Intra-peritoneal interleukin-6 system is a potent determinant of the baseline peritoneal solute transport in incident peritoneal dialysis patients

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Abstract

Background. Interleukin-6 (IL-6) is a key player in modulating inflammation. IL-6 and soluble IL-6 receptor (sIL-6R) complex induces the synthesis and secretion of various chemokines, adhesion molecules and angiogenic molecules. We hypothesized that the baseline peritoneal solute transport rate (PSTR) early after commencing peritoneal dialysis (PD) may depend largely on the IL-6/sIL-6R system. We also hypothesized that the dialysate concentrations of IL-6/sIL-6R could be closely related to local inflammation or angiogenesis in the peritoneal cavity.

Methods. Fifty incident patients with a modified peritoneal equilibration test result within 3 months after commencing PD and without a previous history of peritonitis were enrolled. Clinical parameters such as age, sex, comorbid disease, body mass index, residual renal function and C-reactive protein were assessed. Serum and dialysate markers including CA125, IL-6, sIL-6R, monocyte chemoattractant protein-1 (MCP-1), vascular endothelial growth factor (VEGF) and angiopoietin-2 (Ang-2) were measured and correlated with PSTR.

Results. Dialysate concentrations of IL-6 (r = 0.576, P < 0.001), MCP-1 (r = 0.408, P = 0.003) and Ang-2 (r = 0.408, P = 0.003) correlated with mass transfer area coefficient for creatinine (MTACcr), respectively. Dialysate appearance rate (AR) of albumin correlated with dialysate concentrations of CA125 (r = 0.751, P < 0.001), IL-6 (r = 0.303, P = 0.039), sIL-6R (r = 0.497, P < 0.001), MCP-1 (r = 0.488, P < 0.001), VEGF (r = 0.443, P = 0.004) and Ang-2 (r = 0.488, P < 0.001). Neither MTACcr nor AR of albumin was associated with systemic markers. Multivariate analysis showed that MTACcr is independently associated with dialysate IL-6 and serum albumin. It also showed that AR of albumin is independently predicted by dialysate sIL-6R. Dialysate IL-6 correlated with dialysate concentrations of CA125 MCP-1, VEGF and Ang-2.

Conclusion. Our study from incident PD patients suggested that (i) dialysate the IL-6 system is a potent determinant of baseline PSTR and (ii) elevation of IL-6 in the dialysate is associated with up-regulation of intra-peritoneal inflammatory and angiogenic molecules.

Keywords: albumin appearance rate; interleukin-6; MCP-1; peritoneal dialysis; PSTR

Introduction

Peritoneal solute transport rate (PSTR) is measured by dialysate-to-plasma (D/P) ratios or mass transfer area coefficients (MTACcr) of low molecular weight solutes. High PSTR reflects either a large effective peritoneal surface area, which results from a large number of perfused peritoneal capillaries engaged in the diffusion of the solute, or increased permeability of the peritoneal vasculature.

Some investigators have suggested that baseline PSTR, measured early after the commencement of peritoneal dialysis (PD), is associated with comorbidity or systemic inflammation [1,2]. However, we have recently shown, from a relatively large number of Korean patients, that the baseline PSTR is not associated with markers of systemic inflammation or comorbidity [3]. Therefore, it could be said that these associations are somewhat context and/or population dependent [3–6]. Instead, the pathophysiologic implication of the several intra-peritoneal markers has been investigated with diverse results.

Interleukin-6 (IL-6) is a key player in modulating inflammation. IL-6 is secreted from a variety of cells including macrophages and monocytes at inflammatory sites. It was also shown that the human peritoneal mesothelial cells (HPMC) can release IL-6 upon exposure to the spent...
high (three or more comorbid diseases) risk groups. Low (no comorbid disease), intermediate (one or two comorbid diseases) and high (three or more comorbid diseases) risk groups. 

Patients
Fifty incident patients starting PD between July 2005 and December 2006 were enrolled from Seoul National University Hospital, Seoul, Korea, after having given their informed consent. Approval for the study was obtained from the local ethics committee. In all these patients, the modified peritoneal equilibration test (PET) using 3.86% glucose PD fluid was performed within 3 months after commencing PD. Patients who had peritonitis before or during the PET were excluded. Comorbidity of the patients was assessed on the basis of the Davies comorbidity score [1], which comprises seven comorbid conditions, leading to three risk groups, i.e. low (no comorbid disease), intermediate (one or two comorbid diseases) and high (three or more comorbid diseases) risk groups.

Materials and methods

Dialysate or interleukin-1β (IL-1β) [7]. IL-6 not only stimulates the production of acute phase proteins in response to various stimuli but regulates a temporal switch from acute to chronic inflammation [8]. A soluble form of IL-6 receptor (sIL-6R) is shed from neutrophils by limited proteolysis of membrane IL-6R or translation of alternatively spliced mRNA of IL-6R [9,10]. As a result, the complex of IL-6 and sIL6R can bind to the gp130, a signal-transducing subunit on the cell membrane, which exists on most of the cell types. This way of IL-6 signaling, which is named trans-signaling, compared to classic signaling, enables the IL-6 system to activate cells, including HPMCs [8], that do not normally express IL-6R on the surface membrane. IL-6 and sIL-6R complex induces the synthesis and secretion of CC chemokine, monocyte chemotactrant protein-1 (MCP-1), which attracts monocytes and lymphocytes. It also induces other molecules as well, such as chemokines (MCP-3, IL8), adhesion molecules (ICAM-1, VCAM-1) [11,12] and angiogenic molecule (vascular endothelial growth factor, VEGF) [13,14]. It was shown in a few reports that dialysate IL-6 concentration is associated with PSTR either at baseline [2,15] or among prevalent patients [16] but refuted by other investigators [17].

We have previously shown that the dialysate concentration of IL-6 closely correlates with PSTR in incident patients [15]. Our observation is supported by other investigators as well [2,18]. However, the mechanistic significance of dialysate IL-6 in the development of high PSTR still remains to be elucidated. IL-6 has been described as having both pro- and anti-inflammatory effects [19,20]. IL-6 is also presumed to be a marker of cell vitality [21]. Therefore, whether elevated dialysate concentration of IL-6 in association with high PSTR could represent a state of enhanced peritoneal inflammation or a state of peritoneal mesothelial cell integrity needs to be investigated.

Based on our previous observation that the baseline PSTR early after commencing PD is associated with dialysate IL-6 concentration, we hypothesized that the up-regulation of intra-peritoneal IL-6 from high/fast transporter is linked with up-regulation of downstream inflammatory markers such as sIL-6R and MCP-1 and increased expression of angiogenic molecules. According to the three-pore model of the peritoneal membrane function, we dissociated the small solute transport associated with the small-pore area from the albumin appearance rate (AR) representing large molecular transport.

Calculations
Residual renal function was derived from the glomerular filtration rate (GFR) calculated as the average of renal urea and creatinine clearance. The patient’s normalized body surface area was calculated by the Du Bois and Du Bois equation [24]. Residual renal Kt/V urea was obtained from 24-h collection of urine. Urea distribution volume was calculated using the Watson equation [25]. Peritoneal Kt/V urea and creatinine clearance (Ccr) were derived from 24-h collection of the PD effluent. Total Kt/V urea was calculated by the sum of renal and peritoneal Kt/V urea.

Dialysate AR of each molecule was calculated by the product of its dialysate concentration and drained volume divided by the dwell time and expressed as picograms per minute.

Statistical analysis

Normality of distribution for each variable was assessed by the Kolmogorov–Smirnov test. Variables without a normal distribution were

Peritoneal solute transport rate

Modified PET with 3.86% glucose PD fluid was performed as described in our previous report [3]. The peritoneal transport type of each patient was classified as described elsewhere [22]. Mass transfer area coefficient for creatinine (MTACc) was calculated by the simplified Garred equation [23]. Dialysate-to-plasma concentrations for Cr (D/P Cr) and albumin (D/P Alb) at 4 h were acquired. MTACc and D/P Cr representing small solute transport and AR representing large molecular transport were measured. For comparison, patients were assigned to two groups according to the D/P Cr: high/high average (H/A) and low/low average (L/A) groups.

Biochemical parameters
Dialysate glucose and creatinine concentration was measured as described previously [3]. High sensitivity C-reactive protein (hsCRP) was measured by immunoturbidimetry (latex) with a sensitivity of 0.1 mg/l (normal values <5 mg/l).

Determination of serum and dialysate markers
Samples for the determination of serum IL-6, sIL-6R, MCP-1, IL-1β, TNFα, VEGF and angiopoietin-2 (Ang-2) concentrations were obtained from the serum taken during PET test and immediately frozen at −70°C until analysis. Serum IL-6, IL-1β and TNFα concentrations were analyzed using Bioplex Multiplex Cytokine Assay according to the manufacturer’s instructions (Biorad Laboratories, Hercules, CA, USA). The limits of detection in the serum were defined as 0.17 pg/ml for IL-6, 0.24 pg/ml for IL-1β and 0.70 pg/ml for TNFα, respectively. Serum VEGF concentrations were analysed with enzyme-linked immunosorbent assay (ELISA) with a sensitivity of 4.1 pg/ml by using DuoSet ELISA Development System (R&D Systems, Minneapolis, USA). Serum sIL-6R, MCP-1 and Ang-2 concentrations were measured using Quantikine ELISA Kit (R&D Systems) according to the manufacturer’s instruction.

Samples from the overnight effluent were taken and immediately frozen at −70°C until analysis for the determination of dialysate CA125, IL-6, sIL-6R, MCP-1, TNFα, IL-1β, VEGF and Ang-2. CA125 was determined by using electrochemiluminescence immunoassay with Elecys CA 125 II (Roche Diagnostics, Mannheim, Germany) according to the manufacturer’s instruction. The lower detection limit was 0.6 IU/ml. Dialysate IL-6 concentration was determined using photometric ELISA (Boehringer Mannheim, Mannheim, Germany) with intra- and inter-assay coefficients of variation of 2.6% and 7.1%, respectively. The sensitivity was 7.5 pg/ml. Dialysate TNFα and VEGF concentrations were analysed using Bio-Plex Multiplex Cytokine Assay according to the manufacturer’s instructions (Biorad Laboratories). The limit of detection in the dialysate was defined as 0.82 pg/ml for TNFα and 4.30 pg/ml for VEGF. Dialysate IL-1β concentrations were analyzed with ELISA with a sensitivity of 1.1 pg/ml by using DuoSet ELISA Development System (R&D Systems).

For the determination of sIL-6R, dialysate samples were diluted five times and were measured using Quantikine Human sIL-6R Immunoassay Kit (R&D Systems). Intra- and inter-assay variations were 3.5% and 5.1%, respectively. Dialysate MCP-1 and Ang-2 concentrations were measured using Quantikine ELISA Kit (R&D Systems).

Each assay is considered highly specific for the molecule with no significant cross-reactivity.
Table 1. Patient characteristics (n = 50)

| Age (years) | 46.5 ± 14.0 |
| Male/female | 27/23 |
| Diabetic (n, %) | 14 (28%) |
| BMI (kg/m²) | 22.9 ± 3.9 |
| BSA (m²) | 1.63 ± 0.20 |
| Time on PD (months) | 1.9 ± 1.0 |
| High-sensitivity CRP (mg/l) | 0.3 (0.1–2.4) |
| Etiology of renal failure |
| Diabetes mellitus | 14 (28%) |
| Hypertension | 12 (24%) |
| Glomerulonephritis | 11 (22%) |
| Allograft rejection | 4 (8%) |
| Others | 9 (18%) |
| Davies comorbidity score |
| None (0) | 31 (62%) |
| Intermediate (1–2) | 16 (32%) |
| High (3–7) | 3 (6%) |
| D/P creatinine | 0.72 ± 0.09 |
| D/D₀ glucose | 0.31 ± 0.12 |
| Net UF during PET (ml) | 690 ± 233 |
| Weekly total Kt/V | 2.35 ± 0.67 |
| GFR (l/week/1.73 m²) | 45.0 ± 27.0 |

BMI, body mass index; BSA, body surface area; UF, ultrafiltration; GFR, glomerular filtration rate calculated as the mean of creatinine and urea clearance. Data are shown as mean ± SD or median (interquartile range) if not specified.

either transformed into logarithmic scale for correlation analysis or compared by a nonparametric test. For continuous variables, comparison between two groups was made by using Student’s t test or Mann–Whitney U test. Chi square test was used for categorical variables. Correlation between two continuous variables was analysed by Pearson correlation test. A P value <0.05 was considered statistically significant. SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis.

Table 2. Comparison between L/A and H/A groups

<table>
<thead>
<tr>
<th>L/A (n = 26)</th>
<th>H/A (n = 24)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48.0 ± 15.0</td>
<td>45.0 ± 2.9</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>13/13</td>
<td>14/10</td>
</tr>
<tr>
<td>Diabetes (n, %)</td>
<td>8 (30.8%)</td>
<td>6 (25.0%)</td>
</tr>
<tr>
<td>Time on PD (months)</td>
<td>1.8 ± 0.9</td>
<td>2.0 ± 1.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.1 ± 3.8</td>
<td>21.6 ± 4.0</td>
</tr>
<tr>
<td>GFR (l/week/1.73 m²)</td>
<td>43.1 ± 25.9</td>
<td>47.9 ± 30.0</td>
</tr>
<tr>
<td>Davies comorbidity score (n, %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (0)</td>
<td>16 (61.5%)</td>
<td>15 (62.5%)</td>
</tr>
<tr>
<td>Intermediate (1–2)</td>
<td>8 (30.8%)</td>
<td>8 (33.3%)</td>
</tr>
<tr>
<td>High (3–7)</td>
<td>2 (7.7%)</td>
<td>1 (4.2%)</td>
</tr>
<tr>
<td>High-sensitivity CRP (mg/l)</td>
<td>0.1 (0.1–2.3)</td>
<td>0.8 (0.1–2.5)</td>
</tr>
<tr>
<td>Serum IL-6 (pg/ml)</td>
<td>2.9 (1.4–4.1)</td>
<td>2.8 (1.2–7.7)</td>
</tr>
<tr>
<td>Serum sIL-6R (ng/ml)</td>
<td>53.9 (39.6–66.0)</td>
<td>47.7 (38.3–52.3)</td>
</tr>
<tr>
<td>Serum MCP-1 (pg/ml)</td>
<td>413 (338–475)</td>
<td>381 (317–512)</td>
</tr>
<tr>
<td>Serum VEGF (pg/ml)</td>
<td>38.9 (15.8–72.3)</td>
<td>38.5 (19.5–66.0)</td>
</tr>
<tr>
<td>Serum Ang-2 (pg/ml)</td>
<td>5813 (4737–7819)</td>
<td>6089 (3843–7170)</td>
</tr>
<tr>
<td>Dialysate CA125 (IU/ml)</td>
<td>11.7 (5.5–22.2)</td>
<td>15.4 (11.6–29.5)</td>
</tr>
<tr>
<td>Dialysate IL-6 (pg/min)</td>
<td>123 (56–300)</td>
<td>307 (163–465)</td>
</tr>
<tr>
<td>Dialysate sIL-6R (pg/min)</td>
<td>1558 (678–2446)</td>
<td>935 (437–2831)</td>
</tr>
<tr>
<td>Dialysate MCP-1 (pg/min)</td>
<td>581 (249–925)</td>
<td>746 (464–1293)</td>
</tr>
<tr>
<td>Dialysate VEGF (pg/min)</td>
<td>218 (157–278)</td>
<td>249 (90–345)</td>
</tr>
<tr>
<td>Dialysate Ang-2 (pg/min)</td>
<td>668 (343–1344)</td>
<td>635 (317–1985)</td>
</tr>
</tbody>
</table>

BMI, body mass index; BSA, body surface area; GFR, glomerular filtration rate calculated as the mean of creatinine and urea clearance. Data are shown as mean ± SD or median (interquartile range) if not specified.

Results

Patient characteristics

All patients (n = 50) were ethnic Koreans and older than 18 years. Among them, 27 (54%) were male and 14 (28%) were diabetic. Patients were 46.5 ± 14.0 years old and the patients were receiving peritoneal dialysis for 1.9 ± 1.0 months when modified PET was performed. The causes of ESRD were diabetes in 14 (28%) patients, hypertension in 12 (24%), chronic glomerulonephritis in 11 (22%), allograft rejection in 4 (8%) and other causes in 9 (18%). Only 3 patients (6%) had 3 or more comorbid conditions and 16 patients (32%) had 1 or 2 comorbid conditions (Table 1).

Mean D/P creatinine ratio at 4 h was 0.72 ± 0.09 and mean dialysate-over-initial dialysate concentration ratio (D/D₀) for glucose at 4 h was 0.31 ± 0.12 for the entire population. Mean net ultrafiltration (UF) during the PET test was 690 ± 233 ml. The total Kt/V (peritoneal + renal) was 2.35 ± 0.67 and GFR was 45.0 ± 27.0 l/week/1.73 m².

Comparison between L/A vs H/A

L/A and H/A groups had similar age, sex ratio, diabetes prevalence and the time on PD. No significant differences were observed between the two transport types in terms of body mass index (BMI), GFR, comorbidity assessed by Davies comorbidity score, hsCRP and serum concentrations of various markers such as IL-6, sIL-6R, MCP-1, VEGF and Ang-2. However, dialysate IL-6 were significantly higher in the H/A group (P = 0.002). Dialysate levels of CA125, sIL-6R, MCP-1, VEGF and Ang-2 were not different between the two groups (Table 2). IL-1β and TNFα concentrations in the serum and dialysate were
under the detection limit in the majority of the patients (data not shown) and, thus, not considered for analysis.

**Correlation between dialysate markers and PSTR**

MTAC<sub>cr</sub> correlated with dialysate IL-6 (<i>r</i> = 0.576, <i>P</i> < 0.001), MCP-1 (<i>r</i> = 0.408, <i>P</i> = 0.003) and Ang-2 (<i>r</i> = 0.408, <i>P</i> = 0.003; Table 3). However, MTAC<sub>cr</sub> did not correlate with dialysate levels of CA125, sIL-6R and VEGF.

**Dialysate AR of albumin correlated with CA125 (<i>r</i> = 0.751, <i>P</i> < 0.001), IL-6 (<i>r</i> = 0.303, <i>P</i> = 0.039), sIL-6R (<i>r</i> = 0.497, <i>P</i> < 0.001), MCP-1 (<i>r</i> = 0.488, <i>P</i> < 0.001), VEGF (<i>r</i> = 0.443, <i>P</i> = 0.004) and Ang-2 (<i>r</i> = 0.488, <i>P</i> < 0.001) in the dialysate (Table 3).
Dialysate appearance rates for IL-6, sIL-6R, MCP-1, VEGF, Ang-2 were entered in a multiple linear regression analysis. Age, gender, diabetes, Davies comorbidity grade, CRP, serum albumin, dialysate appearance rates for IL-6, sIL-6R, MCP-1, VEGF, Ang-2 were entered in a multiple linear regression analysis.

No correlation between systemic markers and PSTR

Associations between various markers in the serum and MTAC_{cr} or dialysate AR of albumin were analysed by Pearson correlation analysis. Neither MTAC_{cr} nor dialysate AR of albumin correlated with the serum concentrations of CRP, IL-6, sIL-6R, MCP-1, VEGF and Ang-2 (Table 4).

No correlation between systemic and dialysate markers

As for the molecules analysed in the present study (IL-6, sIL-6R, MCP-1, VEGF and Ang-2), no correlation was found between the systemic and dialysate concentrations of each marker molecule (data not shown).

Multivariate analysis for MTAC_{cr} and albumin appearance rate

Multivariate linear regression models for MTAC_{cr} and albumin AR are given Table 5. Higher MTAC_{cr} was independently predicted by dialysate IL-6 (P < 0.001) and serum albumin (P = 0.005). Higher dialysate AR of albumin was independently predicted by dialysate sIL-6R (P < 0.001; Table 5).

Correlation of dialysate IL-6 with various intra-peritoneal markers

Dialysate level of IL-6 correlated with CA125 (r = 0.346, P = 0.017), MCP-1 (r = 0.619, P < 0.001), VEGF (r = 0.329, P = 0.031), Ang-2 (r = 0.619, P < 0.001) but not with sIL-6R (r = 0.145, P = 0.321) (Figure 1).

Discussion

High PSTR may have two potential etiologies—a large effective peritoneal surface area resulting in a large small-pore area and inflammation (or vascular injury) resulting in increased peritoneal permeability. In the present study, in order to distinguish between these two processes, we dissociated small solute transport rate represented by MTAC_{cr} and protein leak across large pores represented by dialysate albumin AR.

Our results showed that peritoneal small solute transport rate represented by MTAC_{cr} strongly correlated with the dialysate concentrations of IL-6, MCP-1 and Ang-2. Dialysate AR of albumin correlated with the dialysate concentrations of IL-6, sIL-6R, MCP-1, VEGF, CA125 and Ang-2. Elevated concentration of dialysate IL-6 was associated with up-regulation in the dialysate of MCP-1, VEGF, Ang-2 and CA125, respectively. After adjustment, multivariate linear regression analysis showed that MTAC_{cr} was independently predicted by dialysate IL-6 and serum albumin concentration. Dialysate AR of albumin was independently associated with sIL-6R in the dialysate.

Role of the intra-peritoneal markers in determining PSTR has been suggested in several studies. Continuous ambulatory peritoneal dialysis (CAPD) associated peritonitis is usually a localized infection in the peritoneal cavity and is associated with a transient increase in PSTR and low UF volume, accompanied by marked elevation in the effluent fluid of inflammatory or vasoactive substances such as IL-1β, IL-6, transforming growth factor β1 and fibroblast growth factor [26]. In a longitudinal study [17], baseline D/P creatinine was associated with dialysate concentration of locally produced vasoactive substances rather than the systemic markers. In that study, D/PCr change over time was associated with dialysate IL-6.

The present study showed a strong correlation between baseline PSTR with dialysate IL-6. Dialysate IL-6 correlated both with MTAC_{cr} and with albumin AR. Association of PSTR with dialysate IL-6 has been suggested by other researchers as well. Pecoits-Filho et al. [2] reported a strong positive correlation between baseline D/PCr and dialysate IL-6. In their study, the association between baseline D/PCr and dialysate IL-6 was even stronger than that between D/PCr and plasma IL-6. However, unlike ours, the association of IL-6 with sIL-6R or with MCP-1 was not investigated in their study.

IL-6 has been described as having both pro- and anti-inflammatory effects [19,20]. Also, HPMC is one of the principal sources of IL-6 [27,28]. Therefore, the mechanistic meaning of elevated dialysate IL-6 level, whether it indicates a local pro- or anti-inflammatory state or enhanced mesothelial vitality, needs to be elucidated. In our study, elevation of dialysate IL-6 was strongly associated with up-regulation of MCP-1, a downstream inflammatory molecule and angiogenic markers such as VEGF and Ang-2 (Table 3, Figure 1).

It was shown in a murine model that sIL-6R shed from infiltrating neutrophils during bacterial peritonitis complexes with intra-peritoneal IL-6. The IL-6 and sIL-6R complex regulates differential expression of chemokines and induces transition from initial neutrophil-predominant to the more sustained monocyte-predominant phase [8]. MCP-1(CCL2) is a potent chemoattractant and activator of monocytes/macrophages. Release of MCP-1 was increased when HPMCs were stimulated by IL-6 and sIL-6R complex [11]. Intra-peritoneal injection of lipopolysaccharide induced up-regulation of MCP-1, VEGF and other cytokines, resulting in increased peritoneal solute transport rate [29]. Dialysate MCP-1 concentration positively correlated with D/PCr in a cross-sectional analysis with a mixture of stable prevalent patients and new patients [31]. Therefore, correlation of PSTR with dialysate IL-6, sIL-6R and MCP-1 in our study, altogether, suggests that baseline PSTR is associated with a state of pro-inflammatory milieu in the peritoneal membrane.

### Table 5. Multivariate regression analysis for MTAC\textsubscript{cr} and dialysate albumin appearance rate

<table>
<thead>
<tr>
<th>Dependent variable: MTAC\textsubscript{cr}</th>
<th>Standardized coefficient $\beta$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log IL-6</td>
<td>0.517</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>-0.351</td>
<td>0.005</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dependent variable: Albumin appearance rate</th>
<th>Standardized coefficient $\beta$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>log AR sIL-6R</td>
<td>0.585</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Role of the intra-peritoneal markers in determining PSTR**

No correlation between systemic markers and PSTR

As for the molecules analysed in the present study (IL-6, sIL-6R, MCP-1, VEGF and Ang-2), no correlation was found between the systemic and dialysate concentrations of each marker molecule (data not shown).

Multivariate analysis for MTAC\textsubscript{cr} and albumin appearance rate

Multivariate linear regression models for MTAC\textsubscript{cr} and albumin AR are given Table 5. Higher MTAC\textsubscript{cr} was independently predicted by dialysate IL-6 ($P < 0.001$) and serum albumin ($P = 0.005$). Higher dialysate AR of albumin was independently predicted by dialysate sIL-6R ($P < 0.001$; Table 5).

Correlation of dialysate IL-6 with various intra-peritoneal markers

Dialysate level of IL-6 correlated with CA125 ($r = 0.346$, $P = 0.017$), MCP-1 ($r = 0.619$, $P < 0.001$), VEGF ($r = 0.329$, $P = 0.031$), Ang-2 ($r = 0.619$, $P < 0.001$) but not with sIL-6R ($r = 0.145$, $P = 0.321$) (Figure 1).
Fig. 1. Dialysate IL-6 correlated with dialysate CA125 (A), MCP-1 (C), VEGF (D) and Ang-2 (E) but not with sIL-6R (B).
In the present study, the dialysate concentration of IL-6 was 15.3 (median) times higher than the serum counterpart, which is in parallel with other reports [16,31]. This suggests that the dialysate concentration of IL-6 reflects local production of the molecule in the peritoneum. IL-6 was detected in the dialysate of patients without any signs of systemic inflammation [31]. In the present study, dialysate concentrations of IL-6 did not correlate with the serum IL-6 or with CRP (data not shown). Local peritoneal production of IL-6 may reflect chronic inflammatory state of IL-6 or with CRP (data not shown). Local peritoneal production of the molecule in the peritoneum. IL-6 suggests that the dialysate concentration of IL-6 reflects its transport from the systemic circulation rather than its local production in the peritoneum.

VGEF is a potent stimulator of vascular permeability and neoangiogenesis [32]. In our study with incident patients, VGEF in the dialysate correlated with the AR of albumin but not with MTAC,cr. Our data also show a positive correlation between dialysate IL-6 and VGEF, supporting that VGEF is up-regulated by IL-6 [13,14].

Ang-2 is one of the angiogenic factors suspected to play a role in the peritoneal neoangiogenesis in a rat model of experimental encapsulating peritoneal sclerosis [33]. In our study, associations of Ang-2 with both MTAC,cr and albumin AR were revealed (Table 3). This is in keeping with a recent report elsewhere that Ang-2 plays a role in the pathogenesis of vascular leakiness to macromolecules [34].

Dialysate CA125 is a glycoprotein produced constitutively by mesothelial cells [35]. CA125 is considered to reflect peritoneal mesothelial cell mass or turnover [36]. Our study showed an association of CA125 with albumin appearance but not with MTACC,cr. Dialysate CA125 correlated with dialysate IL-6, supporting the fact that HPMC is a major source of IL-6 in the peritoneum.

In the present study, IL-1β and TNFα concentrations in the serum and dialysate were in the lower-than-detectable level in the majority of the patients, which is in accordance with other reports [37,38]. Therefore, the association of PSTR with these two markers could not be assessed in the present study.

Our patient population had much lower comorbid conditions compared with others. Among 50 patients, only three patients (6%) had three or more comorbid conditions while others reported 11% [1]. Median CRP level was 0.3 mg/l (interquartile range 0.1–2.4 mg/l). These observations together suggested a much lower level of systemic inflammation for our patient population compared with others [39]. Differences in the baseline patient characteristics could be one explanation for our finding that systemic factors do not have much influence on the baseline PSTR, which is in contrast with other reports [1,2].

The present study has some limitations—small number of patients selected from a single PD unit. These might have rendered the study to be prone to type 2 errors and centre effect. Therefore, our observations need to be confirmed in a larger prospective study.

Our study employed modified PET using 3.86% glucose PD fluid, although previous reports on peritoneal solute transport rate mostly employed standard PET using 2.26% PD fluid. It was shown elsewhere that no significant differences exist between the two methods in terms of solute transport rates for urea, creatinine and protein [40]. Besides, 3.86% glucose solution provides better information on ultrafiltration and free water transport.

In conclusion, the results from the present study with incident PD patients suggest a dialysate IL-6 system represented by IL-6/sIL-6R/MCP-1 as a potent determinant in baseline PSTR. It also suggests a differential role of individual intra-peritoneal markers in association with MTAC,cr and albumin AR. Elevation of dialysate IL-6 was related to elevation of downstream inflammatory molecule and angiogenic factors in the peritoneum. Future studies in a larger scale are needed to elucidate various serum and/or dialysate markers associated with the peritoneal transport of small molecules and macromolecules.

Conflict of interest statement. None declared.

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