein (CRP) is an acute phase protein (APP) and has been proposed as a surrogate marker for serum IL-6 levels. CRP has also prognostic value in myeloma (1-3).

Patients with MM have an increased susceptibility to infections because of associated hypogammaglobulinemia, and T-cell, NK-cell and monocyte defects. The additional immunosuppressive effect of chemotherapy further increases infection risk. Bacterial infections are one of the main causes of morbidity and mortality in myeloma patients. Therefore, early diagnosis and prompt initiation of broad-spectrum antibiotic therapy are critical in patients with MM (4).

Procalcitonin (PCT) is an APP (5) and has been reported to be elevated in patients with severe systemic infection and sepsis (6). The serum level is correlated with the severity of infection. Elevated PCT level without evidence of infection has been reported in benign liver disease; renal failure; malignant tumors, particularly those arising from the neuroendocrine system; trauma patients; after surgery; and vasculitis (7-12). PCT concentrations are claimed to have a more powerful discriminatory value for bacterial infection than other APPs such as CRP (12).

Our aims were twofold: firstly to determine PCT levels in MM and its relationship with the stage and activity of the disease in patients with MM; and, secondly, to assess the discriminatory value of PCT level in myeloma patients with and without infection

PATIENTS, MATERIALS AND METHODS

Study group

Sixty-nine blood samples from 66 MM patients (30 female, 36 male; mean age 57.6 \pm 1.1) and 25 controls (13 female, 12 male; mean age 57.48 \pm 1.90) were analyzed. Patients with MM were staged according to the system proposed by Durie and Salmon (13). The characteristics of the MM patients are shown in Table 1. Infection was verified by microbiologic identification of an infectious specimen together with clinical presentation and radiological findings. Patients with documented infections were divided into subgroups of patients with major (i.e. pneumonia, sepsis) or minor infection (i.e. upper respiratory tract infection, uncomplicated urinary tract infection).

Table 1. Characteristics of the patients with multiple myeloma.

	n	percent (%)
Multiple myeloma	66	100
MMn	38	57.5
Stage I	4	
Stage II	9	
Stage III	25	
MMp	15	23.0
MMr/r	13	19.5

MMn: new diagnosis multiple myeloma; MMp: myeloma patient in plateau; MMr/r: relapsed and/or refractory multiple myeloma

Blood sampling

All blood samples were taken as soon as the patient was admitted to the hospital and before an antibiotic regimen was started. None of the patients were neutropenic during the blood sampling. Serum samples were obtained following centrifugation at 2500 g for 15 minutes at 4 °C. It was then aliquoted in 0.5 mL vials, deep frozen at -70 °C and thawed just before analysis.

Laboratory assays

Procalcitonin was determined by a commercially available immunoluminetric assay LUMI test PCT kit (Brahms Diagnostica, Berlin Germany). The manufacturer recommends 0.5 ng/mL as the threshold level for PCT. IL-6 (Human IL-6, R&D Systems Quantikine™ HS Minneapolis USA) and IL-1β (Human IL-1β, R&D Systems Quantikine™ HS Minneapolis USA) were quantified by an enzyme-linked immunosorbant assay (ELISA). CRP was assayed by a nephelometric method (Dade-Behring, Amersfoort, The Netherlands). The minimum detectable doses of IL-6 and IL-1β assays were less than 0.094 and 0.1 pg/mL, respectively. Normal ranges for IL-6, IL-1β and CRP were < 10 pg/mL, 2 pg/mL and 4.5 mg/L.

Statistical analysis

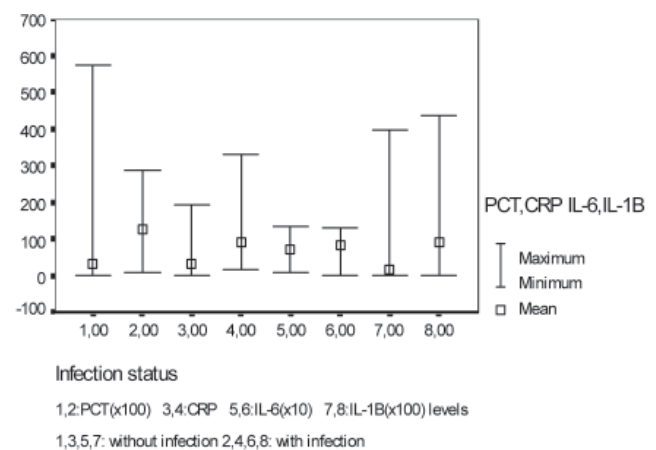
The results were calculated as mean±SE mean (SEM). The Mann-Whitney U test was used to compare the means. The correlation between two parameters was determined by Spearman's rank correlation test. P values lower than 0.05 were considered significant.

Table 2. Mean PCT, IL-6, CRP and IL-1β levels in myeloma patients with and without infection.

Mean±SEM (range)	Patients without infection (n=58)	Patients with infection (n=11)	p
PCT (ng/mL)	0.38±0.15 (0-5.7)	1.42±0.36 (0.1-2.8)	NS
IL-6 (pg/mL)	7.71±0.59 (0.9-13.3)	9.68±1.22 (2.5-13.0)	NS
CRP (mg/L)	33.96±6.07 (1.3-193.0)	86.43±31.94 (13.8-330.0)	<0.01
IL-1β (pg/mL)	0.19±0.02 (0-3.9)	0.94±0.48 (0-4.4)	<0.001

RESULTS

IL-6 levels were elevated in 31 out of the 69 patient samples (45%) and in 1 out of the 25 control samples. CRP levels were elevated in 58 out of the 69 patient samples (84%) and in 1 out of the 25 control samples. IL-1β levels were undetectable in 51 patient samples and in 20 control samples. IL-1β levels were elevated in 4 samples in the patients and in none of the controls. PCT levels were undetectable in 1 patient sample and in 4 control samples. PCT levels were elevated in 9 out of the 69 patient samples and in none of the 25 control samples. From these 9 samples in which PCT levels were elevated, 7 had documented infection, 1 had ankylosing spondylitis, and 1 had elevated liver enzymes without an identifiable cause.

**Figure 1.** PCT, CRP, IL-6 and IL-1β levels in myeloma patients with and without infection.

In myeloma patients PCT levels were lower than 0.5 ng/mL after the exclusion of infections or accompanying diseases that may contribute to elevated PCT levels. Currently the immunoluminetric assay is the only method for determining PCT levels and the threshold level of the test kit used is especially determined to identify patients with systemic bacterial infections (12). Generally PCT levels in healthy controls do not exceed 0.1 ng/mL (8). When we defined the threshold as the upper limit of our controls (0.25 ng/mL) in order to evaluate PCT level merely in myeloma patients without renal failure, 8 patients (18%) had elevated PCT levels. The distribution of patients was as follows: 3 patients with newly diagnosed myeloma (9.6%), 1 patient in the plateau phase (8%), and 4 patients with relapsed/refractory disease (40%).

PCT levels in myeloma patients with and without infection were evaluated to determine the discriminative value of PCT in the infections of these patients (Table 2). Eleven serum samples were obtained during an infectious episode in myeloma patients. Six infectious episodes were classified as major infection and 5 as minor. PCT levels were elevated in 5 major and 2 minor infections. One patient with major infection had radiological evidence of pneumonia but microbiological analysis could not be carried out. Mean PCT levels in myeloma patients with infection, without infection and the controls were 1.42 ± 0.36 , 0.38 ± 0.15 and 0.09 ± 0.02 , respectively. The difference between mean PCT levels in patients with and without infection was insignificant ($p > 0.05$). Among the 69 serum samples analyzed, with 11 infections occurring during sample collection, the sensitivity of PCT for the detection of infection was 64%, and the specificity 82%. The sensitivity of IL-6 and CRP was 72% and 100%, respectively, whereas the specificity for IL-6 and CRP was below 25%. The severity of the infection correlated positively with CRP ($r = 0.31$, $p < 0.01$), IL-1 β level ($r = 0.49$, $p < 0.001$) and PCT ($r = 0.25$, $p < 0.05$), but not with IL-6 level ($p > 0.05$).

DISCUSSION

We investigated PCT levels in MM. In myeloma patients PCT levels were lower than 0.5 ng/mL after the exclusion of infections or accompanying diseases that may contribute to elevated PCT levels. Survival of patients with MM is highly variable and ranges from a few months to many years. Prognostic factors are needed to identify groups of patients with widely different survival rates to assist in counseling and therapeutic strategies. The growth of myeloma cells is regulated by a cytokine network in which IL-6 plays a key role. IL-1 β has a critical role in the pathogenesis of bone disease in MM (14). TNF- β is a survival factor for myeloma cell lines, although less potent than IL-6 (15). These cytokines are also important mediators of acute phase response, which in turn stimulates the synthesis of APP in the liver (16). Studies have shown that serum levels of IL-6 and an APP CRP have prognostic value in myeloma (1,2). Other acute phase proteins, such as α 1-antitrypsin, have prognostic value similar to CRP (3). PCT is an APP and its synthesis is induced by TNF- β and IL-6 in the absence of bacterial products (5). In our study PCT levels were lower than 0.5 ng/mL in MM. However, with a lower threshold, 18% of myeloma patients, especially with relapsed/refractory disease, had elevated PCT levels. Therefore, PCT level in MM needs to be further evaluated using a more sensitive kit and with a lower threshold level (8).

CRP levels have frequently been used to differentiate bacterial from non-bacterial infections (12,17), but in myeloma a significant proportion of patients have elevated levels of CRP unrelated to infection (2,3). Therefore, CRP level in MM is not as useful as other conditions in the discrimination of bacterial infections. In our study, although we observed a significant difference in CRP levels between patients with and without infection, there was a great overlap in CRP values to

a level of 200 mg/L. This was also reflected by the low sensitivity of CRP level in myeloma patients with infection. Serum PCT level has been reported to be high in patients with severe bacterial infections, whereas in local infections and viral infections PCT concentrations are normal or do not exceed 1.5 ng/mL (6). The discriminatory value of PCT concentration in infections has been evaluated in different studies. In some of these studies higher threshold levels for PCT have been proposed to identify infection due to underlying diseases such as renal failure or after surgery (9,11). Eberhard et al. have shown that in patients with systemic autoimmune disease PCT level is useful for detection of systemic bacterial infection, and none of the patients without infection have increased PCT levels (12). Ugarte et al. have concluded that in critically ill patients PCT is not a better marker of infection than CRP and have suggested the concomitant use of both markers (17). The importance of leukocyte count while evaluating PCT level in infections has been emphasized and PCT has proven to be a very sensitive marker of sepsis in non-leukopenic patients (18). In our study, none of our patients had a leukocyte count of lower than $1.0 \times 10^9/L$. After the exclusion of non-infectious conditions that may contribute to elevated PCT levels, 0.5 ng/mL as a threshold for identifying systemic bacterial infections seems to be appropriate for myeloma patients. Local infections could not be properly diagnosed by PCT measurements by this methodology and a test with enhanced sensitivity is necessary to detect localized bacterial infections. These observations show the importance of clinical, radiological and microbiological findings, and PCT levels should be evaluated along with these parameters. PCT level seems to be a good parameter to identify systemic bacterial infections and is a useful marker of the severity of infection in patients with MM if co-existent non-infectious conditions that may contribute to PCT elevation are excluded by appropriate clinical and laboratory investigations.

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