Chronic exposure to peritoneal dialysis (PD) solutions is associated with a low-grade local inflammatory state of the peritoneum. The occurrence of culture-negative peritonitis in some PD patients treated with icodextrin focused our interest on subclinical inflammation in icodextrin-treated patients without peritonitis. The aim of the present study was to compare signs of inflammation in icodextrin-treated patients with the same signs in patients using glucose/lactate–based (GL) dialysis solutions only.

Overnight PD effluents from 19 patients treated with icodextrin and 19 patients treated with GL were investigated for leukocyte count (LC) and differentiation (LD), and for dialysate concentrations of cancer antigen 125 (CA125, the marker of mesothelial cell mass) and hyaluronan (marker of inflammation and tissue remodeling in the peritoneal cavity). Blood cell counts and serum dextran antibodies (DA) were also determined. Total LC in the GL group was significantly lower than that in the icodextrin group. The LD was not different between the two groups, except for the percentage of eosinophils. The blood cell count did not differ between the groups. The median value of DA was similar in both groups. The hyaluronan concentration was markedly higher in the icodextrin group. No significant difference was found for dialysate CA125.

In conclusion, the higher effluent cell count, higher percentage of eosinophils, and higher effluent hyaluronan levels in icodextrin-treated patients are consistent with a greater degree of subclinical inflammation during icodextrin treatment than during GL treatment.

Peritoneal Effluent Markers of Inflammation in Patients Treated with Icodextrin-Based and Glucose-Based Dialysis Solutions

Key words
Inflammation, leukocyte cell count, leukocyte differentiation, icodextrin, glucose/lactate–based dialysis solution

Introduction
In 2001 and 2002, in various European centers, an abnormally high incidence of culture-negative peritonitis was reported in peritoneal dialysis (PD) patients who used icodextrin-based dialysis solution (Extraneal: Baxter Healthcare SA, Castlebar, Ireland). Investigations by the producer pointed to high concentrations of peptidoglycan as the most likely cause of a sterile inflammatory reaction [Personal communication, Baxter Healthcare; and (1)]. That situation focused our attention on the lack of published results regarding normal values for peritoneal effluent cell counts and differentiation in PD patients without peritonitis who are treated with 7.5% icodextrin for the long dwell.

The objective of the present study was to compare signs of local inflammation in stable PD patients treated with icodextrin with the same signs in patients treated with glucose/lactate–based dialysis solution only.

Patients and methods
Long-dwell effluents (median duration: 11 hours; range: 8 – 16 hours) were obtained from 38 stable PD patients (26 men, 12 women; median age: 55 years; age range: 20 – 83 years). The effluents were investigated for leukocyte count and differentiation, and for dialysate concentrations of cancer antigen 125 (CA125, the marker of mesothelial cell mass) and hyaluronan (a marker of inflammation and tissue remodeling). Blood samples were taken at the same time to screen for leukocyte differential cell count and the presence of dextran antibodies.
Of the 38 patients, 19 (GL group) were treated only with commercially available glucose/lactate-based dialysis solutions (Dianeal: Baxter Healthcare); the other 19 used 7.5% icodextrin (Extraneal: Baxter Healthcare) for the long dwell. Of the latter group, 9 patients (icodextrin group 1) used certain batches of icodextrin on which other PD patients had developed culture-negative peritonitis. The remaining 10 patients (icodextrin group 2) started treatment with icodextrin after delivery of new batches (October 2002).

Before inclusion in our study, the patients had been treated with the investigated type of PD solution for at least 1 month, were showing no signs of infection at the time of examination, and had been peritonitis-free for 4 weeks prior to the investigation. The patients in the GL group had been treated with PD for a median period of 23 months (range: 3 – 168 months); those in icodextrin group 1, for a median of 14 months (range: 12 – 32 months); and those in icodextrin group 2, for a median of 15 months (range: 1 – 59 months).

The effluents were cultured using our normal procedures (2), and leukocytes were counted in a Bürker counting chamber. The differential cell counts were performed on cytospin effluents after Jenee–Giemsa staining. The procedure was this: centrifugation for 20 minutes at 1500 rpm, removal of the supernatant, and mixing of the final pellet with 1 mL phosphate-buffered saline. Cytospins of the pellets were prepared using 60 µL of the suspension.

A microparticle enzyme immunoassay, in combination with a monoclonal antibody against CA125 (Abbott Laboratories, Abbott Park, IL, U.S.A.), was used to measure CA125. A radioimmunoassay (Kabi Pharmacia Diagnostics AB, Uppsala, Sweden) was used to determine the peritoneal hyaluronan concentration. The total leukocyte count and differential cell counts in blood were determined by flow cytometry (Technicon H1: Technicon Instruments, Tarrytown, NY, U.S.A.) in blood anticoagulated with ethylenediaminetetraacetic acid (EDTA). Dextran antibodies were measured in serum by an enzyme-linked immunosorbent assay (ELISA) using the dextran 500,000 antigen (Pharmacia, Uppsala, Sweden), anti-human immunoglobulin G antibodies (Dako Corporation, Glostrup, Denmark) and O-phenylenediamine dihydrochloride (Sigma, Steinheim, Germany) as substrate. The quantity of dextran antibodies is expressed as a percentage of a serum pool obtained from 150 healthy subjects.

Statistical analysis
Owing to asymmetric distribution, results are expressed as medians and ranges. The Mann–Whitney test was applied to compare differences between the groups.

Results
Table I presents the dialysate total leukocyte counts, differential cell counts expressed as percentages, and percentages of mesothelial cells. The total leukocyte count and the percentage of eosinophils were lower in the GL group than in the two icodextrin groups ($p = 0.01$). The icodextrin groups were not different from one another. The percentages of other cells were similar among the groups. The absolute counts for all leukocyte types, except basophils, were higher in icodextrin group 1 ($p < 0.03$) than in the GL group; only the eosinophil and monocyte counts were higher in icodextrin group 2 ($p < 0.01$) than in the GL group (Table II). Table III gives the total leukocyte counts and differentiations for blood. No difference was significant.

The median dialysate CA125 concentration was 35 U/mL (range: 7 – 163 U/mL) in the GL group, 36 U/mL (range: 17 – 73 U/mL) in icodextrin group 1, and 21 U/mL (range: 8 – 54 U/mL) in icodextrin group 2 ($p = $nonsignificant$).

The median level of serum dextran antibodies was 25% (range: 6% – 630%) in the glucose group, 23% (range: 2% – 376%) in icodextrin group 1, and 23% (range: 5% – 450%) in icodextrin group 2. The differences were not significant.

The dialysate hyaluronan level was related to the dialysate total leukocyte count ($r = 0.51, p = 0.01$, Figure 1). The hyaluronan concentration was 355 µg/L (range: 117 – 256 µg/L) in the GL group and 682 µg/L (range: 499 – 1298 µg/L) in icodextrin group 1 ($p < 0.01$).

Discussion
Chronic exposure to PD solutions is associated with impairment of peritoneal host defense. Alterations of cellular components within the peritoneal cavity lead to disturbances in the release of pro- and anti-inflammatory mediators (3,4). Normal values for effluent cell counts and differentiation have been published for...
TABLE I  Dialysate leukocyte counts and differential cell counts in effluent from the long dwell in patients treated with 7.5% icodextrin solution as compared with counts in effluent from patients treated only with glucose/lactate–based solutions

<table>
<thead>
<tr>
<th></th>
<th>Glucose/lactate&lt;sup&gt;a&lt;/sup&gt; (n=19)</th>
<th>Icodextrin 1&lt;sup&gt;b&lt;/sup&gt; (n=9)</th>
<th>Icodextrin 2&lt;sup&gt;c&lt;/sup&gt; (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total leukocyte count (&lt;$10^6$/L)</td>
<td>5 (0–80)</td>
<td>25 (10–85)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>30 (0–70)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Macrophages (%)</td>
<td>86 (5–100)</td>
<td>70 (61–90)</td>
<td>76 (56–86)</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>5 (0–90)</td>
<td>9 (0–26)</td>
<td>4 (0–11)</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>6 (0–50)</td>
<td>10 (1–13)</td>
<td>12 (6–14)</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>1 (0–6)</td>
<td>6 (1–8)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6(3–22)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>0 (0–1)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td>Mesothelial cells (%)</td>
<td>2 (1–6)</td>
<td>2 (2–3)</td>
<td>2 (1–4)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Patients who used glucose/lactate–based dialysis solution only.

<sup>b</sup> Stable patients who used certain batches of 7.5% icodextrin on which other peritoneal dialysis patients developed culture-negative peritonitis.

<sup>c</sup> Patients who started icodextrin treatment after omission of the potential causative agent for culture-negative peritonitis.

<sup>d</sup> p = 0.01 versus glucose/lactate group.

TABLE II  Absolute counts of the peritoneal leukocyte population in effluent from the long dwell in patients treated with 7.5% icodextrin or only with glucose/lactate–based dialysis solutions

<table>
<thead>
<tr>
<th></th>
<th>Glucose/lactate&lt;sup&gt;a&lt;/sup&gt; (n=19)</th>
<th>Icodextrin 1&lt;sup&gt;b&lt;/sup&gt; (n=9)</th>
<th>Icodextrin 2&lt;sup&gt;c&lt;/sup&gt; (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophages (&lt;$10^6$/L)</td>
<td>4.7 (0–69.9)</td>
<td>18.3 (14.0–75.9)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>23.9 (0–56)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lymphocytes (&lt;$10^6$/L)</td>
<td>0.1 (0–20.9)</td>
<td>3.6 (0.6–11.4)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.1 (0–7.8)</td>
</tr>
<tr>
<td>Neutrophils (&lt;$10^6$/L)</td>
<td>0.2 (0–27.2)</td>
<td>2.1 (0.2–8.3)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.9 (0–7.8)</td>
</tr>
<tr>
<td>Eosinophils (&lt;$10^6$/L)</td>
<td>0.1 (0–4.2)</td>
<td>1.8 (0.2–5.6)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.0 (0–15.6)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basophils (&lt;$10^6$/L)</td>
<td>0 (0–0.1)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td>Mesothelial cells (&lt;$10^6$/L)</td>
<td>0.2 (0.1–2.5)</td>
<td>0.5 (0.3–0.6)</td>
<td>0.6 (0.4–1.1)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Patients who used glucose/lactate–based dialysis solution only.

<sup>b</sup> Stable patients who used certain batches of 7.5% icodextrin on which other peritoneal dialysis patients developed culture-negative peritonitis.

<sup>c</sup> Patients who started icodextrin treatment after omission of the potential causative agent for culture-negative peritonitis.

<sup>d</sup> p < 0.01.

<sup>c</sup> p = 0.03 versus glucose/lactate group.

TABLE III  Comparison of blood leukocyte populations between patients treated with 7.5% icodextrin and patients treated with glucose/lactate–based dialysis solution for the long dwell

<table>
<thead>
<tr>
<th></th>
<th>Glucose/lactate&lt;sup&gt;a&lt;/sup&gt; (n=19)</th>
<th>Icodextrin 1&lt;sup&gt;b&lt;/sup&gt; (n=9)</th>
<th>Icodextrin 2&lt;sup&gt;c&lt;/sup&gt; (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total leukocyte count (&lt;$10^6$/L)</td>
<td>8 (5–20)</td>
<td>8 (7–9)</td>
<td>8 (6–9)</td>
</tr>
<tr>
<td>Macrophages (%)</td>
<td>7 (5–13)</td>
<td>8 (5–14)</td>
<td>8 (6–14)</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>19 (7–45)</td>
<td>21 (9–23)</td>
<td>20 (12–27)</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>68 (41–87)</td>
<td>68 (64–83)</td>
<td>66 (60–79)</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>3 (0–17)</td>
<td>4 (0–5)</td>
<td>4 (0–9)</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>0 (0–1)</td>
<td>0 (0–1)</td>
<td>0 (0–1)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Patients who used glucose/lactate–based dialysis solution only.

<sup>b</sup> Stable patients who used certain batches of 7.5% icodextrin on which other peritoneal dialysis patients developed culture-negative peritonitis.

<sup>c</sup> Patients who started icodextrin treatment after omission of the potential causative agent for culture-negative peritonitis.
glucose-based dialysis solutions (5–7). In the present study, we investigated those parameters for 7.5% icodextrin. In addition, we analyzed dialysate concentrations of the mesothelial cell mass marker CA125 and the inflammatory marker hyaluronan.

Leukocyte numbers and differentiation in glucose effluents were similar to those reported previously by our group (6). Icodextrin effluents contained significantly more leukocytes. Also, the percentage of eosinophils was markedly higher in icodextrin effluent than in glucose effluent. Nevertheless, the values found for icodextrin were still within the normal ranges reported in previous studies for glucose solutions (6–9).

Icodextrin group 1 was investigated at the time of a culture-negative peritonitis epidemic in icodextrin patients. We therefore could not exclude the possibility that the values obtained might have been influenced by high dialysate peptidoglycan levels. For that reason, we investigated icodextrin group 2, which was studied after the peptidoglycan was removed from the icodextrin dialysis solution. However, the results obtained in icodextrin group 2 were not different from those obtained in icodextrin group 1. That finding makes it unlikely that the cell counts we found were a subclinical manifestation of peptidoglycan-induced culture-negative peritonitis.

Effluent cell counts are generally lower in long-term PD patients than in patients in their first few years of PD (6). Because the duration of PD was similar in the three patient groups, time on PD is excluded as a cause for the differences found.

Dialysate hyaluronan, which is considered a marker of peritoneal inflammation (10), was significantly higher in the icodextrin-treated patients than in patients treated only with glucose solution. Moreover, a correlation was present between effluent hyaluronan and leukocyte count.

The osmotic effectiveness of icodextrin is well established, but the biocompatibility of the solution is controversial. Some studies detected a less injurious impact on membrane function and host defense (11–15); other studies have not observed differences between icodextrin-based and glucose/lactate–based solutions (16–18).

Our data indicate that icodextrin might induce a somewhat greater local inflammatory reaction than does glucose-based solution. However, no differences in effluent concentration of CA125 (the mesothelial cell mass marker) were present among the three groups. Dialysate CA125 is increased during peritonitis (19). Our results therefore indicate that the enhancement of the inflammatory response under icodextrin does not lead to mesothelial cell damage. That finding may explain the results of in vitro studies that show better host defense with icodextrin (11–15). However, differences in the incidence of microbial peritonitis between icodextrin-treated and glucose-only patients have not been reported.

Effluent hyaluronan could not be determined in icodextrin group 2 because production of the relevant radioimmunoassay kits had been discontinued. Nevertheless, based on the similarity between icodextrin group 1 and icodextrin group 2, we assume that the results in icodextrin group 2 would have been similar.

Comparison of blood dextran antibodies revealed no difference between the GL group and the two icodextrin groups. That finding agrees with previous data (20), in which no relationship was found between dextran antibodies and chronic exposure to icodextrin solution.

**Conclusion**

Based on higher cell counts and higher hyaluronan concentrations, we conclude that icodextrin shows some enhancement of the local inflammatory response. That finding was not associated with signs of mesothelial damage. Our findings are unrelated to peptidoglycan-induced culture-negative peritonitis.
References


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Icodextrin is a glucose polymer obtained from starch hydrolysis. It is used as an osmotic agent at 7.5% for peritoneal dialysis (PD). Its use in PD has been associated with several side effects separate from the one reported here, the most frequent being sterile peritonitis. Recently, three mechanisms have been proposed to explain the occurrence of sterile peritonitis: allergy to dextrin, production of anti-dextran antibodies, and impurities introduced during manufacture. Here, we report a peritoneal mononucleosis outbreak that is highly suggestive of being a consequence of the last-mentioned mechanism.

During the period December 2001 to May 2002, a group of 8 Spanish hospitals whose individual PD programs regularly share information and activity reported 29 cases of sterile peritonitis associated with icodextrin use in continuous ambulatory peritoneal dialysis (CAPD) patients [mean age: 60.7 ± 14.47 years; 8 women (27.59%), 21 men (72.41%); mean time on PD: 25.21 ± 35.31 months; mean time on icodextrin: 15.17 ± 11.03 months]. Of the 29 patients, 51.8% showed no symptoms. The remainder presented with mild abdominal discomfort and anorexia. Only 2 patients showed general malaise, severe nausea, fever, and abdominal pain. The initial white cell count in peritoneal effluent was 512 ± 386 cells/mL (45.0% ± 28% neutrophils, 44.92% ± 32.6% mononuclear cells, 7.75% ± 12% eosinophils). In 5 of the patients, we performed an immunophenotype (CD14) study, demonstrating the monocyte nature of 60% – 80% (mean: 70.6%) of the cells. Microbiology cultures were always negative. A rechallenge with the same batches of PD fluid was tried. In 100% of the patients, the clinical and cellular patterns relapsed. No short-term changes in peritoneal function have been observed. The manufacturer informed us that the icodextrin was contaminated with a peptidoglycan.

In this sterile peritonitis outbreak with a simultaneous, similar clinical presentation in a group of patients treated with icodextrin solution (presumably contaminated with peptidoglycan), clinical outcome was, for the most part, mild-to-moderate. Symptoms disappeared immediately after icodextrin withdrawal and relapsed after rechallenge with the relevant fluid batches. Monocyte cell counts predominated during the episode. Although we cannot rule out an allergic cause, the massive peritoneal mononuclear cell recruitment suggests a particular mechanism. This is a new mechanism for peritoneal cell recruitment in PD.

Key words
Mononucleosis, icodextrin, dialysate contamination, peptidoglycan

Introduction
Icodextrin is a glucose polymer obtained from starch hydrolysis. It is used as an osmotic agent (282 mOsm/kg) at 7.5% for peritoneal dialysis (PD), being very useful for long-dwell exchanges. Icodextrin use reduces the need for glucose-containing dialysate, potentially favoring the status of the peritoneal membrane.

The icodextrin molecule is variable in size, with a range of subunits from 4 to 250 (molecular weight: 1,638 Da – 45,000 Da; mean: 16,200 Da; median: 5,800 Da). The molecule structure branches variably by mean of α linkages (less than 10% 1→4, and more
than 85%\textsuperscript{6}) in varying quantities and proportions. Dextrans are composed only of \(\alpha\) linkages\textsuperscript{6}) and have a limited catabolic capacity (1).

Icodextrin PD solution is generally well tolerated, but several side effects separate from the one reported here have been described. The first side effects reported were psoriasis-like skin reactions, epidermolysis, and blistering, which were suggested to be cross-reactivity with dextran (2–4). Interference with plasma determinations of glucose and amylase have also been found. But the most frequent side effect is so-called sterile peritonitis. Recently, three mechanisms have been proposed to explain that condition: allergy to dextrin, production of anti-dextran antibodies, and impurities introduced during fluid manufacture (5).

In the present article, we report a peritoneal mononucleosis outbreak, manifested as sterile peritonitis episodes, that is highly suggestive of being a consequence of the last-mentioned mechanism.

**Patients and methods**

During the first meeting of Grupo Centro de Diálisis Peritoneal (GCDP) in 2002, the existence of several cases of icodextrin-related sterile peritonitis were reported. A decision was taken to collect all of those episodes when they appeared in any of the 26 hospitals in the group. To that purpose, an episode was defined as any increase in peritoneal cell count > 100/mL, whether or not accompanied by abdominal or general symptoms, with no microbiological growth from effluent sampled in appropriate conditions (no antibiotics) from patients using icodextrin. Nephrologists were alerted to observe (independently of their own requirements) patients using icodextrin.

The following 8 hospitals reported a total of 29 episodes: 4 in Fundación Jiménez–Díaz (Madrid), 4 in Hospital Alarcos (Ciudad Real), 3 in Hospital La Paz (Madrid), 1 in Hospital del Aire (Madrid), 1 in Hospital San Pedro de Alcántara (Cáceres), 1 in Hospital Príncipe de Asturias (Alcalá de Henares), 2 in Hospital San Carlos (Madrid), and 13 in Hospital La Princesa (Madrid). All of the episodes were reported to the Spanish health authorities.

The methodology for peritoneal effluent culture is similar in all of the hospitals. However, the cell count methodology is different: some methods are automated (and distinguish lymphocytes from monocytes, all of them referred to as “mononuclear”) and some methods are manual, with direct observation (and unable to separate mononuclear cells from one another).

**Results**

The mean age of the affected patients was 60.7 ± 14.47 years (range: 25 – 85 years). Eight (27.59%) were female, and 21 (72.41%) were male. Mean time on dialysis was 25.21 ± 35.31 months (range: 1 – 145 months), and the mean time on icodextrin was 15.17 ± 11.03 months (range: 1 – 52 months). Of the 29 patients, 28 were being treated with CAPD, and 1 with optimized continuous peritoneal dialysis (OCPD), with a diurnal icodextrin exchange of the CAPD type. The icodextrin batches involved were R25JI7G40, O2A10G40, O2A31G42, R29K29G42.

Clinical manifestations (mild abdominal pain and anorexia) were present in 14 of 29 patients (48.2%). Two patients presented with peritoneal rebound, fever, and nausea. The other patients showed no symptoms at all. The manifestations were independent of hospital, dialysate batch, demographic characteristics, and initial cell count.

With regard to the peritoneal effluent cell count, the cell count during the initial outbreak showed varying results, according to the methodology used. (The automated device used in one of the hospitals revealed that most of the cells were mononuclear. As a result of the varying approaches, the mean results reported here have the potential for misinterpretation.) A mean of 512 ± 386 cells/mL (45.4% ± 28% neutrophils, 44.92% ± 36% mononuclear cells, 7.75% ± 12% eosinophils) were detected. An initial mononuclear predominance was present in 20 of 29 patients (69%); 1 patient later changed to eosinophil predominance. The other 9 patients (31%) started with neutrophil predominance (>60% of the count); 3 later changed to mononuclear cell predominance.

In 29.1% of patients, the syndrome resolved in less than 24 hours after icodextrin withdrawal. In 29.1% of patients, resolution came in 1 – 3 days; and in 29.1%, in 4 – 7 days. In 12.5% of the cases, the syndrome lasted for more than 7 days. For 5 patients, we have no information about outcome. Half of the patients received empiric intraperitoneal antibiotics (most of them before the outbreak was recognized). After the second meeting of the GCDP, antibiotic treatment was restricted to doubtful cases, so that the rest of the patients did not receive empiric antibiotics.
Severe Peritoneal Mononucleosis with ICO in CAPD

The 13 cases at our center (all of them at a mild grade of severity, and most of them undetected by the patient) represented 100% of our icodextrin users. To clarify the peritoneal effluent cell count, we asked 6 patients to reintroduce an isolated icodextrin exchange from the same batch that provoked the phenomenon. A similar clinical picture unequivocally followed re-instillation of icodextrin. Immuno-phenotypic characterization by flow cytometry confirmed the monocytic nature of the cells in 5 of 6 cases (60% – 80% CD14+; mean: 70.6%; Figure 1).

The manufacturer (Baxter Healthcare SA, Castlebar, Ireland) found that the icodextrin batches involved were contaminated by a peptidoglycan.

Discussion

In the present article, we describe an outbreak of sterile peritonitis caused by contamination of several batches of icodextrin fluid for CAPD. The principal peritoneal reaction was mononuclear cell infiltration. This occurrence should be added to the previously known causes of sterile peritonitis in PD.

We were not able to establish a cause–effect relationship between the contaminant (peptidoglycan) and the particular peritoneal cell recruitment. What we observed is that, in the cases in which polymorphonuclear neutrophilic granulocytes (PMNs) were not involved, the reaction seemed to be self-limiting. It disappeared, leaving no markers of damage, after icodextrin withdrawal.

Monocyte chemoattraction is mediated by specific molecules, mainly monocyte chemoattractant protein-1 (MCP-1) (6). That molecule may mediate the particular monocyte migration related to peptidoglycan contamination of icodextrin. The mechanism goes beyond our initial aim, which was simply to describe a new model in peritoneal cell recruitment. That model was previously observed after addition of intraperitoneal granulocyte macrophage–colony stimulating factor (GM-CSF) in vivo (7). The characteristic that the model has in common with the present situation is the maintenance of cell integrity, which preserves peritoneal tissue from the catastrophic consequences of rupture of white cells.

In other words, we can consider monocyte recruitment induced by peptidoglycan to be a chemotactic phenomenon instead of an inflammatory condition. However, the data revealed by a peritoneal biopsy study in an identical situation might question the benignity of the process (8). Denudated mesothelium, submesothelial edema, and monocyte infiltration confirm that the process has some anatomic repercussions. Because the biopsy was taken after brief use of icodextrin, we may not have been able to observe more profound changes. The process is defined immunologically as type IV hypersensitivity reaction, mediated by dendritic cells.

In clinical terms, most of our patients showed a transitory reaction, totally dependent on the presence of the contaminated icodextrin, with no functional repercussions at medium term. In contrast, prolonged use of the contaminated fluid was associated with a severe reaction in 2 cases. In those cases, the peritoneal polymorphonuclear cell recruitment and systemic repercussions are suggestive of a classical inflammatory process.

To summarize, the extended use of contaminated icodextrin may generate a change in the initial response to the contaminant, changing into a true peritonitis process with all of its potential sequelae. We strongly recommend using a cytology diagnosis to differentiate peritoneal mononucleosis in its early stage, considering that removing icodextrin at that stage assures a benign and quiet outcome.

Conclusions

In the present article, we describe a sterile peritonitis outbreak with simultaneous, similar clinical presentation in a group of patients treated with icodextrin.
presumably contaminated with peptidoglycan. Clinical outcome was mild-to-moderate in most of the patients, with immediate disappearance of the symptoms after icodextrin withdrawal. Symptoms relapsed upon rechallenge with batches of the fluid involved, and monocytes predominated in the cell counts during the episodes. Although we cannot rule out an allergic reaction, the massive recruitment of peritoneal mononuclear cells suggests a particular mechanism. This is a new mechanism for peritoneal cell recruitment in PD.

References

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Bacteria from the exit site of the peritoneal dialysis (PD) catheter and from contaminated dialysis fluids can grow into microcolonies in biofilm. Those bacteria are implicated in recurrent peritonitis in patients undergoing treatment with PD. The present article highlights new strategies that are designed to alter the major factors regulating biofilm formation and that thereby reduce biofilm-related infections in PD patients.

Key words
Biofilm bacteria, peritonitis, catheter biomaterials, antibiotic lock

Introduction
Biofilm formation on peritoneal catheters is common (1). The primary factors that regulate biofilm formation in peritoneal dialysis (PD) are (A) skin bacteria that colonize PD catheters, gaining entrance to the sterile peritoneal cavity from the exit site and or from touch contamination of PD fluid during daily dialysis exchange procedures; (B) catheter biomaterials that allow skin bacteria to adhere and to grow in biofilm microcolonies; and (C) a PD environment that provides optimum conditions for growth, proliferation, and dissemination of biofilm bacterial microcolonies from the catheter surface. The subject has recently been reviewed in detail (1).

Discussion
The strategies outlined below are useful for intervening in and preventing biofilm-related infection in PD patients.

Reduce access by skin bacteria
The primary strategy is to minimize access by skin bacteria to catheter surfaces:

- Use proper exit-site care and prophylactic antibiotics (for example, mupirocin) locally at the exit site (2,3)
- Screen for Staphylococcus carrier state in patients with recurrent catheter infections, and use prophylactic antibiotics for carriers (4,5)
- Avoid touch contamination of PD fluid by using disconnect systems such as the twin-bag system (6,7)
- Use the Moncrief–Popovich catheter implantation procedure when possible in PD (8). Use of the Moncrief–Popovich procedure reduces biofilm growth on catheter surfaces and exit-site infection (randomized study) in PD (8). The procedure is widely used even with routine catheters and has been termed the “AV [arteriovenous] fistula of PD treatment” (8).

Biofilm culture and antibiotic sensitivity
Early removal of a biofilm-colonized dialysis catheter and subsequent replacement with a new catheter is required to cure recurrent catheter-related infection in PD and to protect the peritoneum of the PD patient. The procedure is often arbitrarily done without proper culture of biofilm growth from PD effluents. Over time, biofilms develop antibiotic resistance as they develop a mature biofilm matrix, and, in those cases, catheter removal is indicated. But early biofilms are sensitive to antibiotics (9), and the catheter can be salvaged by proper antibiotic treatment. For that reason, it is preferable to perform a parallel culture of PD effluent with the routine microbiologic (planktonic) culture, together with a biofilm culture in the same clinical materials and a comparison of antibiotic sensitivities [minimal inhibitory concentrations (MICs)] between the two cultures. If the biofilm MIC is less than two dilutions different from the routine (planktonic) culture, early biofilm formation with mild antibiotic resistance is indicated. Antibiotic treatment should be continued, and
catheter removal will not be required. However, if the biofilm MIC is significantly different from the planktonic culture (>2 dilution difference), mature biofilm formation with high antibiotic resistance is present. Patients whose catheter-related infections show such a result are at high risk of treatment failure with continuation of routine doses of antibiotics. Catheter removal and replacement by a new catheter is appropriate in that subgroup (10).

The same strategy can be applied to determine the treatment end-point for antibiotic use in catheter-related infections in hemodialysis (HD) and PD patients alike. Several assays to culture biofilm growth from dialysis and other biologic fluids have been described in the literature, but neither the nephrology community nor the dialysis industry has used them to manage biofilm-related infections in dialysis patients (1).

**Drugs that penetrate biofilm layers**

In recent literature, the use of certain antibiotics (such as rifampin) and thrombolytic agents (such as streptokinase or urokinase, which penetrate biofilm matrix and allow antibiotics to act on biofilm bacteria) have been described for successful treatment of biofilm-related infections in dialysis patients.

Rifampin exhibits exceptional antimicrobial activity against staphylococcal biofilms, with a synergistic effect by addition of other antibiotics (11). Rifampin is therefore already recommended as a part of the antibiotic protocol for treatment of recurrent peritonitis in PD (12,13). However, use of rifampin is sometimes restricted, because it interacts with heparin, insulin, and several other drugs that are commonly used in patients on maintenance dialysis.

Thrombolytic agents such as streptokinase and urokinase also lyse biofilms and allow antibiotic penetration into biofilms. Those agents have been successfully used for recurrent peritonitis (14) and for catheter salvage in PD patients who develop two or more peritonitis episodes and cannot tolerate hemodialysis. Both drugs have similar efficacy. Drawbacks include adverse drug reactions with streptokinase. Also, urokinase is not always commercially available because of manufacturing problems. To circumvent those limitations, recombinant tissue plasminogen activator (tPA) has been used successfully in patients with relapsing peritonitis (15).

Biofilms can also be eradicated by instilling a highly concentrated solution of antibiotics (“antibiotic lock”) into the catheter lumen (16). According to the study protocol, the antibiotic solution was instilled into the catheter lumen at the end of each consecutive dialysis session. The solution was left to dwell until the beginning of the next dialysis session. A protocol for the use of antibiotic lock in conjunction with systemic antibiotics has recently been described for successful management of HD catheter-related infections (16). The concentration of the antibiotics in the lumen reached a level 100-fold higher than the corresponding systemic therapeutic concentration in plasma (16). The protocol permits eradication of biofilms and achievement of a bacteriologic cure without replacement of the catheter (16).

**New catheter biomaterials**

Silastic catheter materials are prone to biofilm formation. Research is in progress to develop new biomaterials that would prevent bacterial colonization. Among the new materials tested, hydroxyapatite-coated and silver-coated catheters seem to be promising (17). Clinical data for hydroxyapatite-coated catheters are still lacking. Silver-coated PD catheters are biocompatible, prevent bacterial colonization, and have antibacterial activity as well (17). However, silver is released from the catheter into the pericatheter tissues and adjoining blood vessels (17), raising concern that toxicity (argyria) could develop with long-term use. The release of silver from the coated catheters may indicate technical problems in laser-coating the silver onto Silastic catheters. Clinical use of silver-coated PD catheters cannot be considered safe pending further technical refinement in the quality of the coating (17).

**Alterations in the dialysis environment**

Clinical and research data indicate that biofilm growth and dissemination from PD catheters occur easily in a PD environment of conventional glucose-based dialysis solutions. No information is available about the influence of new PD solutions (for example, polyglucose and amino-acid solutions) on biofilm growth (1).

**Future research**

New treatment strategies for recurrent biofilm-related infections are under investigation in animal models. Those strategies include use of liposomal ciprofloxacin hydrogel-coated silicone in a rat model of persistent Pseudomonas peritonitis (18); use of a heparin-coated...
PD catheter, which improved exit-site healing in another rat model of PD (19); and use of a heptapeptide, RNAIII-inhibiting peptide, which slows the growth of staphylococcal biofilms on silicone and polyurethane dialysis catheters (20).

Adsorption of host proteins or other host molecules from blood plasma or serum can activate adhesion of bacteria on catheter surfaces to form heterogeneous biofilms with fibrin clots and other eukaryotic cells (1). Subsequent dislodgment of those biofilms may induce chronic and distant tissue infections. That area of research needs to be explored.

References
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Application of mupirocin to the nares or catheter exit site and frequency of mupirocin administration in continuous ambulatory peritoneal dialysis (CAPD) patients remain controversial. The objective of our study was to evaluate, using a historical control group, the efficacy on CAPD-related infections of once-weekly application of mupirocin at the catheter exit site. We instructed 18 CAPD patients, who did not initially use prophylactic antibiotic treatment, about once-weekly application of mupirocin ointment to the exit site as part of their exit-site care. We recorded the incidence of catheter-related infections, the causative micro-organisms, and the rate of catheter loss.

We observed 17 acute exit-site infections (AESIs: 0.45 episodes/patient-year) before mupirocin treatment and 2 AESIs (0.06 episodes/patient-year) after treatment. The relative rate of AESI reduction was 86%. Before application of mupirocin, 52% of AESIs were attributable to Staphylococcus aureus; after mupirocin administration, no AESIs were staphylococcal. Peritonitis episodes were also reduced from 21 before mupirocin treatment (0.56 episodes/patient-year), to 9 after mupirocin administration (0.29 episodes/patient-year). The relative rate of peritonitis reduction was 48%. Once-weekly application of mupirocin to the exit site resulted in a reduction in exit-site infections and peritonitis episodes comparable to those obtained with daily application.

Key words
Mupirocin, exit-site care, exit-site infections, peritonitis, Staphylococcus aureus

Introduction
Infections are of great concern in the management of peritoneal dialysis (PD). Introduction of new delivery systems and advances in connection technology have resulted in a reduction in the rate of catheter-related infections. Although many infections are treated successfully with antibiotics, some still complicate PD and cause technical failure. Recurrent and severe peritonitis can also result in sclerosing peritonitis and a switch from PD to hemodialysis.

The micro-organism that most commonly causes catheter-related infections in continuous ambulatory peritoneal dialysis (CAPD) patients is Staphylococcus aureus. That bacterium is commonly isolated from hands, groin, and (particularly) the nares of patients. Carriage of S. aureus is related to infections in CAPD patients. Prophylactic antibiotics (neomycin sulfate, mupirocin, rifampin, trimethoprim/sulfamethoxazole, and ciprofloxacin) have been administered to eradicate S. aureus colonization and to prevent catheter-related infections (1,2). Reductions in infection rates have been achieved by nearly all antibiotics; however, because of side effects and the potential for development of antibiotic resistance, topical antibiotics (mupirocin) have been substituted for systemic ones in the past decade (3,4).

Mupirocin binds to bacterial isoleucyl transfer RNA synthetase and inhibits bacterial protein synthesis. At low concentrations, mupirocin is bacteriostatic; topical administration has a bacteriocidal effect for S. aureus.

Several investigators have evaluated the effect of administration of mupirocin ointment to the exit site and nares, either daily or 2 – 5 times per week, in CAPD patients. They showed a statistically significant reduction in acute exit-site infections (AESIs) and peritonitis rates (1–6). However, to date, outcome from
regular once-weekly application of mupirocin to the exit site in CAPD patients is unknown.

The objective of our study was to evaluate, using a historical control group, the efficacy on CAPD-related infections of once-weekly application of mupirocin at the catheter exit site.

**Patients and methods**
The study population included 18 patients receiving CAPD treatment between November 1996 and August 2002. We instructed those patients about once-weekly application of mupirocin ointment to their exit sites as part of exit-site care. The patients had not initially used that treatment. They now were asked to apply, by themselves, mupirocin 2% ointment (Bactroban: GlaxoSmithKline, Istanbul, Turkey) as a thin film to the exit site.

Evaluation of exit sites occurred at the time of the monthly outpatient visit, according to the classification described by Twardowski et al. Diagnosis of peritonitis was made according to the presence of two of the following three findings: abdominal pain, cloudy dialysate, and demonstration of micro-organisms upon dialysate culture. The incidence of exit-site infections and peritonitis before the mupirocin treatment period, the organisms isolated at those times, and the causes of transfer to other renal replacement therapies were retrospectively investigated.

After mupirocin prophylaxis was started, we recorded the incidence of catheter-related infections, the causative micro-organisms, and the rate of catheter loss. Patients were followed up for occurrence of side effects related to mupirocin application (burning, itching, erythema).

Statistical analysis was carried out using descriptive statistics.

**Results**
Table I shows the demographic data (age, sex, duration of CAPD therapy, and cause of renal failure) for the study population. The average period of follow-up was 20 ± 12 months from the start of mupirocin application. No patient was lost to follow-up or transferred to another renal replacement modality during study period. We encountered no side effects related to mupirocin use. We observed 17 AESIs (0.45 AESI episodes/patient–year) before the start of mupirocin treatment, and 2 AESIs (0.06 AESI episodes/patient–year) after. The relative rate of reduction in AESIs was 86%. Before mupirocin application, 52% of AESIs were attributable to S. aureus. After mupirocin administration was started, no AESI was staphylococcal. Episodes of peritonitis were also reduced from 21 before mupirocin treatment (0.56 episodes/patient–year) to 9 after mupirocin administration (0.29 episodes/patient–year). The relative rate of peritonitis reduction was 48%. Table II and Figure 1 show the number and the percentages of AESIs and peritonitis episodes by micro-organisms isolated before and after treatment with mupirocin.

**Discussion**
Previous reports showed that prophylactic administration of mupirocin ointment reduces catheter-related infections in CAPD patients. However, how frequently mupirocin should be applied and whether it should be applied to the exit site or nares, to all patients or only to S. aureus carriers, remain controversial. Bernardini et al. (2) showed that daily administration of mupirocin ointment to the exit site is as effective as cyclic oral rifampin in reducing staphylococcal peritonitis and exit-site infections: peritonitis and exit-site infections respectively declined by 33% and 55% relative to historical controls. Better success rates (69% and 91% respectively) were obtained by Thodis et al. (5), who compared daily or thrice-weekly applications of mupirocin to the exit site with a historical control group. In a prospective, historically-controlled study, Casey et al. (6) demonstrated that daily administration of mupirocin to the exit site reduces rates of peritonitis and AESIs (31% and 49%, respectively).

The present study obtained results comparable to those of Thodis and colleagues. Our results suggest that once-weekly application of mupirocin to the exit site produced a 48% reduction in the rate of peritonitis.
and an 86% reduction in the rate of AESIs. According to our study, once-weekly administration seems to be equivalent to daily or thrice-weekly application.

Another finding of the present study is a reduction in infections attributable to micro-organisms other than S. aureus (including culture-negative infections). Although mupirocin has antibacterial activity against other bacteria in vitro, the finding of activity in vivo is an uncommon finding shared only with the study from Thodis et al. (5).

Administration of mupirocin has beneficial effects on catheter-related infections, but long-term therapy with mupirocin can lead to the emergence of resistant staphylococci. In a study by Annigeri and colleagues, resistance rates after 4 years of mupirocin use were reported to be only 3% (8). All of the patients in whom resistance occurred were treated with intermittent (1–4 times weekly) mupirocin. No mupirocin resistance was seen in daily (5–7 times weekly) users (8). But the limited number of studies analyzing resistance to mupirocin in CAPD patients means that the question of whether frequency of mupirocin administration affects the emergence of mupirocin resistance remains speculation.

In recent years, Perez–Fontán et al. (9) have reported higher rates of mupirocin resistance (0% – 12.4%) and an associated increase in the risk of exit-site infection in CAPD patients. In our department, we isolated a mupirocin-resistant S. aureus strain in only 1 of 36 CAPD patients who used the antibiotic once weekly for 2 years (Unpublished data).

### Conclusions

Once-weekly application of mupirocin to the exit site in CAPD patients causes reductions in AESIs and peritonitis episodes comparable to those seen with daily application. Application of mupirocin to the exit site may also reduce the rate of non staphylococcal infections. Future studies concerning mupirocin prophylaxis are likely to be related to mupirocin resistance.

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