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Original Article

Diagnostic and prognostic significance of procalcitonin and endotoxin in peritoneal dialysis-related peritonitis

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Abstract: Objective: To identify potential markers for detecting and monitoring inflammation in patients with peritoneal dialysis (PD)-related peritonitis, several inflammation markers, including procalcitonin (PCT), endotoxin and C-reactive protein (CRP), were evaluated in patients with PD-related peritonitis. Methods and Results: In this study, 50 cases of patients with PD-related peritonitis were recruited during 2011-2014, and 50 patients who had continuous ambulatory peritoneal dialysis but did not develop any infection were included as the control group. Serum samples were first collected to compare basal levels of PCT, endotoxin and CRP between PD-related peritonitis patients and control group. Then a prospective study was developed in PD-related peritonitis patients to compare the levels of PCT, endotoxin and CRP before and 2 weeks after antibiotic treatments. Meanwhile, PD fluid samples were collected for dialysate nucleated cell counts and the culture of effluent. Our results showed that levels of hemoglobin and serum albumin were significantly decreased while PCT, endotoxin, CRP and dialysate nucleated cell counts were elevated in patients with PD-related peritonitis, compared to the control group. After antibiotic treatment, levels of serum PCT, endotoxin and CRP were significantly decreased compared to basal levels in patients with PD-related peritonitis. Thirty-five cases (70%) of patients with PD-related peritonitis yielded positive results of bacterial culture, among which 21 cases (60% of 35 cases) were identified to be Gram-positive bacteria, 12 cases (34.26% of 35 cases) were Gram-negative bacteria and 2 cases (5.71%) were fungi. Importantly, in PD-related peritonitis patients, patients with positive bacterial culture showed greater levels of PCT and endotoxin, compared to patients with negative bacterial culture. Conclusions: Our results supported that serum PCT and endotoxins may be useful markers for monitoring inflammatory status and predicting responses to antibiotic treatment in PD-related peritonitis.

Keywords: Peritoneal dialysis, peritonitis, procalcitonin, endotoxins

Introduction

Peritoneal dialysis (PD)-related peritonitis is the leading complication of peritoneal dialysis, and isassociated with a significant risk of mortality. Monitoring of systemic inflammation provides valuable insights for clinical management of PD-related peritonitis [1], it is thus of importance to identify novel inflammatory markers in PD-related peritonitis patients.Previous studies showed that C-reactive protein (CRP) is correlated with the disease progression and outcomes of PD-related peritonitis, however, the increase of CRP could be secondary to the acute infection [2-4]. Several studies implied the importance of endotoxin in PD-related peritonitis [5, 6], but how endotoxin is changed and predicts the clinical outcomes of PD-related peritonitis remain unclear.

Meanwhile, recent studies showed that level of serum procalcitonin (PCT), the precursor of calcitonin, is correlated with infection and showed good sensitivity and specificity for the early diagnosis of systemic bacterial infection in PD patients [7-10], however, other studies reported that PCT is not changed in patients with long-term PD related peritonitis [11, 12] and may only serve as an adjunct to traditional inflammation markers in PD-related peritonitis patients [13]. These controversies indicated that more studies are needed to confirm the role of PCT in PD-related peritonitis patients. In this study, we analyzed levels of PCT, CRP and endotoxin in 50 patients with PD-related peritonitis before and after clinical management. Our results will provide valuable insights for identification of diagnostic and prognostic markers for PD-related peritonitis patients.

Table 1. Clinicopathologic characteristics of control and peritonitis patients

Items	Control group (N=50)	Peritonitis group (N=50)	*P* value
Age (y)	61.8±24.5	65.8±18.3	>0.05
Serum creatinine levels (μmol/L)	818±248	879±198	>0.05
Hemoglobin (g/L)	87.3±18.4	63.1±17.9[a]	<0.05
Plasma albumin (g/L)	35.1±19.2	26.6±18.6[a]	<0.05
KT/V	1.88±0.34	1.76±0.51	>0.05

a, P<0.05, compared to control group.

Table 2. Micro-organisms identified in samples from the peritonitis patients (%)

Micro-organisms	Number of isolates	Ratio (%)
Gram-positive	21	60.00
Staphylococcus epidermidis	5	14.29
Staphylococcus aureus	13	37.14
Staphylococcus haemolyticus	2	5.71
Enterococcus faecalis	1	2.86
Gram-negative	12	34.26
Acinetobacter Baumannii	1	2.86
Klebsiella	2	5.71
Escherichia coli	9	25.71
Fungi	2	5.71
Candida albicans	1	2.86
Aspergillus	1	2.86
Total	35	100.00

Materials and methods

Patients' characteristics

This prospective study was approved by the ethics committee of First People's Hospital of Hangzhou. All patients gave written informed consent. The patients' information was collected during June 2011-June 2014. Double-cuffed Tenckhoff catheter and dialysis fluid (Baxter, Shanghai, China) were used in PD for all the patients. The primary diseases which cause the patients to receive PD were: chronic glomerulonephritis (N=32), diabetic nephropathy (N=27), benign nephrosclerosis (N=28), lupus nephritis (N=2), systematic sclerosis (N=1), obstructive nephropathy (N=5) and gouty nephropathy (N=5).

Definition of peritonitis and antibiotic treatments

Diagnosis of peritonitis was made according to the guidelines of International Society for Peritoneal Dialysis: 1) Presences of cloudy effluent and/or abdomen pain and/or fever; 2) Effluent cell count white blood cells (WBCs) more than 100/μL with at least 50% polymorphonuclear neutrophilic cells; 3) Effluent culture is positive. Patients were diagnosed as peritonitis if two of the above three presentations were observed [1]. Combination treatments of 1st and 3rd generation cephalosporin were immediately initiated after diagnosis of peritonitis, and further optimized according to the antibiotics sensitivity of effluents.

Blood samples

Blood samples were collected before administration of antibiotics and two weeks after antibiotic treatment. Levels of WBC, PCT, CRP, endotoxin and albumin were measured. Effluents of PD-related peritonitis patients were collected for routine testes and cultured in aerobic and anaerobic environments respectively before the antibiotics treatment. In certain cases, effluents were collected one more time 2 weeks after the antibiotics treatment to confirm the results. CRP was quantified with turbidimetric inhibition immunoassay (OLYMPUS-2700, automatic clinical chemistry analyzer). Endotoxin was analyzed with Kinetic turbidimetric assay. PCT was measured with Immune chemiluminescence (Lumitest kit, BRAHMS Diagnostic, Berlin, Germany).

Statistical analysis

All the data were analyzed with SPSS17.0 and expressed as mean ± SEM. Unpaired t test was used for comparison between two groups. P<0.05 was considered statistically significant.

Results

Anemia and hypoproteinemia in patients with PD-related peritonitis

Fifty patients with PD-related peritonitis, including 26 male patients and 24 female patients, were recruited for this study. Patients were 61.8±24.5 years old and had an average dialysis term of 58.23±28.19 months. Meanwhile, 50 age and sex-matched patients who were receiving PD but did not have any history of

Table 3. Levels of serum PCT and endotoxin in control and peritonitis patients

Items	Control Group (N=50)	Patients with PD-related peritonitis	
		Before treatment (N=50)	2-week after treatment (N=50)
Serum PCT (ng/mL)	0.28±0.12	9.78±5.58[b]	0.29±0.11[c]
Endotoxin (ng/L)	4.56±3.81	23.45±14.66[b]	5.16±2.97[c]
Effluent WBCs ($\times 10^6$/L)	12.23±8.20	1921.32±456.71[b]	18.12±7.42[c]
Serum CRP (mg/L)	5.27±2.78	141.1±60.18[b]	7.72±3.86[c]
Blood WBC ($\times 10^9$/L)	6.08±2.59	17.44±7.81[b]	6.27±3.38[c]

b, compared with control group, P<0.01; c, compared with levels before treatment, P<0.01.

Table 4. Levels of serum PCT, endotoxin in peritonitis patients with different bacterial culture results

Items	Positive bacterial cultures (N=35)	Negative bacterial cultures (N=15)
Serum PCT (ng/mL)	12.24±6.12[d]	1.98±0.58
Endotoxin (ng/l)	31.45±12.52[d]	10.41±4.57
Effluent WBCs ($\times 10^6$/L)	2313.00±636.34	2289.00±598.27
Serum CRP (mg/L)	155.00±40.82	151.00±54.63
Serum WBC ($\times 10^9$/L)	18.76±8.41	17.71±9.28

d, compared with PD-related peritonitis patients without infection P<0.01.

peritonitis were recruited as the control group (average age: 65.8±18.3 years old, 25 male and 25 female patients). Both PD-related peritonitis patients and control group patients showed equal adequacy of dialysis (KT/V, dialyzer clearance/min) and serum creatinine levels; however, the patients with PD-related peritonitis showed significantly decreased levels of blood hemoglobin and plasma albumin (**Table 1**).

Types of Micro-Organism infection in patients with PD-related peritonitis

PD effluents yielded positive results in 35 patients (70% of 50 patients) (**Table 2**). Twenty-one (60% of 35 patients) cases were Gram-positive strains which showed resistance to penicillin and ampicillin but showed 100% sensitivity to vancomycin and imipenem-cilastatin sodium. Twelve (34.26%) were identified to be Gram-negative strains, which showed high sensitivity to ampicillin/sulbactam and 100% sensitivity to meropenem and imipenem-cilastatin sodium. Two cases (5.71%) were fungi infection and showed sensitivity to fluconazole and itraconagole.

Levels of PCT, endotoxin and CRP and clinical outcomes of the patients

Compared with control group, PD-related peritonitis patients showed significantly elevated serum PCT (9.78±5.58 ng/mL vs. 0.28±0.12 ng/mL), endotoxin, CRP, levels of blood and effluent WBCs (**Table 3**). After 2-week antibiotics treatment, levels of serum PCT CRP, and blood WBCs were significantly decreased, compared to levels before treatment in PD-related peritonitis patients. Antibiotic treatments were efficient to relive clinical symptoms in 49 patients who showed normal levels of serum PCT, endotoxin, CRP and blood WBCs after treatment. Two patients with fungi infection discontinued PD and switched to hemodialysis, one of whom died of hypotension combined with multi-organ failure. One patient with acinetobacter baumannii infection showed resistance to several antibiotics initially, but showed improved symptoms after administration of antibiotics treatment and hemodialysis.

Levels of PCT and endotoxin in patients with PD-related peritonitis and positive/negative bacterial cultures

Compared to patients withPD-related peritonitis and negative bacterial cultures, levels of PCT and endotoxin were significantly elevated in patients with PD-related peritonitis and positive bacterial cultures, while CRP, blood and effluent WBCs did not show differences between two groups. Importantly, levels of PCT in all the patient with PD-related peritonitis and positive bacterial cultures (N=35) were greater than 2 ng/mL (**Table 4**).

Discussion

As the most common complication of PD and the major reason of PD discontinuation, PD-related peritonitis leads to severe septic shock

and death. Etiological diagnosis is time consuming and could not provide any information for severity of infection, it is thus of importance to identify an early inflammatory marker for patients with PD-related peritonitis. PCT has been widely used in sepsis patients to predict the presences of bacterial infection and evaluate the progression of treatment. Because serum PCT is not elevated in the presence of virus, parasites or mycoplasma infection, it has been considered as an ideal marker to distinguish bacterial infection and virus infection[14]. Elevation of PCT has been observed in diffuse general peritonitis and is related with increased risk of septic shock and death [15]. Furthermore, PCT have shown both high specify and sensitivity in evaluating severity and prognosis of bacterial infection [16]. Yang SK et.al. reported that PCT showed better diagnostic property than CRP in patients with bacterial peritonitis (sensitivity: 0.83, 95% CI: 0.76-0.89; Specify: 0.92, 95% CI: 0.87-0.96) [17]. However, another study reported that basal level of PCT is changed in patients with chronic kidney diseases due to individual heterogeneity and limitation of quantification methods, suggesting that clinical performances need to be combined with PCT quantification in diagnosis [18].

We observed that serum albumin and hemoglobin were significantly decreased in patients with PD-related peritonitis, suggesting the presence of hypoalbuminemia and anemia, this may be caused by theincreasedperitoneal protein leakage in peritonitis [19]. Meanwhile, we identified that 35 cases (70% of 50 patients) yielded positive results of bacterial cultures, among which 21 cases were identified to be Gram-positive strains, including Staphylococcus epidermidis and Staphylococcus aureu etc. This result indicates that it is of great importance to educate the PD patients regarding knowledge of personal hygiene, house cleanliness as well as the basic but necessary prevention to nasal cavity-carried Staphylococcus.

Our results showed that serum PCT, endotoxin, CRP, blood WBCs and effluent WBCs were significantly elevated in patients with PD-related peritonitis compared to control group. Endotoxins are normally released by the outer membrane of gram-negative bacteria, and are capable of causing proinflammatory response and endotoxemia. Importantly, we noticed that endotoxin levels were significantly increased in patients with Gram-positive bacterial infection, which may cause increased gastrointestinal permeability and gastrointestinal symptoms patients which we observed in these patients (data not shown).

Our study demonstrated that serum PCT and endotoxin levels were significantly elevated in patients with PD-related peritonitis and positive effluent culture results, importantly, after antibiotic treatments, both PCT and endotoxin were significantly decreased to levels which is close to the control group, suggesting that serum PCT level can potentially act as a clinical marker to evaluate the infection severity and effects of anti-biotics in peritonitis patient. Our results showed that 35 patients had a PCT level at more than 2 ng/mL, which is at the equal level in sepsis patients, indicating that these patients had an increased risk in developing sepsis and effective antibiotics should be administrated as early as possible. Serum PCT was suggested to distinguish Gram-negative, Gram-positive and Fungi infection, which is supported by greater levels of serum PCT in Gram-negative bacteria infection patients compared to gram-positive bacterial infection [20]. Meanwhile, our results suggested that PCT and endotoxin may serve as better predictors of positive dialysate fluid cultures than serum CRP or peripheral WBC counts. One limitation of this study is that this study has a small patient size and was performed in a single center,so future studies will need to confirm the role of PCT and endotoxins in large patient population in multiple centers.

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Disclosure of conflict of interest

None.

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References

[1] Li PK, Szeto CC, Piraino B, Bernardini J, Figueiredo AE, Gupta A, Johnson DW, Kuijper EJ, Lye WC, Salzer W, Schaefer F and Struijk DG. Peritoneal dialysis-related infections recommendations: 2010 update. Perit DialInt 2010; 30: 393-423.

[2] Zalunardo NY, Rose CL, Ma IW and Altmann P. Higher serum C-reactive protein predicts short and long-term outcomes in peritoneal dialysis-associated peritonitis. Kidney Int 2007; 71: 687-692.

[3] Lam MF, Leung JC, Lo WK, Tam S, Chong MC, Lui SL, Tse KC, Chan TM and Lai KN. Hyperleptinaemia and chronic inflammation after peritonitis predicts poor nutritional status and mortality in patients on peritoneal dialysis. Nephrol Dial Transplant 2007; 22: 1445-1450.

[4] Lai KN, Lai KB, Lam CW, Chan TM, Li FK and Leung JC. Changes of cytokine profiles during peritonitis in patients on continuous ambulatory peritoneal dialysis. Am J Kidney Dis 2000; 35: 644-652.

[5] Ishizaki M, Oikawa K and Miyashita E. Usefulness of the endotoxin test for assessing CAPD peritonitis by gram-negative organisms. Adv Perit Dial 1996; 12: 199-202.

[6] Cho Y, Hawley CM and Johnson DW. Clinical causes of inflammation in peritoneal dialysis patients. Int J Nephrol 2014; 2014: 909373.

[7] Karlsson S, Heikkinen M, Pettila V, Alila S, Vaisanen S, Pulkki K, Kolho E and Ruokonen E. Predictive value of procalcitonin decrease in patients with severe sepsis: a prospective observational study. Crit Care 2010; 14: R205.

[8] Herget-Rosenthal S, Marggraf G, Pietruck F, Husing J, Strupat M, Philipp T and Kribben A. Procalcitonin for accurate detection of infection in haemodialysis. Nephrol Dial Transpl 2001; 16: 975-979.

[9] Herget-Rosenthal S, Klein T, Marggraf G, Hirsch T, Jakob HG, Philipp T and Kribben A. Modulation and source of procalcitonin in reduced renal function and renal replacement therapy. Scand J Immunol 2005; 61: 180-186.

[10] Dumea R, Siriopol D, Hogas S, Mititiuc I and Covic A. Procalcitonin: diagnostic value in systemic infections in chronic kidney disease or renal transplant patients. Int Urol Nephrol 2014; 46: 461-468.

[11] van Esch S, Krediet RT and Struijk DG. Prognostic factors for peritonitis outcome. Contrib Nephrol 2012; 178: 264-270.

[12] Lam MF, Leung JC, Lam CW, Tse KC, Lo WK, Lui SL, Chan TM, Tam S and Lai KN. Procalcitonin fails to differentiate inflammatory status or predict long-term outcomes in peritoneal dialysis-associated peritonitis. Perit DialInt 2008; 28: 377-384.

[13] Guz G, Colak B, Hizel K, Reis KA, Erten Y, Bali M and Sindel S. Procalcitonin and conventional markers of inflammation in peritoneal dialysis patients and peritonitis. Perit DialInt 2006; 26: 240-248.

[14] Simon L, Gauvin F, Amre DK, Saint-Louis P and Lacroix J. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. ClinInfect Dis 2004; 39: 206-217.

[15] Pupelis G, Drozdova N, Mukans M and Malbrain ML. Serum procalcitonin is a sensitive marker for septic shock and mortality in secondary peritonitis. AnaesthesiolIntensive Ther 2014; 46: 262-273.

[16] Ruiz-Alvarez MJ, Garcia-Valdecasas S, De Pablo R, Sanchez Garcia M, Coca C, Groeneveld TW, Roos A, Daha MR and Arribas I. Diagnostic efficacy and prognostic value of serum procalcitonin concentration in patients with suspected sepsis. J Intensive Care Med 2009; 24: 63-71.

[17] Yang SK, Xiao L, Zhang H, Xu XX, Song PA, Liu FY and Sun L. Significance of serum procalcitonin as biomarker for detection of bacterial peritonitis: a systematic review and meta-analysis. BMC Infect Dis 2014; 14: 452.

[18] Grace E and Turner RM. Use of procalcitonin in patients with various degrees of chronic kidney disease including renal replacement therapy. ClinInfect Dis 2014; 59: 1761-1767.

[19] Dong J, Chen Y, Luo S, Xu R and Xu Y. Peritoneal protein leakage, systemic inflammation, and peritonitis risk in patients on peritoneal dialysis. Perit DialInt 2013; 33: 273-279.

[20] Brodska H, Malickova K, Adamkova V, Benakova H, Stastna MM and Zima T. Significantly higher procalcitonin levels could differentiate Gram-negative sepsis from Gram-positive and fungal sepsis. Clin Exp Med 2013; 13: 165-170.