To clarify the influence of neutral dialysate (ND) on peritonitis, we examined changes in peritoneal permeability and in various markers of the coagulation and fibrinolytic system in effluent and the correlations between peritoneal permeability and those markers in peritoneal dialysis (PD) patients using ND. We evaluated 14 patients (8 men, 6 women; mean age: 58.6 ± 12.0 years) who started PD using ND. The peritoneal equilibration test (PET) was performed to assess dialysate-to-plasma ratio for creatinine (D/P Cr) as peritoneal permeability. Coagulation markers [thrombin–antithrombin complex, fibrin monomer (FM), prothrombin fragment 1+2 (F1+2)] and fibrinolytic markers (fibrin degradation products, D-dimer) in effluent were also measured. At 2 years, FM in effluent was significantly lower ($p = 0.006$). The other markers and the D/P Cr did not change significantly.

At the initiation of PD and at 2 years, D/P Cr was significantly correlated with F1+2 ($r = 0.70$ and $0.76$ respectively, $p < 0.01$). Furthermore, multiple regression analysis showed that only F1+2 was correlated with D/P Cr at 2 years ($r = 0.79$, $p = 0.004$). These results suggest that ND has little influence on coagulation and fibrinolytic markers in effluent. In addition, F1+2 is a useful marker for peritoneal permeability in PD patients using ND.

**Key words**
Peritoneal permeability, prothrombin fragment 1+2, F1+2

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the correlations between peritoneal permeability and those markers in PD patients using ND.

**Methods**

We evaluated 14 patients (8 men, 6 women) who, as their first renal replacement therapy, started PD using ND from January 2008. Their mean age was 58.6 ± 12.0 years (range: 38 – 71 years). The causes of end-stage renal disease were diabetic nephropathy in 7 patients, chronic glomerulonephritis in 4 patients, nephrosclerosis in 2 patients, and polycystic kidney disease in 1 patient. Mean body mass index in this group was 23.4 ± 3.9. Peritonitis was observed in 2 patients one time each. We excluded patients who received drugs that affect the coagulation and fibrinolytic system.

Incremental PD, defined as dialysis at the minimum dose sufficient to maintain a continuous Kt/V urea at 1.7 or above, was applied in all patients. Of the 14 patients, 12 were on continuous ambulatory PD, and 2 were on automated PD. The mean daily dialysate fill volume was 4.7 ± 1.0 L, and the mean daily dwell time was 12.9 ± 3.7 hours at PD initiation.

The standard peritoneal equilibration test (PET) invented by Twardowski (9) was performed every 6 months after PD start. The dialysate-to-plasma ratio of creatinine (D/P Cr) was calculated from the 4-hour effluent and 2-hour plasma values. It is recommended that a PET be done 4 weeks after recovery from peritonitis (10). Thus, in the 2 patients who developed peritonitis during this study, a PET was performed 2 months after recovery from peritonitis. In all patients, the D/P Cr was measured at least twice.

**Assay of markers**

In the peritoneal effluent, we measured, as markers for ongoing coagulation, prothrombin fragment 1+2 (F1+2), which is generated during enzymatic conversion of prothrombin into thrombin; the complexes between thrombin and its inhibitor, antithrombin III (TAT); and fibrin monomer (FM), the product of the coagulation cascade. We also determined fibrin degradation products and D-dimer as fibrinolytic markers. All effluent markers were compared at PD initiation and at 2 years.

Thrombin–antithrombin complex was measured by fluorescent immunoassay (Mitsubishi Chemical Medience Corporation). Prothrombin fragment 1+2 was measured using ELISA, according to the manufacturer’s description (Siemens, Tokyo, Japan). Other biochemical parameters were measured using standard laboratory methods.

**Statistical analysis**

Data are expressed as mean ± standard deviation. For comparisons of paired data, a nonparametric Wilcoxon signed-rank test was used. For correlations between continuous variables, a Spearman rank correlation test was used. Multivariate regression analysis was performed to clarify the factors that contributed to the D/P Cr. A p value less than 0.05 was considered statistically significant. All statistical analyses were performed using the SPSS software application (version 18: SPSS Japan, Tokyo, Japan).

**Results**

At 2 years, the D/P Cr had not changed significantly compared with the D/P Cr at the PD initiation. In effluent, FM was significantly lower at 2 years (18.4 ± 7.0 µg/mL at initiation vs. 9.2 ± 3.7 µg/mL at 2 years, p = 0.006). However, other coagulation and fibrinolytic factors showed no significant changes (Table I). The D/P Cr was significantly correlated with F1+2 at PD initiation (r = 0.70, p = 0.006). Similarly, the D/P Cr was significantly correlated with F1+2 at 2 years (r = 0.76, p = 0.007, Figure 1). Furthermore, in a multivariate regression analysis with D/P Cr as the dependent variable and diabetes (present), dwell time, and fill volume as the independent variables, only F1+2 was significantly correlated with D/P Cr at 2 years (r = 0.79, p = 0.004).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dialysate vintage</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D/P Cr</td>
<td>0.60±0.09</td>
<td>0.59±0.12</td>
</tr>
<tr>
<td>TAT (ng/mL)</td>
<td>6.7±5.9</td>
<td>3.9±3.8</td>
</tr>
<tr>
<td>FM (µg/mL)</td>
<td>18.4±7.0</td>
<td>9.2±3.7</td>
</tr>
<tr>
<td>F1+2 (pmol/L)</td>
<td>160±103</td>
<td>113±86</td>
</tr>
<tr>
<td>D-Dimer (µg/mL)</td>
<td>8.1±9.0</td>
<td>5.8±13.0</td>
</tr>
<tr>
<td>FDPs (ng/mL)</td>
<td>4959±1952</td>
<td>4071±1724</td>
</tr>
</tbody>
</table>

TAT = thrombin–antithrombin complex; FM = fibrin monomer; F1+2 = prothrombin fragment 1+2; FDPs = fibrin degradation products.
Discussion
The most reliable diagnosis of peritoneal damage is obtained by peritoneal biopsy. It is generally assumed that alterations in peritoneal function are related to structural changes in the peritoneal membrane (11). However, because the biopsy procedure is invasive, it cannot be performed repeatedly. It is therefore necessary to find high-sensitivity, specific, and reproducible markers for peritoneal damage in effluent.

Recently, several studies have aimed to clarify peritoneal inflammation status by measuring various markers in effluent. In the present study, we focused on coagulation and fibrinolytic factors as indicators of peritoneal damage. During the process of tissue injury, mesothelial and endothelial cells produce cytokines that stimulate the release of tissue factor from monocytes and macrophages. Tissue factor increases local thrombin generation, which activates platelets and endothelium to produce growth factors and thus enhances neoangiogenesis and local fibrosis (12). In the peritonea of PD patients, various stimulations promote the coagulation cascade and the formation of fibrin (13). Peritoneal mesothelial cells have fibrinolytic activity and therefore prevent peritoneal adhesions and fibrin deposition (14).

It has been reported that high levels of F1+2 are found in plasma from PD patients, indicating low-grade activation of the coagulation system (15). Goedde et al. reported that intra-abdominal fibrin formation increased in the effluent of stable PD patients (16). Those authors suggested that high levels of coagulation and fibrinolytic factors and related antigens in effluent from patients without peritonitis cannot be explained by transport from plasma into the peritoneal cavity and that the measured levels may reflect a high rate of intra-abdominal fibrin turnover. Similarly, Homma et al. reported that turnover of fibrin in effluent increased in stable PD patients and stabilized after transfer to hemodialysis (8). However, their study was cross-sectional and did not observe the patients over time. Moreover, the PD patients in that study were using acidic dialysate. By contrast, the present study was performed as an observational cohort study in PD patients using ND. In our study, the D/P Cr, coagulation factors (with the exception of FM), and fibrinolytic factors were not significantly changed after 2 years of PD. We believe that the lesser peritoneal damage is attributable to either or both of the neutral pH of the dialysate and lesser glucose exposure in incremental PD. Although several coagulation and fibrinolytic markers were correlated with the D/P Cr in the univariate regression analysis at initiation or at 2 years (data not shown), only F1+2 was significantly correlated with D/P Cr both at initiation of PD and at 2 years. Furthermore, the multivariate regression analysis showed that only F1+2 was correlated with D/P Cr at 2 years. These results suggest that F1+2 is a useful marker for peritoneal permeability in PD patients using ND.

It is difficult to diagnose the peritoneal damage leading to EPS in only 1 examination. A comprehensive

![Figure 1](image)

**Figure 1** Correlation between dialysate-to-plasma ratio of creatinine (D/P Cr) and prothrombin fragment 1+2 (F1+2) (A) at initiation of peritoneal dialysis and (B) 2 years later.
decision based on several examinations, including peritoneal biopsy and biomarkers in effluent is therefore recommended. In the present study, we found a significant correlation between peritoneal permeability and coagulation factors in effluent. However, there are limitations to the interpretation of the results, because we could not determine whether the elevation of F1+2 in effluent with the increase in D/P Cr was a result only of enhanced peritoneal permeability or also because of a peritoneal inflammatory reaction. Future studies will therefore be necessary to clarify the correlation between the coagulation system and various inflammatory markers such as IL-6.

Conclusions
Our results suggest that ND had little influence on peritoneal permeability and on coagulation and fibrinolytic markers in effluent. In addition, F1+2 was a useful marker of peritoneal permeability in PD patients using ND.

Disclosures
The authors have no financial conflicts of interest to disclose.

References

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