PART ONE

Peritoneal Kinetics and Anatomy
Kinetics of Carboxy-Terminal Propeptide of Type I Procollagen in Pediatric Patients Undergoing Peritoneal Dialysis

Beata Leszczynska, Maria Roszkowska—Blaim

Carboxy-terminal propeptide of type I procollagen (PICP) is used as a marker of bone formation and, presumably, also peritoneal fibrosis. The aim of the present study was to assess the effect of dialysate PICP level (PICP_d) on serum PICP level (PICP_s).

The study group consisted of 20 patients divided into two groups: group A consisted of 10 children without peritonitis, mean age 13.6 ± 5 years, 3 on continuous ambulatory peritoneal dialysis (CAPD) and 7 on automated peritoneal dialysis (APD); and group B consisted of 10 children with peritonitis (last episode having occurred at least 2 months before the study), mean age 13.9 ± 2 years, 8 on CAPD and 2 on APD. Osmolarity of the dialysate fluid in groups A and B was similar (375.7 ± 42 mOsm/L and 363.1 ± 16 mOsm/L respectively). The PICP_s and PICP_d levels, PICP index (PICP_s/PICP_d), PICP peritoneal clearance (ClPICP), and PICP mass transfer (MTPICP) were measured in both groups.

Mean PICP_s was higher in group B, but the difference was not significant. No significant differences were seen in the mean values of PICP_d, PICP index, ClPICP, and MTPICP between groups. Levels of PICP_d correlated positively with ClPICP (r = 0.75, p < 0.001) and negatively with MTPICP (r = −0.89, p < 0.0001). No associations were seen between PICP_s and PICP_d, ClPICP, and MTPICP. No associations were observed between osmolarity and ClPICP and MTPICP.

Serum PICP level in children undergoing peritoneal dialysis does not depend on peritoneal PICP level.

Key words
PICP, children

Introduction
Carboxy-terminal propeptide of type I procollagen (PICP) is used as a marker of bone formation, because it reflects the amount of type I collagen synthesized in osteoblasts in a 1:1 ratio (1,2). Peritoneal fibroblasts probably also produce PICP, which may make PICP a marker of peritoneal fibrosis in patients undergoing peritoneal dialysis (3). Therefore, the aim of the present study was to assess the effect of dialysate PICP level on serum PICP level.

Patients and methods
We studied 20 children, aged 6—19.5 years (mean: 13.75 ± 3.5 years), with end-stage renal disease (ESRD) treated with continuous ambulatory peritoneal dialysis (CAPD) or automated peritoneal dialysis (APD). The children were divided into two groups depending on history of dialysis-related peritonitis (DRP). Group A included 10 children aged 13.6 ± 5 years with no history of dialysis-related peritonitis (3 on CAPD, 7 on APD). Mean duration of dialysis therapy was 18.3 ± 21 months, and osmolarity of dialysis fluid was 375.7 ± 42 mOsm/L. Seven children had some residual diuresis, ranging from 50 mL to 3500 mL daily (mean: 1014.5 ± 1165 mL daily). Group B included 10 children aged 13.9 ± 2 years with a history of at least 1 episode of DRP, but no episodes during the last 2 months before the study (8 on CAPD, 2 on APD). Mean duration of dialysis therapy was 33 ± 13.9 months, and osmolarity of dialysis fluid was 363.1 ± 16.1 mOsm/L. Eight children had residual diuresis ranging from 150 mL to 1500 mL daily (mean: 655 ± 710 mL daily).

Continuous ambulatory peritoneal dialysis was performed using twin-bag dialysis fluid sets (Baxter Healthcare Corporation, Deerfield, IL, U.S.A.). Dialysis fluid containing 1.36%—3.86% glucose was exchanged 4—6 times daily (1000 mL per square meter of body surface area per exchange). Osmolarity was individually adjusted to keep the patient’s dry weight constant. Automated peritoneal dialysis was performed at night, using a Baxter cycler and 5—8 dialysate fluid exchanges in 12 hours. Some children
required an additional exchange during the day. The content and the volume of the dialysis fluid were both similar to that in CAPD patients.

Serum PICP level (PICP<sub>s</sub>) and PICP level in 24-hour effluent dialysate collection (PICP<sub>d</sub>) were measured in all children. Samples of dialysate for PICP measurements were frozen at —20˚C until assay. Blood samples were drawn at 10'h, after an overnight fast following the end of the 24-hour dialysate fluid collection. Based on PICPs and PICP<sub>d</sub> values, the following parameters were calculated:

1. PICP index (3): \( I = \frac{\text{PICP}<sub>d</sub>}{\text{PICP}<sub>s</sub>} \)

2. PICP clearance (4):

\[
\text{Cl}_{\text{PICP}} (\text{mL/min}) = \left( \frac{\text{PICP}<sub>d</sub>}{\text{PICP}<sub>s</sub>} \right) \frac{V}{T}
\]

where PICP<sub>d</sub> is the PICP level in the 24-hour dialysate effluent collection (µg/L); PICP<sub>s</sub> is the serum PICP level (µg/L); \( V \) is the volume of 24-hour dialysate fluid collection (mL); and \( T \) is the 24-hour total exchange time (minutes).

3. PICP mass transfer (4):

\[
\text{MT}_{\text{PICP}} (\text{mg/24h}) = C_1 V_1 - C_2 V_2
\]

where \( C_1 \) is the PICP level in the inflow dialysate fluid; \( C_2 \) is the PICP level in the outflow dialysate; \( V_1 \) is the volume of the inflow dialysate fluid; and \( V_2 \) is the volume of the outflow dialysate.

Serum and dialysate PICP levels were measured by the radioimmunologic method (RIA) with Orion Diagnostica kits (SF-02101: Espoo, Finland). Levels of PICPs were interpreted using normal values for given age as reported by Trivedi et al (5): 330±130µg/L for ages 4—16 years and <160µg/L for ages >16 years.

Statistical analysis was performed using the Student t-test for normally distributed variables and the Wilcoxon test for non normally distributed variables. Correlations between parameters were analyzed using linear regression. A \( p \) value less than 0.05 was considered statistically significant.

Results
We found no differences between groups in regard to age of the studied patients, duration of dialysis therapy, residual diuresis, and 24-hour osmolarity of dialysis fluids used. In group B, 5 children had a history of 1 episode of DRP. 4 had a history of 2 episodes, and 1 child had a history of 3 previous episodes of DRP. Table 1 shows the mean serum and dialysate PICP levels, the PICP mass transfer, and the PICP peritoneal clearance in groups A and B.

In group A, PICPs was above the normal range in 7 patients, below the normal range in 1 patient, and within the normal range in the remaining 2 patients. In group B, PICPs was above the normal range in 6 patients, below the normal range in 1 patient, and within the normal range in the remaining 3 patients. Mean PICPs was higher in group B as compared with group A; however, the difference was not statistically significant. No significant differences were seen in mean PICP<sub>d</sub> level, PICP index, Cl<sub>PICP</sub>, and MT<sub>PICP</sub> between groups A and B. No children in group A had a higher PICP<sub>d</sub> level than PICP<sub>s</sub> level (PICP index < 1); in group B, the PICP index was >1 in only 2 children with a history of DRP.

Because no significant differences were seen between the groups, correlations between the parameters were tested in the pooled population. No correlation was seen between PICP<sub>d</sub> and PICP<sub>d</sub> and between PICP<sub>s</sub> and Cl<sub>PICP</sub> or MT<sub>PICP</sub>. We found a positive correlation only between PICP<sub>d</sub> and Cl<sub>PICP</sub> (\( r = 0.75, p < 0.001 \)) and a negative correlation only between PICP<sub>d</sub> and MT<sub>PICP</sub> (\( r = -0.89, p < 0.0001; \) Figures 1 and 2). Osmolarity of the inflow and outflow dialysate fluid had no effect on Cl<sub>PICP</sub>, MT<sub>PICP</sub>, or PICP index.

Discussion
Our study showed higher serum and dialysate PICP levels in most children in group B, although the differences between the mean values in the two groups were not significant. Digenis et al (3,6,7) and Gerakis et al (8) also found higher PICP<sub>d</sub> levels in adults with a history of DRP, as did other researchers (9,10).

In our study, PICP<sub>d</sub> levels in group B were not related to the number of previous episodes of DRP. For example, the PICP index in a patient with 3 episodes of DRP was below 1, while in 2 other children (including one with 1 previous episode of DRP and another with 2 previous episodes of DRP), the PICP index was above 1.

We found no significant difference in mean PICP index values between groups A and B. In our study, no relationship was seen between duration of peritoneal dialysis, osmolarity of the dialysate fluids used, and serum and dialysate PICP levels, which may sug-
gest that measurements of PICP level have no value as a marker of peritoneal fibrosis in patients with a history of dialysis-related peritonitis. That suggestion is also supported by the lack of difference in ClPICP and MTPICP between the groups.

A positive correlation between PICPd and ClPICP clearance and a negative correlation between PICPd and MTPICP may suggest transfer of PICP molecules through the peritoneal membrane. Such a transfer would be quite surprising, as PICP is a protein with a high molecular weight (100,000 Da) and was found not to be able to cross the peritoneal membrane (1,11). The PICPd level likely reflects the relationship between a large peritoneal surface and the extent of subendothelial connective tissue able to synthesize type I collagen (11).

Lack of a significant difference in PICPs level relative to a history of DRP in children undergoing peritoneal dialysis does not refute a role for PICP as a marker of bone formation in such patients. Transport of PICP from the peritoneum to the circulation via the lymphatic vessels seems to have little effect on PICPs level (2,8).

**Table 1** Mean values of carboxy-terminal propeptide of type I procollagen (PICP) in serum (PICPs) and dialysate (PICPd) and PICP clearance (ClPICP) and mass transfer (MTPICP) in two groups of children on peritoneal dialysis

<table>
<thead>
<tr>
<th></th>
<th>PICPd (µg/L)</th>
<th>PICPs (µg/L)</th>
<th>PICP index</th>
<th>ClPICP (mL/min)</th>
<th>MTPICP (mg/24h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>346.8±141.7</td>
<td>93.8±55.7</td>
<td>0.29±0.16</td>
<td>1.5±1.15</td>
<td>-0.68±0.53</td>
</tr>
<tr>
<td>Group B</td>
<td>465±135.7</td>
<td>192.4±166.9</td>
<td>0.51±0.55</td>
<td>1.8±1.5</td>
<td>-1.08±0.85</td>
</tr>
</tbody>
</table>

*p* Value 0.07 NS NS NS NS NS

NS = nonsignificant.

**Conclusion**

Our study shows that peritoneal synthesis of type I collagen has no major influence on PICPs level in children undergoing chronic peritoneal dialysis.

**References**


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Estimation of lymphocyte subset counts (SLCs) is a useful tool in diagnosing nutrition and immune changes in continuous ambulatory peritoneal dialysis (CAPD) patients. Administration of recombinant human erythropoietin (rHuEPO) or angiotensin-converting enzyme inhibitors (ACEIs) can influence SLCs. Our aim was to evaluate the relationship between rHuEPO and ACEI doses and SLCs in the course of CAPD.

In the group of studied patients (n = 55), 34 patients were taking rHuEPO and 38 patients were taking ACEIs. In 35 patients, enalapril was the ACEI used. Seven patients were taking rHuEPO, but not ACEIs; 11 patients were taking ACEIs, but not rHuEPO; 27 patients were taking rHuEPO and ACEIs both; and 10 patients were receiving neither rHuEPO nor ACEIs.

Flow cytometry was used to estimate CD3, CD4, CD8, CD19, and CD16+56 antigens.

In the study group, a correlation was seen between dialysis duration and rHuEPO dose (r = 0.395). No correlation was seen between CAPD duration and ACEI dose, but it was seen for total rHuEPO and ACEI doses (r = 0.327). A negative correlation was also seen between dialysis duration and CD19 cell count (r = -0.313). In patients taking only ACEIs (n = 11), a negative correlation was seen between total ACEI doses and CD16+56 cell count (r = -0.710). In patients who were not receiving rHuEPO or ACEIs, negative correlations were seen between dialysis duration and total lymphocyte count (r = -0.727), CD3 cell count (r = -0.706), CD4 cell count (r = -0.636), and CD8 cell count (r = -0.764).

In conclusion, rHuEPO and ACEIs can influence the total lymphocyte count or lymphocyte subset counts the natural changes being disturbed with prolongation of CAPD treatment. The possibility of this influence should be taken into account when evaluating lymphocyte counts as indices of nutrition and immune status.

Key words
Lymphocyte, erythropoietin, angiotensin-converting enzyme inhibitors

Introduction
A decrease in total lymphocyte count (TLC) during the course of continuous ambulatory peritoneal dialysis (CAPD) is an indicator of disturbances in immune response and nutrition status (1—3) and is mentioned as a prognostic index of mortality in CAPD patients (4—6). Studies by Palop et al (7) revealed a significant decrease not only in TLC, but also in B lymphocyte count, during the course of CAPD as compared with the start of treatment. Our data indicate that lymphocyte subset counts (SLCs), except that for natural killer cells, decrease over the course of CAPD treatment, and that the decrease in SLCs can be seen earlier than the fall in TLC (8,9).

In the present study, we undertook to determine whether recombinant human erythropoietin (rHuEPO) and angiotensin-converting enzyme inhibitors (ACEIs), both commonly used in CAPD patients, can influence SLCs. Previous studies have indicated that rHuEPO (10—16) and ACEIs (17) influence TLC and SLCs in non dialyzed or hemodialyzed patients.
mic patients immediately before initiation of CAPD treatment (group 0: 6 women, 9 men; age 56.2 ± 15.6 years). The underlying kidney diseases were chronic glomerulonephritis (19 cases), chronic pyelonephritis (12 cases), diabetic nephropathy (10 cases), hypertensive nephropathy (8 cases), polycystic kidney disease (3 cases), gout nephropathy (1 case), and unknown etiology (2 cases).

The CAPD patients were divided into four groups, depending on dialysis duration. Group I consisted of 15 patients (3 women, 12 men; age: 54.5 ± 13.4 years) treated for 6—12 months (mean: 9.3 ± 1.6 months); group II, of 15 patients (4 women, 11 men; age: 50.2 ± 14.7 years) treated for 13—24 months (mean: 17.9 ± 2.7 months); group III, of 15 patients (7 women, 8 men; age: 48.5 ± 9.2 years) treated for 25—36 months (mean: 26.9 ± 2.9 months); and group IV, of 10 patients (3 women, 7 men; age: 50.0 ± 9.6 years) treated for more than 36 months (mean: 40.9 ± 4.9 months). In 12 patients treated with CAPD through 15.7 ± 8.1 months, the examinations were repeated at 29.3 ± 8.6 months of CAPD therapy.

Before the start of dialysis, none of the 55 CAPD patients was receiving rHuEPO, but ACEIs were being administered to 15 patients. In the course of CAPD, 34 patients took rHuEPO and 38 patients took ACEIs.

In groups I to IV, the percentages of patients who were continuously or temporarily taking subcutaneous doses of rHuEPO were 40%, 60%, 73%, and 80% respectively. The rHuEPO doses, calculated for the entire CAPD course, including periods without rHuEPO, were 2884 U, 3177 U, 6695 U, and 6279 U per CAPD month, respectively. Patients treated with rHuEPO were simultaneously taking an oral drug containing iron (210 mg Fe²⁺ daily) and folic acid (0.70 mg daily). Mean serum ferritin level was 548 ± 784 ng/mL (median: 231 ng/mL; range: 180—3830 ng/mL).

In 35 of the 55 CAPD patients, enalapril was used as the ACEI; 1 patient was receiving captopril, and 2 were receiving perindopril. In group I, 80% of the patients were using ACEIs (dose: 243 mg). In group II, the percentage of ACEI recipients was 73% (dose: 275 mg); in group III, 80% (dose: 211 mg); and in group IV, 73% (dose: 309 mg). Doses of ACEI are also expressed per CAPD month, including periods of CAPD treatment without ACEI administration.

Other drugs and supplements were administered depending on clinical indications.

In the entire group of 55 CAPD patients, 7 patients took rHuEPO, but never received ACEIs; 11 patients took ACEIs, but never received rHuEPO; 27 patients took rHuEPO and ACEIs both; and 10 patients received neither rHuEPO nor ACEIs.

Percentage SLCs were determined by flow cytometry, using the commercially available monoclonal antibodies CD3, CD4, CD8, CD19, CD16+56 (Becton Dickinson, San Jose, CA, U.S.A.). Granulocytes and monocytes were excluded from calculations with the help of monoclonal antibodies CD45 and CD14 (Becton Dickinson). Absolute counts of lymphocytes were calculated using white blood cell count, estimated by routine procedures (stained smears, chamber method). In our laboratory, the normal range for TLC is 1.5—10⁹/L — 3.5—10⁹/L; for CD3 cells (pan T cells), it is 1.1—10⁹/L — 1.7—10⁹/L; for CD4 cells (helper T cells), it is 0.7—10⁹/L — 1.4—10⁹/L; for CD8 cells (cytotoxic-suppressor T cells), it is 0.5—10⁹/L — 0.9—10⁹/L; for CD19 (B cells), it is 0.2—10⁹/L — 0.4—10⁹/L; and for CD16+56 cells (natural killer cells), it is 0.2—10⁹/L — 0.4—10⁹/L. The normal CD4/CD8 ratio is 0.8—2.2.

Descriptive data are reported as mean ± 1 standard deviation of the mean. The TLC and SLCs in all patient groups were compared to their respective normal ranges and to each other using the Kruskal—Wallis test. Comparative results were considered significant if the p value was below 0.05. Correlations between dialysis duration, SLCs, and rHuEPO and ACEI doses were checked using the Spearman test.

Results

The lowest TLC and SLC values were observed immediately before CAPD initiation. After 6—12 months of CAPD therapy, TLC, CD3 count, CD16+56 count, and CD4/CD8 ratio were in the normal range. In the subsequent years of CAPD therapy, CD4, CD8, and CD19 cell counts were below the normal range, but mean TLC and CD3 count remained in the normal range. Counts of CD16+56 cells exceeded the upper limit of normal in patients treated for more than 36 months. Significant differences were seen only between the TLC and SLCs obtained immediately before the start of CAPD and the results obtained during CAPD therapy. No significant differences were seen between the TLC or SLCs obtained in the course of CAPD (Table I). Repeated examinations (performed
in 12 patients after approximately 14 months of CAPD therapy) also did not reveal significant differences in TLC or SLCs (Table II).

In the entire group of CAPD patients, a correlation was seen between dialysis duration and rHuEPO doses used from the start of CAPD until the time when the SLC estimations were performed ($r^2 = 0.049, p = 0.006, n = 55$). Another correlation was seen between total rHuEPO and ACEI doses ($r^2 = 0.279, p = 0.039, n = 55$). A significant negative correlation was also seen between dialysis duration and CD19 cell count ($r^2 = -0.319, p = 0.018, n = 55$).

Table III presents absolute numbers for TLC and SLCs in CAPD patients in the four groups depending on drug (rHuEPO, ACEI) administration. In patients taking ACEIs, but not receiving rHuEPO ($n = 11$), a negative correlation was seen between total ACEI doses and CD16+56 cell count ($r^2 = -0.709, p = 0.014$).

In patients who were receiving neither rHuEPO nor ACEIs, negative correlations were seen between dialysis duration and TLC ($r^2 = -0.709p = 0.022, n = 10$), CD3 cell count ($r^2 = -0.680p = 0.030, n = 10$), and CD8 cell count ($r^2 = -0.757p = 0.011, n = 10$).

Discussion
Use of rHuEPO increases as renal function declines and serum creatinine concentration rises (18). The significant positive correlation between dialysis duration and rHuEPO dose in our patients also reflects the usual practice of increasing the dose of rHuEPO during the course of CAPD therapy. Despite that increase, a worsening of immune and nutrition parameters is observed with prolongation of dialysis treatment, including a decrease in lymphocyte count (1—7) and in CD4/CD8 ratio (19). In patients on maintenance hemodialysis, increased lymphocyte apoptosis (leading to a reduced number of lymphocytes in the peripheral blood) has been shown (20, 21).

Table 1 Total lymphocyte count (TLC) and lymphocyte subset counts in peripheral blood from uremic patients immediately before and during continuous ambulatory peritoneal dialysis (CAPD)

<table>
<thead>
<tr>
<th>Lymphocytes (10⁹/L)</th>
<th>Immediately before CAPD start (n=15)</th>
<th>6—12 (n=15)</th>
<th>13—24 (n=15)</th>
<th>25—36 (n=15)</th>
<th>37—52 (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLC</td>
<td>1.02±0.47²</td>
<td>1.91±0.72²</td>
<td>1.51±0.41²</td>
<td>1.52±0.47²</td>
<td>1.82±0.76²</td>
</tr>
<tr>
<td>CD3</td>
<td>0.77±0.38²</td>
<td>1.37±0.57²</td>
<td>1.11±0.36²</td>
<td>1.12±0.33²</td>
<td>1.17±0.53²</td>
</tr>
<tr>
<td>CD4</td>
<td>0.31±0.16²</td>
<td>0.62±0.31²</td>
<td>0.52±0.19²</td>
<td>0.50±0.19²</td>
<td>0.46±0.24²</td>
</tr>
<tr>
<td>CD8</td>
<td>0.27±0.19²</td>
<td>0.37±0.20²</td>
<td>0.30±0.14²</td>
<td>0.34±0.15²</td>
<td>0.37±0.17²</td>
</tr>
<tr>
<td>CD19</td>
<td>0.06±0.04²</td>
<td>0.16±0.10²</td>
<td>0.09±0.04²</td>
<td>0.11±0.08²</td>
<td>0.09±0.06²</td>
</tr>
<tr>
<td>CD16+56</td>
<td>0.16±0.14²</td>
<td>0.35±0.15²</td>
<td>0.28±0.13²</td>
<td>0.24±0.15²</td>
<td>0.42±0.36²</td>
</tr>
<tr>
<td>CD4/CD8 ratio</td>
<td>1.52±1.21</td>
<td>1.81±1.74²</td>
<td>1.87±1.83²</td>
<td>1.63±1.83²</td>
<td>1.30±1.37²</td>
</tr>
</tbody>
</table>

² Mean value below normal range.
³ Mean value above normal range.

Table 2 Total lymphocyte count (TLC) and lymphocyte subset counts in peripheral blood from continuous ambulatory peritoneal dialysis patients who underwent repeated examinations

<table>
<thead>
<tr>
<th>Lymphocytes (10⁹/L)</th>
<th>First examination</th>
<th>Second examination</th>
<th>Correlation coefficient between first and second examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLC</td>
<td>1.58±0.67</td>
<td>1.43±0.41</td>
<td>0.466</td>
</tr>
<tr>
<td>CD3</td>
<td>1.16±0.56</td>
<td>1.08±0.31²</td>
<td>0.635</td>
</tr>
<tr>
<td>CD4</td>
<td>0.13±0.12²</td>
<td>0.08±0.04²</td>
<td>0.463</td>
</tr>
<tr>
<td>CD8</td>
<td>0.55±0.31</td>
<td>0.49±0.17²</td>
<td>0.543</td>
</tr>
<tr>
<td>CD19</td>
<td>0.32±0.16</td>
<td>0.31±0.13</td>
<td>0.281</td>
</tr>
<tr>
<td>CD16+56</td>
<td>0.26±0.07³</td>
<td>0.24±0.15</td>
<td>0.181</td>
</tr>
<tr>
<td>CD4/CD8 ratio</td>
<td>1.80±0.68</td>
<td>1.80±0.92</td>
<td>0.769</td>
</tr>
</tbody>
</table>

³ Mean value below normal range.
Altered numbers of immune cells contribute to immunologic abnormalities (3), depressed erythropoiesis (22), increased infection rates (23), and poor outcome (4). Early detection of immunologic disturbances and features of malnutrition may initiate clinical intervention, resulting in more effective treatment with peritoneal dialysis. Repeated determination of B and T lymphocyte subset counts seems to be helpful in the early diagnosis of such disturbances.

In our study, a significant negative correlation between CAPD duration and TLC or SLCs was shown, especially in patients receiving neither rHuEPO nor ACEIs. Administration of drugs can influence a natural decrease in TLC or SLCs observed in the course of CAPD. Numerous data indicate an increasing effect of rHuEPO on TLC (24) and on counts of CD3 (14), CD4 (16), CD8 (15), and CD16 (13) cells, as well as on CD4/CD8 ratio (11—13,16,19). A study by Yorioka et al (13) demonstrated that rHuEPO enhances the proliferation of human B cells and B-cell lines. Deterioration of nutrition, usually associated with more severe anemia, leads to an increase in rHuEPO dose. We suspect that the nearly stable (nonsignificantly decreased) CD4/CD8 ratio in the course of CAPD treatment reflects the effect of rHuEPO, which protects from a significant decrease in CD4/CD8 ratio occurring with worsening nutrition status. A direct relationship between rHuEPO dose and TLC or SLCs was not shown, however.

Natural killer cells are derived from bone marrow. They play a role in defense against infection (25). Their cytotoxic function is bone-marrow and cytokine dependent (26,27). In the examined patients, we observed an increase in the number of natural killer cells over the normal range with length of time on CAPD. That finding accords with the study performed in CAPD patients by Palop et al (3). Natural killer cells and their precursors are more resistant to immunosuppressive agents than are other immunocompetent disturbances (28,29), so that suppressive uremic toxins affect the number and function of T lymphocytes more effectively than they affect the number and function of natural killer cells (29,30). In patients on peritoneal dialysis, episodes of peritonitis and exit-site and tunnel infection may stimulate production of natural killer cells. In hemodialyzed patients, the percentage of natural killer cells was significantly higher compared with a control group, but the absolute number of those cells was analogous to that found in healthy volunteers (11,12). That difference between peritoneal dialysis and hemodialysis patients might be related to the greater number of infectious episodes in peritoneal dialysis patients.

In our patients taking ACEIs but not rHuEPO, a significant negative correlation was shown between total ACEI dose and natural killer cell count. An earlier study demonstrated that administration of ACEIs (causing an increase in serum bradykinin concentration) might decrease T lymphocyte proliferation (17). In addition, ACEIs can abolish a positive effect of rHuEPO on bone marrow. Patients treated with ACEIs may require higher rHuEPO doses in the course of peritoneal dialysis (31) or hemodialysis (32,33). On the other hand, a positive correlation between rHuEPO and ACEI dose can indicate a necessity for increments in antihypertensive drug doses owing to an increase in blood pressure related to rHuEPO administration or to difficulties in proper dehydration of patients when the peritoneal membrane permeability increases in the course of CAPD.

### Table III: Total lymphocyte count (TLC) and lymphocyte subset counts in peripheral blood from continuous ambulatory peritoneal dialysis patients grouped according to drug administration

<table>
<thead>
<tr>
<th>Lymphocytes (10⁹/L)</th>
<th>Drug administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rHuEPO only (n=7)</td>
</tr>
<tr>
<td>TLC</td>
<td>1.57±0.36</td>
</tr>
<tr>
<td>CD3</td>
<td>1.14±0.30</td>
</tr>
<tr>
<td>CD4</td>
<td>0.46±0.13a</td>
</tr>
<tr>
<td>CD8</td>
<td>0.40±0.12a</td>
</tr>
<tr>
<td>CD19</td>
<td>0.09±0.02a</td>
</tr>
<tr>
<td>CD16+56</td>
<td>0.30±0.09</td>
</tr>
</tbody>
</table>

* Mean value below the normal range.

rHuEPO = recombinant human erythropoietin; ACEI = angiotensin-converting enzyme inhibitor.
Conclusion
In the course of CAPD, rHuEPO and ACEIs can influence total lymphocyte count or lymphocyte subset counts the natural changes being disturbed with prolongation of CAPD treatment. The possibility of such an influence should be taken into account when evaluating lymphocyte counts as indices of immune and nutrition status.

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References


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Based on previous work, we hypothesized that the alterations in tissue hyaluronan concentration ([HA]) brought about by chronic treatment of the peritoneum would result in corresponding changes in solute and water transport. To address that hypothesis, we carried out daily intraperitoneal (IP) injections for 2 months in 200—300 g rats of 30—40 mL of a sterile solution via peritoneal catheter tunneled to a subcutaneous port in the neck. Solutions used were: 4% N-acetylglucosamine (NAG), 4% mannitol (M), 4% glucose (G), and control group (C).

After 2 months, each animal underwent transport studies, using plastic chambers affixed to the parietal peritoneum of the abdominal wall, to determine: small-solute mass, osmotic filtration, and albumin flux in response to 450—500 mOsm/kg. After each animal was humanely killed, the tissue below the chamber was removed and analyzed for [HA].

A 50% enhancement in [HA] in tissue was seen in NAG-treated animals as compared with animals in the other groups. Results for small-solute transport (one-way ANOVA, \( p > 0.6 \)) and osmotic filtration (one-way ANOVA, \( p > 0.2 \)) both demonstrated no significant differences among groups. The albumin flux (mean ± standard error of the mean) in the control group (C) was significantly higher (0.36 ± 0.03 µL/min/cm²) than in the three treatment groups, but no difference was seen among the treatment groups (NAG: 0.25 ± 0.03 µL/min/cm²; M: 0.26 ± 0.03 µL/min/cm²; G: 0.29 ± 0.03 µL/min/cm²; one-way ANOVA, \( p = 0.064 \)).

We conclude that increasing [HA] by 50% in parietal peritoneal tissue does not bring about major changes in transperitoneal transport.

Key words
Hyaluronan, osmotic ultrafiltration, albumin transport

Introduction
Previously, we demonstrated that acute enhancement of the hyaluronan content ([HA]) of abdominal wall tissue resulted in a decrease in the hydraulic conductivity through the tissue, and that decrease of [HA] in the tissue resulted in a marked increase in hydraulic conductivity and movement of large solutes (1). Studies by others (2,3) have demonstrated that increasing [HA] by chronic administration has improved net ultrafiltration and decreased protein loss. Wu and colleagues (4) showed that peritoneal transport characteristics improve with the chronic administration of N-acetylglucosamine (NAG), substituted for dextrose in PD solutions. N-Acetylglucosamine is one of the two components of hyaluronan and resulted in increased alcian blue staining for total glycosaminoglycans within the tissue.

In the present work, we set out to test the hypothesis that NAG enhances [HA] in the subperitoneal tissue. After exposing the peritoneal cavity daily to a variety of solutions, including a solution of NAG, we attempted to directly link [HA] in tissue with transport phenomena.

Materials and methods

Animals and implantation of tunneled catheters
All procedures were performed in accordance with the University of Rochester’s Committee on the Use and Care of Animals.

Female Sprague—Dawley rats, 200—225 g (± 40), were anesthetized with intramuscular injections of sodium pentobarbital (60 mg/kg). Using aseptic technique, an incision was made in the skin of the posterior neck and in the skin overlying the linea alba in the abdominal wall. Blunt dissection of each area was performed. A Silastic catheter connected to a small subcutaneous (SC) chamber (Rat-O-Port: Access Technologies, Skokie, Illinois, U.S.A.) was tunneled from the neck under the skin to the abdomen. A trocar was used to penetrate the abdominal wall, and the cath-

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eter tip was inserted into the peritoneal cavity and secured with a purse stitch in the abdominal wall. The skin overlying the abdomen was closed with wound clips. The chamber in the neck was secured to the underlying muscle with an absorbable suture.

Prior to closure of the skin over the chamber, 1 mL of a solution containing 5 mg cefazolin was administered via the catheter, followed by 1 mL containing 10 U heparin. The animal was returned to its cage and carefully monitored until recovery. Over the next week, each animal was examined for infection or complications from the surgery.

Chronic injections
After 1 week, wound clips were removed and daily 30—40 mL injections (varied according to the animal’s size) were administered for 2 months via the SC port while the animal was under halothane gas anesthesia. The sterile solutions used were a Krebs—Ringer—bicarbonate (KRB) solution containing 4% mannitol (group M), a KRB solution containing 4% glucose (group G), and a KRB solution containing 4% N-acetylglucosamine (group NAG). Controls (C) included (i) age-control animals receiving no treatment and no catheter; (ii) catheter-control animals who had a catheter placed, but no treatment; and (iii) animals injected with KRB alone. The three C subgroups had matched transport and tissue characteristics and were therefore grouped together for comparisons. Complications were minimized with the use of titanium ports and a 7 French catheter. All sterile solutions were handled within a laminar air-flow hood, and the skin over the subcutaneous port was carefully prepped with Betadine before each injection. Other than the initial dose given at catheter placement, antibiotics were not administered prophylactically.

Transport studies
After 2 months of daily injections, each animal was anesthetized with intramuscular (IM) sodium pentobarbital (60 mg/kg). Intravenous and intra-arterial catheters were placed; a tracheostomy was performed; the animal’s temperature was maintained between 35°C and 38°C; blood pressure was monitored to assure that the mean was greater than 80 mmHg. A laparotomy was performed, and a plastic chamber [diameter approximately 1.3 cm, volume approximately 3 mL, see (5) for details] was affixed to the serosa of the abdominal wall.

In the first 90 minutes, a hypertonic solution (4% mannitol in Krebs—Ringer—bicarbonate, approximately 500 mOsm/kg) containing 14C-mannitol was placed in the chamber. The volume and tracer concentrations were measured for 90 minutes to determine the volume flux (flow rate / Area_chamber) into the chamber and the mass of mannitol remaining in the chamber after 90 minutes. At 90 minutes, fluorescein isothiocyanate conjugate (FITC)—albumin was administered intravenously, and the plasma concentration and chamber volume and concentration were measured versus time over an additional 180 minutes. From those measurements, the albumin flux (mass transfer rate / plasma concentration / Area_chamber) was calculated.

After the animals were humanely killed, the tissue under the chamber was collected, and a portion was weighed, digested, and processed for measurement of [HA] as in our previous publication (6).

Statistics
Results are reported as mean ± standard error of the mean. Specific effects were tested using one-way ANOVA.

Results
The [HA] of the tissue under the chamber had only slight variations among the control, mannitol, and glucose groups (962 ± 73 µg/g dry tissue for C to 1169 ± 69 µg/g for G). However, the NAG solution increased the [HA] by 50% above that of the control animals (1428 ± 69 µg/g). No significant differences were seen among the groups for small-solute mass at 90 minutes of dwell in the chamber (0.88—0.90, p > 0.6) or for the osmotic flux (G: 41.4 ± 10.4 µL/min/cm²; C: 72.6 ± 10.9 µL/min/cm²; p > 0.2). Albumin appearance was approximately the same for each of the three treatment groups (NAG: 0.25 ± 0.03 µL/min/cm²; M: 0.26 ± 0.03 µL/min/cm²; G: 0.29 ± 0.03 µL/min/cm²), while the control group had a higher rate of albumin appearance. The one-way ANOVA for that data was not significant at p = 0.064.

Discussion
In the search for more biocompatible solutions, many groups have developed chronic animal models of peritoneal dialysis to test new solutions or additives to currently used solutions. Typically, an indwelling catheter is used to administer daily injections of solutions (2). At specified times, a procedure similar to the
4-hour peritoneal equilibration test is carried out to measure urea and creatinine dialysate-to-plasma ratios, protein loss, and net ultrafiltration. In some studies, samples of the mesentery (2) or omentum (7) are collected at the end of the transport measurements to analyze [HA] and to examine the changes in morphology brought about by the chronic administration of the test solution. In those studies, therefore, it is assumed that the tissue sampled is representative of the tissue in contact with the dialysis fluid during the transport measurements. We have recently shown in rodents that, even with maximal volumes in the peritoneal cavity, only about 40% of the surface is exposed to fluid during a dwell (8). Therefore, the assumption that tissue analysis can be linked to the observed transport phenomena is questionable.

In this study, we directly linked the hyaluronan content in parietal peritoneal tissue to the observed transport. We used an injection technique similar to that of Zweers and colleagues (7) to expose the peritoneal tissue to several different treatment solutions, each having a high osmolality (approximately 500 mOsm/kg) and a normal pH. The chamber technique isolates a portion of the parietal peritoneum, and transport experiments are carried out which determine small-solute transport, albumin transport, and the flow of water from the tissue as a result of the osmotic gradient. At the end of the experiment, the tissue directly under the chamber is analyzed for [HA].

Despite inducing significant changes in [HA] with the use of NAG, we saw no major changes in transport of small solutes or osmotically induced water in comparison with solutions having comparable osmotic pressure, or with controls. We observed larger differences between the treatment and control groups in albumin transport, but no significant differences were seen among the treatment groups. The present experiments differ from previous studies in that they provide a direct link between transport and tissue structure. A limitation is that only one type of tissue can be measured in each animal. Thus, other tissues (such as the intestine) could account for the varying results found in other studies (4).

References

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