Epithelial-to-mesenchymal transition of mesothelial cells is an early event during peritoneal dialysis and is associated with high peritoneal transport

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Ultrafiltration (UF) failure is a consequence of long-term peritoneal dialysis (PD). Fibrosis, angiogenesis, and vasculopathy are causes of this functional disorder after 3–8 years on PD. Epithelial-to-mesenchymal transition (EMT) of mesothelial cell (MC) is a key process leading to peritoneal fibrosis with functional deterioration. Our purpose was to study the peritoneal anatomical changes during the first months on PD, and to correlate them with peritoneal functional parameters. We studied 35 stable PD patients for up to 2 years on PD, with a mean age of 45.3 ±14.5 years. Seventy-four percent of patients presented loss of the mesothelial layer, 46% fibrosis (>150 μm) and 17% in situ evidence of EMT (submesothelial cytokeratin staining), which increased over time. All patients with EMT showed myofibroblasts, while only 36% of patients without EMT had myofibroblasts. The number of peritoneal vessels did not vary when we compared different times on PD. Vasculopathy was present in 17% of the samples. Functional studies were used to define the peritoneal transport status. Patients in the highest quartile of mass transfer area coefficient of creatinine (Cr-MTAC) (>11.8 ml min⁻¹) showed significantly higher EMT prevalence (P=0.016) but similar number of peritoneal vessels. In the multivariate analysis, the highest quartile of Cr-MTAC remained as an independent factor predicting the presence of EMT (odds ratio 12.4; confidence interval: 1.6–92; P=0.013) after adjusting for fibrosis (P=0.018). We concluded that, during the first 2 PD years, EMT of MCs is a frequent morphological change in the peritoneal membrane. High solute transport status is associated with its presence but not with increased number of peritoneal vessels.

KEYWORDS: epithelial-to-mesenchymal transition; mesothelial cell; peritoneal high transport; submesothelial fibrosis; peritoneal biopsy

Chronic peritoneal dialysis (PD) for end-stage renal disease treatment has been used for more than 30 years. Nowadays, the expansion of PD continues to be limited by the membrane incapacity to perform diffusive and/or convective transport over the long term. Water, sodium, and small solute transports can all be affected by this limitation. The worst functional consequence is UF failure, which results in extracellular volume overload, increased cardiovascular risk, and the restriction for technique continuity. Functional deterioration of the peritoneum is related to the damage induced by components of PD fluids, most likely pH, glucose, and glucose degradation products. Therefore, during the last decade many efforts have been made to improve the biocompatibility of fluids. Such an improvement of biocompatibility is expected to result in less peritoneal damage. Recently, important contributions from cell biology and histopathology studies have helped us to understand the pathophysiology of the peritoneal membrane response. Epithelial-to-mesenchymal transition (EMT) of MC has been identified as a key process leading to peritoneal fibrosis with functional deterioration. Concerning human histopathology studies, it must be remarked that most peritoneal biopsy studies have been based on PD patients with long-term treatment and peritoneal functional problems, specifically UF failure. As a consequence, advanced morphopathological changes such as fibrosis, angiogenesis, and vasculopathy are probably overrepresented. These lesions are the main cause of functional disorder after 3–8 years on PD. Obtaining peritoneal biopsies from short- to medium-term PD patients with no functional anomalies is not easy. Except for renal transplantation, there are few other opportunities to have access to a noninjured peritoneal membrane. The understanding of pathologic
processes leading to advanced peritoneal anatomical-functional disorders requires recognition of the earlier key points. Knowledge of premature peritoneal changes might reveal information sufficient to interpret the primary response to PD. The main objective of the present study was to examine such an initial phase of PD treatment. For this purpose, we have explored the peritoneal anatomical changes appearing during the first months on PD, and correlated these findings with peritoneal functional parameters determined in the same period.

**Table 1 | Peritoneal functional data of the whole series**

<table>
<thead>
<tr>
<th>Patients</th>
<th>35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peritonitis episodes</td>
<td>7</td>
</tr>
<tr>
<td>Days of peritonitis</td>
<td>2.7 ± 2</td>
</tr>
<tr>
<td>Urea-MTAC (ml min⁻¹)</td>
<td>19.7 ± 6</td>
</tr>
<tr>
<td>Cr-MTAC (ml min⁻¹)</td>
<td>8.7 ± 4.6</td>
</tr>
<tr>
<td>UF (ml per 4 h)</td>
<td>871 ± 283</td>
</tr>
<tr>
<td>D/P creatinine</td>
<td>0.7 ± 0.09</td>
</tr>
</tbody>
</table>

D/P, dialysate/plasma; Cr-MTAC, mass transfer area coefficient of creatinine; Urea-MTAC, mass transfer area coefficient of urea; UF, ultrafiltration.

**RESULTS**

**Analysis of data from the complete series of biopsies**

Since the low number of patients at first year (n = 15) does not permit a deeper analysis, we performed the analysis of the overall group (35 patients) as a whole (Table 1).

**Histopathological findings**

**Mesothelial layer.** Seventy-four percent of patients presented partial or total loss of the mesothelial layer. Forty percent of them showed no mesothelium at all.

**Submesothelial zone: epithelial-to-mesenchymal transition features.** Sixteen patients (46%) showed some degree of submesothelial thickness (>150 μm) or fibrosis (the terms submesothelial thickness and fibrosis are used in this paper indistinctly) (Figure 1). Patients with submesothelial thickening had similar mean time on PD than patients without fibrosis (13.9 ± 6.4 vs 13.8 ± 7 months, P = 0.97). The prevalence of submesothelial fibrosis did not vary during time on PD when we analyzed the four semesters (Figure 2). In situ evidence of EMT was present in six patients (17%). Mean time on PD was not statistically significantly different between patients with or without EMT (15 ± 8.6 vs 13.6 ± 6.3 months, P = 0.56). There was a trend to a higher prevalence of EMT in the fourth semester on PD, but the low number of patients does not permit the statistical analysis to be performed (Figure 3). Submesothelial thickness was not associated with the presence of EMT: 83% patients with EMT had fibrosis, but 38% patients without EMT also showed fibrosis (P = 0.07).

Forty-seven percent of the biopsies showed myofibroblasts. All patients with EMT showed myofibroblasts, while only 36% of patients without EMT had myofibroblasts (P = 0.006). One-third of patients with myofibroblasts (α-smooth-muscle actin +) also showed EMT.

**Vessel density.** The number of peritoneal vessels did not vary when we compared different times on PD (Figure 4).

**Vasculopathy.** Mild degree of vasculopathy was present in six patients (17% of the samples). Moderate degree was present only in 3% of patients, with no cases with severe vasculopathy. Patients with and without vasculopathy showed similar mean time on PD (13.6 ± 4.6 vs 13.9 ± 7 months, P = 0.93). The prevalence of vasculopathy was similar in different semesters of treatment (Figure 5). There

![Figure 1 | Biopsy findings in normal and high transport patients.](image)

(a and c) Biopsy samples from patients with normal transport that show no relevant fibrosis (a, hematoxylin–eosin, original magnification × 100), and absence of cytokeratin + submesothelial fibroblasts (c, immunoperoxidase, original magnification × 150). (b and d) Samples from high transport patients and show fibrosis (b, hematoxylin–eosin, original magnification × 100) and cytokeratin + submesothelial fibroblasts (d, immunoperoxidase, original magnification × 150). (e and f) Samples from normal and high transport patients show a similar number of vessels (three and two, respectively, hematoxylin–eosin, original magnification × 100).

![Figure 2 | Prevalence of submesothelial fibrosis according to different semesters.](image)

The prevalence of submesothelial fibrosis was similar in the different semesters.
was no association between vasculopathy and fibrosis (83% patients with vasculopathy had fibrosis and 38% patients without vasculopathy had fibrosis; \( P = 0.073 \)) or EMT (33% of patients with vasculopathy had EMT and 14% patients without vasculopathy had EMT; \( P = 0.26 \)). Fifty percent of patients with vasculopathy had myofibroblasts, and 46% patients without vasculopathy had myofibroblasts; \( P = 1.00 \). Table 2 summarizes the prevalence of different peritoneal lesions along the time observed.

**Morphofunctional correlations**
The different peritoneal lesions were related to the peritoneal functional parameters divided into quartiles. Patients in the highest quartile of mass transfer area coefficient of creatinine (Cr-MTAC) and the median value of UF were considered the reference. Table 3 shows the prevalence of peritoneal lesions in the four quartiles of Cr-MTAC and UF values.

**Small solute peritoneal transport.** Patients in the highest quartile of Cr-MTAC (\( > 11.8 \text{ ml min}^{-1} \)) showed significantly higher prevalence of EMT (\( P = 0.016 \)) (Figure 6) and similar presence of myofibroblasts, fibrosis, and vasculopathy (\( P = 1.00, \text{NS} \)) than the other quartiles. However, all showed a similar prevalence of fibrosis (first quartile, 30%; second quartile, 50%; third quartile, 56%; and fourth quartile, 50%), as well as a similar number of peritoneal vessels (first quartile: \( 3 \pm 1 \text{ vessels per field, } n = 8 \); second quartile: \( 4.7 \pm 2 \text{ vessels per field, } n = 2 \); third quartile: \( 4.4 \pm 1 \text{ vessels per field, } n = 2 \); and fourth quartile: \( 4.3 \pm 2 \text{ vessels per field, } n = 6 \)) (NS).

We found no relation between the presence of EMT and previous peritonitis, since one of the six patients with EMT (17%) had a previous episode of peritonitis in contrast to 6 of the 29 patients (21%) without EMT (\( P = 0.82 \)). Days of peritonitis did not influence these results either (2 days in EMT group vs 2.8 days in non-EMT, NS)

**Ultrafiltration capacity.** No correlation was found between the peritoneal lesions evaluated and UF capacity. When we compared patients over and under the median value of UF (820 ml per 4 h), we found a trend to a higher prevalence of fibrosis in patients with lower UF capacity (over the median: 29.4% and under the median: 61.1%) (\( P = 0.06 \)). No statistical differences were found in the prevalence of EMT (\( > \text{median: 5.9%, } < \text{median: 27.8%} \) (NS), myofibroblast presence (\( > \text{median: 44%, } < \text{median: 50%} \) (NS), vasculopathy (\( > \text{median: 18%, } < \text{median: 17%} \) (NS), and number of vessels (\( > \text{median: 3.45} \pm 1.2 \text{ vessels per field, } n = 8 \); \( < \text{median: 4.12} \pm 1.8, \ n = 10 \) (NS).

**Logistic regression analysis.** Table 4 shows the univariate analysis data (unadjusted odds ratio) for the presence of EMT in peritoneal biopsies. In the multivariate analysis, the highest quartile of Cr-MTAC (\( > 11.8 \text{ ml min}^{-1} \)) remained as an independent factor predicting the presence of EMT (odds ratio 12.4; confidence interval: 1.6–92; \( P = 0.013 \)) after adjusting for fibrosis (\( P = 0.018 \)). None of the variables included in our study significantly predicted the presence of submesothelial thickness or vasculopathy in peritoneal biopsies.

**Analysis of data from biopsies taken during the first year**
The six patients with biopsies obtained during the first 6 months on PD with no peritonitis showed two cases (33%)...
with submesothelial fibrosis, one (16%) with EMT tissue data, none with vasculopathy and a normal number of vessels (mean 4.45 ±1.2 vessels per field). In these patients, Cr-MTAC ranged from 8.7 to 16.6 ml min⁻¹, and UF capacity ranged from 500 to 1800 ml per 4-h 3.86% glucose dwell time. Since this group consists of few patients, we examined the data from the 15 patients studied within the first year. The inclusion of two patients who have suffered peritonitis, showed fibrosis and EMT tissue data, and three cases (20%) with vasculopathy. Therefore, although specific, this detection method has low sensitivity since only a portion of transdifferentiated MCs will be detected.

With mesothelial-to-mesenchymal transition, in vivo, by the presence of fibroblastic-like cells located in the submesothelium that express mesothelial markers such as cytokeratins. During their conversion into myofibroblasts, MCs gradually lose their location, morphology, and immunophenotype. What we detect using immunohistochemistry against cytokeratin is a subset of myofibroblasts or transitional cellular forms that still retain cytokeratin expression, reflecting their mesothelial origin. When final conversion has occurred, myofibroblasts will have lost the expression of cytokeratin and other mesothelial markers. Therefore, although specific, this detection method has low sensitivity since only a portion of undifferentiated MCs will be detected.

Mesothelial cell detachment was seen in 74% of the peritoneal biopsies. Almost half of the tissue samples showed peritoneal morphological change more common in those patients with higher solute transport status, and that such higher transport status was not associated with an increase in the number of vessels. A new interpretation of the mechanisms associated with fast peritoneal solute transport at early PD stages arises from these data.

Mesothelial-to-mesenchymal transition is defined, in vivo, by the presence of fibroblastic-like cells located in the submesothelium that express mesothelial markers such as cytokeratins. During their conversion into myofibroblasts, MCs gradually lose their location, morphology, and immunophenotype. What we detect using immunohistochemistry against cytokeratin is a subset of myofibroblasts or transitional cellular forms that still retain cytokeratin expression, reflecting their mesothelial origin. When final conversion has occurred, myofibroblasts will have lost the expression of cytokeratin and other mesothelial markers. Therefore, although specific, this detection method has low sensitivity since only a portion of undifferentiated MCs will be detected.

DISCUSSION

This study, based on peritoneal biopsies performed during the first 2 years on PD, showed that EMT of MCs is a frequent peritoneal morphological change more common in those patients with higher solute transport status, and that such higher transport status was not associated with an increase in the number of vessels. A new interpretation of the mechanisms associated with fast peritoneal solute transport at early PD stages arises from these data.

Mesothelial-to-mesenchymal transition is defined, in vivo, by the presence of fibroblastic-like cells located in the submesothelium that express mesothelial markers such as cytokeratins. During their conversion into myofibroblasts, MCs gradually lose their location, morphology, and immunophenotype. What we detect using immunohistochemistry against cytokeratin is a subset of myofibroblasts or transitional cellular forms that still retain cytokeratin expression, reflecting their mesothelial origin. When final conversion has occurred, myofibroblasts will have lost the expression of cytokeratin and other mesothelial markers. Therefore, although specific, this detection method has low sensitivity since only a portion of undifferentiated MCs will be detected.

Mesothelial cell detachment was seen in 74% of the peritoneal biopsies. Almost half of the tissue samples showed...
some degree of submesothelial thickening. The two main features of EMT, that is cytokeratin and α-smooth-muscle actin + submesothelial fibroblasts, were present in 17 and 47% of biopsies, respectively. All biopsies with cytokeratin + fibroblasts also showed myofibroblasts, while 36% of patients with no cytokeratin + fibroblasts had evidence of myofibroblastic differentiation ($P = 0.006$). One-third of patients with myofibroblasts (α-smooth-muscle actin + ) also showed EMT.

A mild-to-moderate grade of vasculopathy was present in 17% of the series, and its association with submesothelial thickening and myofibroblast was sporadic. Longer time on PD was associated with submesothelial fibrosis only when EMT was also present. Analyzing the EMT findings over time, a remarkable higher prevalence of these findings at the fourth semester was demonstrated. It can be assumed that time on PD is a risk factor for submesothelial fibrosis only when EMT was present. Analyzing the EMT findings over time, a remarkable higher prevalence of these findings at the fourth semester was demonstrated. It can be assumed that time on PD is a risk factor for submesothelial thickening associated with EMT after the first year. In fact, our previous findings in biopsies taken at longer PD periods confirmed a progressive increase in EMT incidence (up to 48%). Myofibroblast presence in biopsies was very erratic over the time examined. Although they are characteristics of EMT process, fibroblasts from other origins must be participating in the process as well.27–29

Vasculopathy was absent during the first semester and present to a mild degree in the other periods examined. Its presence at these early stages reinforces the role of this lesion in the peritoneal changes secondary to PD. Vessel density (to be discussed also in transport discussion) remained stable over the periods examined. This observation suggests that vessel number remains similar during the first 2 years of a noncomplicated PD. In other words, the angiogenesis expected in later PD stages, has not yet started.

**Data on solute transport and biopsy findings**

One of the main concerns in peritoneal function is the status of the fast transporter. To avoid bias in the management of Cr-MTAC values in the study of its relationship with biopsy findings, we have divided this parameter into quartiles. In spite of the shortness of the series, we have had sufficient number of patients in each quartile to compare the biopsy findings. Figure 6 shows a remarkably different prevalence of EMT in fast transporters (the highest quartile of Cr-MTAC $> 11.8$ ml min $^{-1}$), approximately fivefold higher than in the other groups. The cut-off value of 11.8 ml min $^{-1}$ determines a true relationship with the presence of EMT in the biopsy, confirmed in the univariate and multivariate analyses. In consequence, we can firmly corroborate the association between higher solute transport and EMT in the biopsy. This association did not exist with submesothelial thickness per se. This seems to indicate qualitative differences in the composition of the thickened submesothelial zone. To confirm this transport–anatomical relationship, it was necessary to know whether or not the vascular density was consistent with the expectation that the higher the transport, the larger the number of vessels.21,30

Contrary to this paradigm, our patients have demonstrated that the sole presence of EMT and related fibrosis, with no increase in the number of capillaries, is sufficient to lead to a high transporter status. Other authors have found that angiogenesis is not necessarily associated with noncomplicated higher peritoneal transport at later PD stages (4–6 years).31 Evidently, peritoneal lesions over PD time should be different in quality and quantity and probably are associated. The present data suggest that the early changes in response to PD are preceded by identifiable cell and extracellular matrix changes in the submesothelial compact zone. The increase in collagen and fibronectin, prior to the increase of vessel number, can be sufficient. In fact, submesothelial thickening is the more constant finding detected in other peritoneal biopsy studies.31,32 Animal models, in which TGF-β transfection of MC is induced, reproduce the sequence of phenomena that seems to apparently occur in humans. These data have demonstrated that TGF-β transfection of rat MC causes peritoneal sclerosis, with previous development of angiogenesis, both processes anteceded by the EMT of MCs during the first 4 days after transfection.33 The process in humans, as it does in the transfection model, should start by the EMT-induced change of MCs secondary to TGF-β effects and continue with submesothelial zone modifications. The EMT process starts by the loss of tight junctions (E-cadherin) by MC with their subsequent detachment into effluent and migration toward the submesothelial compact zone.34–36 For this last purpose, cells develop migrating and invasive capabilities, in which vascular endothelial growth factor (VEGF) is involved.37–39

Our previous study has demonstrated the specific high capacity of VEGF production by transitional MCs in this situation.40 Once cells are homed at submesothelial levels, they acquire capability of producing extracellular matrix components, collagen and fibronectin, and expand the surrounding submesothelial zone.41 The presence of high tissue levels of VEGF also causes vasodilation of peritoneal capillaries. This phenomenon may be coresponsible for the association between VEGF and high transporter status.42,43 Unfortunately, capillary dilation status cannot be quantified in biopsy due to simple technical reasons. To explain the mechanism through which the submesothelial EMT-related fibrosis influences peritoneal transport of solutes we have other considerations. The qualitative change of the peritoneal interstitium composition probably modifies the number and size of peritoneal pores. The potential absence of glycosaminoglycans, synthesized by normal MCs, and their replacement by collagen and fibronectin explain the great change. Interstitial changes have been sufficient to modify peritoneal transport in PD animal models with chronic inflammation.44 We propose that what it is conceptually accepted for a normal membrane, the similarity of the transport in both directions, is modified by the structural change. In the new situation, a constellation of novel phenomena, including more and greater interstitial medium-size pores, relative absence of glycosaminoglycans, and some degree of vasodilation caused...
by high presence of VEGF in tissue, might explain the rupture of the paradigm.

The UF capacity data from our patients complement this information, although in a less expressive manner. Patients with lower UF than the median demonstrated a greater prevalence of submesothelial fibrosis and EMT, both within the limit of statistical significance. The presence of myofibroblasts and vasculopathy was not related to UF capacity. Such capacity is the result of hydraulic permeability to water and the maintenance of osmotic gradient through the membrane. The peritoneal change observed in our patients may affect both conditions in different grades leading to an unpredictable consequence on the UF capacity. The manipulation of solute and water transport by intraperitoneal nonfractionated heparin suggests that the change of peritoneal membrane composition results in functional modifications.\(^{45}\)

The limitations of our study include that it is cross-sectional and the shortness of the series that requires the contribution of other groups interested in the understanding of the peritoneal membrane change per stages, not only at the end of the process such as is generally considered the peritoneal biopsy registry in its current conception.

Prior mild peritonitis did not affect the results of this analysis, because they were not relevant for the presence of EMT (nonsignificant differences). A potential contribution in EMT of more aggressive peritonitis episodes may not be excluded. For future research in this field, progress in the knowledge of interstitium composition, collagen, fibronectin, and presence of glycosaminoglycans and their relationship to membrane transport are mandatory.

In conclusion, our data obtained from peritoneal biopsies performed during the first 2 years on PD demonstrate that the first morphological change in peritoneum that appears as a consequence of PD is submesothelial thickening partially caused by the EMT of MCs. This phenotype change is associated with an increase in peritoneal solute transport independent of the number of capillaries present in the tissue.

### MATERIALS AND METHODS

#### Patients

**Inclusion criteria.**
- Stable patients up to 2 years on PD

**Exclusion criteria.**
- Systemic or local inflammatory condition
- Patients with PD treatment in a previous stage
- Peritoneal samples obtained from hernia sacs
- Patients with declared functional membrane failure

**Groups of patients.** The whole series was constituted of 35 patients (20 men and 15 women) with a mean age of 45.3 ± 14.5 years (range: 20–77). Twenty-six patients were on automated peritoneal dialysis and nine on continuous ambulatory peritoneal dialysis at the time of peritoneal biopsy. Three patients used low glucose degradation product dialysis solutions, one bicarbonate solution and the remaining patients were using lactate standard solution; 17 patients were using icodextrin. Ninety-seven percent of the patients were hypertensive. Sixty-six percent were on ACE treatment, 37% with \(\beta\)-blockers, and 23% with angiotensin II antagonists. The cause of renal failure was glomerulonephritis \((n = 9)\), chronic pyelonephritis \((6)\), systemic disease \((4)\), polycystic kidney disease \((2)\), nephroangiosclerosis \((1)\), hereditary \((1)\), nephrophthophis \((1)\), and nephrocalcinosis \((1)\). Ten patients had renal disease of unknown origin. The mean time on PD was 13.8 ± 6.6 months. Fifteen patients were on PD less than 1 year. Six patients were biopsied during their first 6 months \((2, 3, 3, 4, 5,\) and 5 months, respectively) of treatment. None of these six patients had suffered peritonitis. The remaining nine patients were biopsied during their first 12 months on PD \((7, 8, 9, 11, 11, 11, 11, 12,\) and 12 months). Two of these patients experienced an episode of peritonitis, which lasted 1 and 2 days, respectively. These 15 patients constituted the first year group. The other 20 patients were biopsied during their second year on PD, and the analysis was performed with the whole 35-patient group.

Ten parietal peritoneal biopsies obtained from nonrenal patients with no abdominal pathology were used as normal controls. Seven of them were renal donors and three autopsy cases.

#### Peritoneal samples

The parietal peritoneal samples were obtained during renal transplantation in most cases \((29\) patients, 77\%) or during other abdominal interventions: nephrectomy \((n = 2)\), catheter insertion \((1)\), gastrectomy \((1)\), omentectomy \((1)\), and polypectomy \((1)\).

#### Pathologic analysis

**Biopsy collection and processing.** Parietal peritoneal samples were obtained from the anterior abdominal wall. Each sample measured 10–25 × 10–25 mm. To avoid mesothelial artificial detachment, they were carefully manipulated and immediately fixed with neutral-buffered 3.7% formalin \((pH 7.3)\) for 12–24 h. To avoid retraction they were gently attached to a flat surface. After fixation, samples were cut and embedded in paraﬃn, and then cut into 3-\(\mu\)-m sections. When preparing the paraffin blocks, special efforts were made to orientate the samples perpendicular to the cutting surface. Sections were stained with hematoxylin-eosin and Masson trichromic. For immunohistochemistry, paraffin sections were mounted on precoated slides, deparaffinized and rehydrated, and incubated with 3% hydrogen peroxide to block endogenous peroxidas activity. Antigen retrieval was performed using a citric acid solution \((pH 6)\), which was heated with a microwave. Indirect immunohistochemical studies were performed by means of a dextran-polymer conjugate technique \((EnVision^+; DakoCytomation, Glostrup, Denmark)\). Monoclonal antibodies AE1/AE3 \((cytokeratins)\) and \(\alpha\)-smooth muscle actin \((both from DakoCytomation)\) were used in this study. For visualization, diaminobenzidine was used as a chromogen.

**Sample analysis.** Morphological data regarding mesothelial status, thickness of submesothelial compact zone, hyalinizing vasculopathy, and vascular density were recorded. Density of MCs was measured using a semiquantitative scale \((grade 3, normal; grade 0, total absence)\). The thickness of the compact zone was measured with a micrometer ocular. The mean of three different measures of representative zones was obtained: when less than 150 \(\mu\)m, it was considered normal; if between 150 and 350 \(\mu\)m, it was considered as a moderate thickening; results greater than 350 \(\mu\)m were regarded as intense thickening. Using immunohistochemistry, we explored the presence of submesothelial cytokeratin + fibroblast-like cells and \(\alpha\)-smooth muscle actin + fibroblasts \((myofibroblasts)\). EMT was
defined by the presence of submesothelial fibroblast-like cells expressing cytokeratin. The evaluation of vessels was performed using a method similar to that described by Numata et al. Photomicrographs recording between 10 and 20 microscopy fields of each specimen (×100 magnification) were used for the quantitative analysis of vessels. The total number of cross-sections of vessels per peritoneal field (relative microvessel number) was examined. Hyalinizing vasculopathy was measured using the four grade system described by Honda et al., grade 0, no abnormalities; grade 1, mild thickening without stenosis of the lumen; grade 2, moderate thickening with partial luminal stenosis; and grade 3, intense thickening with marked stenosis and luminal distortion or complete occlusion.

Peritoneal function studies

A peritoneal transport kinetic study was performed to calculate the peritoneal Cr-MTAC (ml/min·1.73m2), based on the creatinine dialysate/plasma ratios at five consecutive peritoneal 4-h dwells, using a previously described mathematical model. The MTAC value is considered to represent exclusively the small solute diffusive transport across the membrane. To avoid a predefined normality, the obtained values were divided into quartiles. The highest quartile is considered that of the faster, higher transporters.

Net ultrafiltration rate (UFR rate, ml) was estimated by the net negative balance (weighing bags during the kinetic study prior infusion and after drainage), using a 2-l hypertonic glucose infusion and after drainage, using a 2-l hypertonic glucose solution. The Textbook of Peritoneal Dialysis, 2nd edn. Kluwer Academic Publishers: Dordrecht, 1996; 348: 403–413.

Ethical issues

The procedures were in accordance with the ethical standards of the Institutional Committee on Human Experimentation and with the Declaration of Helsinki Principles 1975 (and as revised in 1983). All patients were informed about the collection of cells from peritoneal effluent and peritoneal biopsy, and signed the informed consent.

Statistical analysis

Data are expressed in mean±s.d. A P-value less than 0.05 is considered statistically significant. Comparisons of proportions between groups were made using the Fischer exact test and comparisons of means with the nonparametric Mann-Whitney U-test. Univariate and multivariate logistic regression analyses were used to investigate factors associated with the presence on peritoneal biopsy of the EMT, submesothelial fibrosis, and vasculopathy. We used the statistical program SPSS, version 11 (SSPS Inc., Chicago, IL, USA). The quartiles of peritoneal functional parameters have been related to histological data to compare the prevalence of each lesion in every quartile.

DISCLOSURE

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