Hyperleptinaemia and chronic inflammation after peritonitis predicts poor nutritional status and mortality in patients on peritoneal dialysis

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Abstract

Background. The serum leptin level is elevated in patients undergoing peritoneal dialysis (PD) and associated with a loss of lean body mass. The nutritional status of PD patients may further be worsened following peritonitis. We investigated the association between hyperleptinaemia, inflammation and malnourishment in PD-related peritonitis.

Methods. We conducted a prospective study on PD patients who developed peritonitis. Blood samples were obtained as baseline (D0) before the onset of peritonitis, and once peritonitis developed, leptin, adiponectin (ADPN) and other inflammatory markers were collected, on day 1 (D1), day 7 (D7) and day 42 (D42) of peritonitis. Patients were followed-up for any censor event or 1 year after peritonitis.

Results. Forty-two patients with a mean age of 62.9 ± 13.2 years were recruited. Fourteen (33.3%) were diabetic. The serum leptin levels increased significantly from baseline to day 1 and 7, but fell back to the premorbid state at day 42. In contrast, the ADPN level decreased from a baseline value of 15.60 ± 10.4 μg/ml to 13.01 ± 8.1 μg/ml on day 1 (P = 0.01) but rose to 14.39 ± 8.9 μg/ml on day 7 (P = 0.28) and 13.87 ± 7.9 μg/ml on day 42 (P = 0.21). High-sensitivity C-reactive protein (hs-CRP) increased significantly from baseline to day 1, 7 and even at day 42. The lean body mass (LBM) and nutritional markers decreased significantly after peritonitis. For patients with high hs-CRP (>3.0 mg/l) at day 42, there was a higher mortality rate than for those with lower hs-CRP (<3.0 mg/l, P = 0.02), even if they were in clinical remission of peritonitis.

Conclusions. Our study confirmed an increase in serum leptin during acute peritonitis and a prolonged course of systemic inflammation after apparent clinical remission of peritonitis. These factors related to the persistent chronic inflammation may contribute to the development of malnourishment and poor survival rate.

Keywords: hyperleptinaemia; inflammation; malnutrition; peritonitis

Introduction

Chronic inflammation remains an important morbidity factor in patients with end-stage renal disease (ESRD). High mortality and morbidity relating to chronic inflammation are often due to associated cardiovascular events [1,2]. The inflammatory response is evidenced by elevated acute phase proteins, such as C-reactive protein (CRP). Studies examining a single cross-sectional measurement of CRP showed that it is a powerful indicator of all mortality factors as well as cardiovascular death in patients on dialysis treatment [2]. The possible link between the CRP level and atherosclerosis suggests CRP acts not only as an associated inflammatory factor with proatherogenic cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) but also as a direct mediator of vascular disease [3]. On the contrary, adiponectin (ADPN) was found to be a negative predictor of cardiovascular morbidity in patients with ESRD. There was a 3% risk reduction for each 1 μg/ml increase in plasma ADPN levels [4].

Protein-energy malnutrition is also commonly found in dialysis patients with inflammation. Increased release or activation of inflammatory cytokines suppresses appetite and causes muscle proteolysis and hypoalbuminaemia [5]. Stenvinkel et al. [6] first suggested that malnutrition following initial inflammation was due to hyperleptinaemia as peritoneal dialysis (PD) patients losing lean body mass (LBM) had higher initial CRP levels and an elevated leptin level when compared with patients who gained LBM during PD treatment.
The nutritional status of dialysis patients may even further deteriorate when patients have active infection. Dialysis patients with sepsis, independent of their dialysis mode, with sepsis were associated with higher annual mortality rates than the general population, even after stratification for age, race and diabetes mellitus (DM) [7]. The processes of active inflammation secondary to infection probably cause loss of muscle mass and decreases in albumin level, resulting in poor nutritional status. Our previous study showed that cytokines in peritoneal effluent such as IL-1, IL-6 and transforming growth factor-β (TGF-β) remained higher than those in a non-infective effluent throughout a 6-week post-peritonitis period despite clinical remission [8]. This subclinical inflammation may have an adverse effect on patient outcome.

In this study, we hypothesize that there is a strong association between infection and systemic inflammation via hyperleptinaemia and ADPN which results in poor nutritional status and subsequent higher mortality following PD-related peritonitis. We prospectively examine the nutritional status and clinical outcome of long-term PD patients after an episode of peritonitis.

**Subjects and methods**

This study adhered to the Declaration of Helsinki and approval by the ethics committee of our institutions, and all patients gave written informed consent. We recruited patients on continuous peritoneal dialysis (CPD) who developed acute PD-related peritonitis from a university-affiliated dialysis centre during the period between October 2003 and March 2005. Peritonitis was defined as turbid PD fluid with a total white cell count greater than 100/mm³ in which more than 50% are polymorphonuclear cells. Relapsing peritonitis, defined as development of peritonitis within 4 weeks of completion of antibiotics for a prior episode with the same organism or one sterile episode, was counted as the same peritonitis episode. Exclusion criteria included age younger than 18 years, concurrent malignancy and concurrent severe or chronic medical illness. Patients on intermittent PD or having started PD treatment <1 month before were also excluded.

Baseline data including age, sex, body weight, underlying renal disease, PD system and presence of comorbid diseases such as DM, cardiovascular disease (CVD), hepatitis B and hepatitis C infection were recorded. CVD was defined as presence of ischaemic heart disease, congestive heart failure, cerebrovascular disease or peripheral vascular disease. The body mass index (BMI) was computed from the formula $\text{BMI} = \frac{\text{weight (kg)}}{\text{height (m)}^2}$. The lowest adequacy dose, residual renal function, and nutritional markers before the peritonitis episode were recorded. The normalized protein nitrogen appearance rate (nPNA), according to Randerson, Chapman and Farrell, and the LBM according to Keshaviah based on creatinine kinetics were estimated from the 24-h dialysate and urine results of the latest adequacy assessment before peritonitis. Blood samples of all PD patients were also obtained before onset of peritonitis and were taken as baseline (D0) for inflammatory and nutritional markers.

**Inflammatory markers**

Patients were recruited for study when they developed peritonitis. Blood samples were collected for measurement of inflammation markers, namely; IL-6, TNF-α and high-sensitivity C-reactive protein (hs-CRP) on day 1, day 7 and day 42 after the onset of PD-related peritonitis. Serum IL-6 was determined by an ELISA system from Bender MedSystems (Vienna, Austria) with a detection limit of 1.6 pg/ml. The serum concentration of TNF-α was measured by commercial ELISA (R & D System, Minneapolis, MN, USA) with detection limits of 0.5 pg/ml. hs-CRP was measured by a particle-enhanced immunoturbidimetric assay (Roche Diagnostics, GmbH, Mannheim, Germany) using anti-CRP mouse monoclonal antibodies coupled to latex microparticles. The assay was standardized against CRM 470 Reference Preparation for Proteins in Human Serum (RPPHS) and has a detection limit of 0.1 mg/l.

**Nutritional parameters and serum markers**

Blood samples for adiponectin, leptin, albumin and prealbumin levels were collected on day 1, day 7 and day 42 after the onset of peritonitis. Subjective global assessment (SGA) for nutritional status was performed on day 1 and day 42 after onset of peritonitis. The serum albumin and lipid profile were determined by standard methods. The serum concentration of ADPN was measured by commercial ELISA kit (Biosource, Camarillo, CA, USA) with detection limits of 0.25 ng/ml for adiponectin. Leptin was determined by a commercial ELISA kit (Biosource, Camarillo, CA, USA) with a detection limit of 20 pg/ml. Prealbumin was measured by a rate-nephelometric assay automated on the IMMAGE Immunochemistry System (Beckman-Coulter, Fullerton, CA, USA) with a detection limit of 0.01 g/l.

**Treatment of peritonitis**

Treatment of peritonitis based on our standard treatment of first-line antibiotics (cefazolin and tobramycin). Patients were switched to second-line antibiotics if the peritonitis was refractory to first-line treatment after 5 days of appropriate antibiotics. There was no routine treatment for nutritional build-up for patients suffering from peritonitis, other than short-term supplementary parenteral nutrition was given to those who had severe anorexia from the peritonitis.

**Clinical events**

Clinical parameters, morbidity, mortality and nutrition status were documented. Study endpoints included death, transfer to permanent haemodialysis (HD) and withdrawal from the study. Events for ‘patient survival’ included death and for ‘technique survival’ included permanent haemodialysis. The patients were followed prospectively for at least 1 year after the episode of peritonitis and the censor date was 1 year after recruitment of the last patient in March 2005.
Statistical analysis

All data were expressed as mean and SD unless otherwise specified. Statistical difference was analysed with Student’s t-test, chi-square test and Mann–Whitney (non-parametric) test as appropriate. Survival curves were analysed by the Kaplan–Meier method and compared by log-rank test. A P-value of 0.05 was taken as the level of statistical significance. Statistical calculation was performed with the SPSS 11.0 software.

Results

Patient characteristics and baseline PD prescription

Forty-two patients (20 female) with a mean age of 62.9 ± 13.2 (SD) years were recruited. Fourteen patients (33.3%) were diabetic and 13 (31.0%) had symptomatic cardiovascular disease. The body weight and duration of PD at the time of peritonitis were 59.8 ± 11.5 kg and 4.35 ± 4.2 years, respectively. Patients were then followed-up prospectively for a median of 17 months (range, 3–30) after the episodes of peritonitis. The demographic, adequacy and biochemical parameters at the time of peritonitis are shown in Table 1.

Responsiveness of peritonitis

Thirty (71.4%) and two (4.8%) patients responded to first-line and second-line intraperitoneal antibiotic treatment, respectively. Three (7.1%) had a relapse of peritonitis but subsequently responded to another course of intraperitoneal antibiotics. Seven patients (16.7%) required catheter removal because of refractory peritonitis. Two subsequently failed reinsertion of the peritoneal catheter and had to switch to long-term HD.

Mortality

No patient died within 3 months of the related peritonitis episode but 14 (33.3%) were dead by the censor date and four (9.5%) received kidney transplantation. Eight of 14 deaths (57.1%) were due to cardiovascular disease, two due to sepsis and four due to other causes. The 1- and 2-year patient survival rates were 79.3 and 64.0%, respectively. For patients without evidence of a relapse of peritonitis, the survival rate of 27 patients with high hs-CRP (>3.0 mg/l) at day 42 were lower than those 12 patients with low hs-CRP (<3.0 mg/l) (P = 0.02, Figure 1). For patients still remaining on PD after peritonitis with no relapse of peritonitis, the survival rate of 22 patients with high hs-CRP (>3.0 mg/l) at day 42 was also lower than those 10 patients with low hs-CRP (<3.0 mg/l) (P = 0.02).

IL-6, TNF-α, and hs-CRP levels following peritonitis

These cytokines or protein increased significantly on day 1 when compared with baseline levels. The baseline blood samples were obtained at the time of recruiting patients prospectively for peritonitis study. The time interval between the baseline blood sampling and the onset of peritonitis episode was 9.7 ± 5.5 months. IL-6 remained elevated at day 1, 7 and 42 (Table 2). The hs-CRP, illustrated in Figure 2, increased significantly from 8.83 ± 13.60 mg/l at baseline to 128.5 ± 85.5 mg/l on day 1 (P < 0.001), 49.3 ± 75.8 mg/l on day 7 (P < 0.001), and 40.9 ± 80.0 mg/l on day 42 (P < 0.001). The hs-CRP levels correlated with IL-6 levels at baseline (R = 0.321, P = 0.049), day 1 (R = 0.449, P = 0.003), day 7 (R = 0.637, P < 0.001) and day 42 (R = 0.631, P < 0.001) after the onset of peritonitis. In contrast, TNF-α rose transiently on day 1 (1.02 ± 0.33 pg/ml, P = 0.002) but fell to baseline levels on day 7 (0.86 ± 0.19 pg/ml, P = 0.43) and day 42 (0.79 ± 0.22 pg/ml, P = 0.46). By excluding patients with a relapse of peritonitis and refractory peritonitis required peritoneal catheter removal, 22 (68.8%) out of 32 patients were found to have hs-CRP >3.0 mg/l at day 42 despite clinical remission of peritonitis.

Table 1. Demographic and adequacy data of patients

<table>
<thead>
<tr>
<th></th>
<th>Patients, n = 42</th>
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<tbody>
<tr>
<td>CAPD (%)</td>
<td>41 (97.6)</td>
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<tr>
<td>CCPD (%)</td>
<td>1 (2.4)</td>
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<tr>
<td>Residual renal function ml/min/1.73 m²</td>
<td>0.0 (0.0–6.1)</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>23.6 ± 3.8</td>
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<tr>
<td>Haemoglobin g/dl</td>
<td>9.0 ± 1.4</td>
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<tr>
<td>Lymphocyte count x10⁶/l</td>
<td>1.4 ± 0.5</td>
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<tr>
<td>Total Kt/V</td>
<td>1.91 ± 0.32</td>
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<tr>
<td>Dialysate Kt/V</td>
<td>1.64 ± 0.32</td>
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<tr>
<td>Total CrCl (l/wk/1.73 m²)</td>
<td>66.2 ± 24.0</td>
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<tr>
<td>Peritoneal CrCl (l/wk/1.73 m²)</td>
<td>46.7 ± 10.4</td>
</tr>
<tr>
<td>nPCR (g/kg/day)</td>
<td>1.01 ± 0.24</td>
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<td>D/P Cr at 4 h</td>
<td>0.71 ± 0.1</td>
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Fig. 1. Comparison of cumulative survival of patients according to hs-CRP levels at day 42. The difference in survival rate between patients with high hs-CRP at day 42 (>3.0 mg/l) and patients with low hs-CRP (<3.0 mg/l) was statistically significant (P = 0.02).
**Table 2.** Serial changes of inflammatory cytokines and nutritional parameters

<table>
<thead>
<tr>
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<th>Baseline</th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 42</th>
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<tbody>
<tr>
<td>Hs-CRP (mg/L)</td>
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<td></td>
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<tr>
<td>Prealbumin (g/L)</td>
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<tr>
<td>BW (kg)</td>
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<tr>
<td>Albumin (g/l)</td>
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<td>T. Cholesterol (mmol/l)</td>
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<tr>
<td>HCO₃ (mmol/l)</td>
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<tr>
<td>Leptin (ng/ml)</td>
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<tr>
<td>Adiponectin (μg/ml)</td>
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<tr>
<td>TNF-α (pg/ml)</td>
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<td>IL-6 (pg/ml)</td>
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</table>

**Nutritional parameters**

The leptin level initially increased from 10.09 ± 4.7 ng/ml (baseline) to 12.29 ± 4.8 ng/ml (day 1, *P < 0.001) and 11.19 ± 5.1 ng/ml (day 7, *P = 0.02) but finally fell to 10.43 ± 5.4 ng/ml (day 42, *P = 0.7). However, the gender difference in leptin levels was found to be not significant at all time points (female vs male, baseline: 11.51 ± 3.9 vs 8.68 ± 5.0 ng/ml, *P = 0.06; day 1: 12.45 ± 4.2 vs 12.14 ± 5.4 ng/ml, *P = 0.8, day 7: 11.83 ± 4.4 vs 10.60 ± 5.7 ng/ml, *P = 0.4, day 42: 11.70 ± 5.0 vs 9.33 ± 5.6 ng/ml, *P = 0.2). In contrast, the ADPN level decreased from a baseline value of 15.60 ± 10.4 μg/ml to 13.01 ± 8.1 μg/ml at day 1 (*P = 0.01) but rose to 14.39 ± 8.9 μg/ml at day 7 (*P = 0.28) and 13.87 ± 7.9 μg/ml at day 42 (*P = 0.21). The ADPN and leptin levels correlated inversely at day 1 (*R = −0.448, *P = 0.003), day 7 (*R = −0.420, *P = 0.006) and day 42 (*R = −0.343, *P = 0.028), but not at the baseline (*R = −0.304, *P = 0.064).

Serum albumin dropped significantly at day 1 and day 7, of PD-related peritonitis, when compared with values at non-infective periods. The serum albumin at day 42 also showed a trend of lower levels compared with the baseline. Prealbumin behaved similarly to serum albumin, falling from 0.39 ± 0.10 g/l at baseline to 0.25 ± 0.08 g/l at day 1 (*P < 0.001). However, the prealbumin level at day 42 still remained lower than the baseline value (0.32 ± 0.11 g/l, *P < 0.001) despite the patient’s clinical recovery from peritonitis (Figure 2).

The body weight did not differ from the baseline throughout the 6 weeks of peritonitis. However, the LBM fell significantly in 32 patients who responded to intraperitoneal antibiotic treatment (33.0 ± 10.8 kg vs 29.6 ± 8.1 kg, *P < 0.001). SGA of patients also suggested a poorer nutritional status after peritonitis (day 1, median: 5, range: 3–7 vs day 42, median: 4, range: 2–6, *P < 0.001).

**Discussion**

In this study, we showed that even with apparent clinical remission of PD-related peritonitis, dialysis patients, after an episode of peritonitis, may still be affected by prolonged systemic chronic inflammation. The significantly prolonged inflammation contributed to a poorer nutritional status and higher mortality. The finding is consistent with our previous study that the level of cytokines such as IL-1, IL-6 and TGF-β, in the peritoneal effluent remained higher than that in non-infective effluent throughout the 6-week post-peritonitis period, despite clinical remission [8]. It was true that the serum levels of many other inflammatory markers were decreased back to baseline; however, the hs-CRP remained significantly higher by day 42.

CRP as a marker of inflammation well predicts both all-cause and cardiovascular mortality in ESRD patients treated with HD [2] or PD [9]. Individual races have different prevalence rates of elevated CRP [10]. Different dialysis treatment modes may induce variable inflammatory response. In PD treatment, the main causes for acute inflammation are PD-related peritonitis and exit site infection [11]. Here, we found that our PD patients had prolonged elevation of hs-CRP suggestive of chronic inflammation even 6 weeks after apparent remission of the peritonitis. In the subsequent follow-up over a median period of 17 months, one-third of the patients died, in which cardiovascular events were responsible in 57% of the cases. Our findings are in accordance with recent reports that CRP is
independently predictive of higher cardiovascular mortality [1,2]. In addition to a marker of inflammation, CRP can act as a direct mediator of atherosclerosis [3]. It induces the synthesis and release of vascular adhesion molecule-1, E-selectin, and monocyte chemotactrant protein-1 in endothelial cells [12]. Therefore, the prolonged inflammation likely potentiates atherogenesis and increases the risk of cardiovascular events. Amongst various proinflammatory markers, the hs-CRP is most helpful as a clinical parameter for monitoring the inflammation in patients with peritonitis, based on its strong correlation with IL-6, and persistency in serum level when compared with other inflammatory markers such as TNF-α, which only increases transiently on day 1.

Previous study revealed plasma ADPN levels were lower among patients who experienced new cardiovascular events than among event-free patients [4]. As in our patients after peritonitis, the ADPN levels remained low on day 42 (13.87 ± 7.9 μg/ml) when compared with the baseline (15.60 ± 10.4 μg/ml), though the difference did not reach statistical significance. This may partly explain why our patients had more frequent cardiovascular events.

Other than persistent low-grade inflammation, as suggested by elevated hs-CRP, subclinical malnutrition may be another factor for the high mortality in our study. Chronic inflammation with atherosclerosis is closely related to malnutrition, forming the malnutrition–inflammation–atherosclerosis (MIA) syndrome [13]. Anorexia is part of the response to any sepsis and may contribute to malnourishment in post-peritonitis period. As shown in our study, the IL-6 levels remained significantly increased on day 1 and day 7 and correlated with hs-CRP levels at all time points. IL-6, as well as other cytokines, may regulate the food intake via neural and humoral pathways [14]. Hence, the presence of chronic inflammation, despite apparent clinical remission of peritonitis, was likely to contribute to the poorer nutritional status in our patients as reflected in the loss of LBM, poor SGA and low prealbumin levels.

The underlying mechanism for malnourishment remains to be not fully understood and is likely to be multifactorial. In stable PD patients, protein loss in the dialysate and the feeling of fullness due to dialysis fluid in abdomen contribute to a malnourished status. Cheung et al. [15] recently showed that uraemia-associated cachexia is caused by leptin signalling through the hypothalamic melanocortin receptor in a uraemia mice model that exhibited a syndrome of cachexia characterized by decreased food intake, increased metabolic rate and loss of LBM. The animal data support the clinical observation that patients newly introduced to PD, experiencing loss of LBM, are also found to have hyperleptinaemia and high CRP levels [5]. The hyperleptinaemia may be secondary to chronic hyperinsulinaemia in response to high dialysis glucose content [16] or other inflammation responses [17]. Increased leptin expression following bacterial peritonitis has been demonstrated in a mouse model [18]. Hyperleptinaemia has been shown to produce anorexia and loss of LBM without inducing an acute phase response or protein wasting [19]. As we found no correlation between leptin and hs-CRP levels at different time points, it is plausible that hyperleptinaemia may act as an independent contributory factor in the development of malnutrition in patients with peritonitis. In addition to the effect on nutritional status, leptin is also associated with several markers of activated coagulation (fibrinogen, D-dimer, VWF and factor VIII), that may be associated with a higher incidence of cardiovascular events [20]. Our proposal of a hypothetical mechanism of a link between chronic inflammation following peritonitis and malnutrition is shown in Figure 3.

So far, there is no valid and specific anti-inflammatory measure for dialysis patients with chronic inflammation. Nonetheless, we recommend that patients, complicated by peritonitis, should be monitored for CRP at day 42 even with apparent clinical remission. In our study, 22 (68.8%) out of 32 patients were found to have hs-CRP > 3.0 mg/l, which conforms to the 2003 American Heart Association/CDC statement on hs-CRP and cardiovascular risk. It suggested that PD patients complicated by peritonitis may associate with high risk of chronic inflammation and atherosclerosis. Therefore, for patients with high CRP, a careful search for infectious processes and preventive measures for CVD are needed. There is also no established guideline for nutritional replenishment for PD patients complicated by peritonitis. It is logical to improve the
nutritional intake in these PD patients by providing frequent but small meals, draining the dialysate just before meals, and considering total parental nutritional supplementation or enteral tube feedings if the first two manoeuvres fail.

Our study has some limitations. First, we could not isolate individual factors contributory to the malnourishment in our patients. As the nutritional status of our patients was related to the severity of the peritonitis, the appetite or degree of anorexia was only assessed by the SGA. Furthermore, we have to emphasize that the creatinine kinetics is not a reliable method for measuring LBM. Nevertheless, in the setting of peritonitis, other nutritional measurements may also have varying degrees of inaccuracy. The longitudinal monitoring of the changes in nutritional markers may be more helpful in the clinical management of PD patients complicated by peritonitis.

In conclusion, PD patients, complicated by peritonitis, require additional attention for nutritional support and screening for persistent inflammation. Aggressive intervention for nutritional support and anti-inflammatory therapy are warranted such that the vicious circle like MIA syndrome can be abolished.

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Conflict of interest statement. None declared.

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