Longitudinal Study of Small Solute Transport and Peritoneal Protein Clearance in Peritoneal Dialysis Patients

Zanzhe Yu,†* *Mark Lambie,*‡* *and Simon J. Davies*‡*

Summary

Background and objectives Peritoneal protein clearance (Pcl) is determined by both effective (small pores) membrane area and relative capillary leakiness (large pores). It is not known how these two components change with duration of peritoneal dialysis (PD) in the context of progressive membrane injury and differential attrition of patients with higher Pcl, which has been associated with increased mortality risk in several studies.

Design, setting, participants, & measurements Patients treated continuously from 2000 to 2011 for a minimum of 4 years were selected from the longitudinal prospective Stoke PD Study. Pcl, membrane area (peritoneal solute transport rate [PSTR]), dialysis prescription, and residual renal function were measured every 6 months, along with comorbidity and peritonitis events. Multilevel multivariate analysis was used to determine associations with Pcl over time, taking into account within-subject correlations.

Results From 280 incident patients, 335 datasets were analyzed from 49 patients receiving treatment for 4 years. Pcl correlated with PSTR at baseline (R=0.61; P<0.01), but over time there was progressive uncoupling of this relationship (year 4, R=0.28; P=0.05) with increasing PSTR (0.66–0.74; P<0.01) and stable Pcl (78.4–81.9 ml/d; P=0.7). Multivariate analysis found that age, PSTR, daily ultrafiltration, and sodium removal were significant predictors of Pcl when adjusted for sex, comorbidity, glucose exposure, and residual renal function. Peritonitis was associated with increased PSTR but a similar pattern of uncoupling.

Conclusion There is a progressive dissociation of the small- and large-pore pathways with time on PD, which would be in keeping with a switch from local inflammation early on to progressive fibrosis, combined with increased vascular surface area. Measuring longitudinal changes in Pcl may complement membrane function tests used to monitor progressive injury.

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*Institute for Science and Technology in Medicine, Keele University, United Kingdom; †Department of Nephrology, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China; and ‡Department of Nephrology, University Hospital of North Staffordshire, Stoke-on-Trent, United Kingdom

Correspondence: Dr. Simon Davies, Department of Nephrology, University Hospital of North Staffordshire, Newcastle Road, Stoke-on-Trent, Staffordshire, ST4 6QG United Kingdom. Email: simondavies1@compuserve.com

Introduction

Peritoneal dialysis (PD) leads to changes in membrane morphology (1) and function (2,3) over time. In a proportion of patients, it causes ultrafiltration failure and probably predisposes them to the much rarer complication of encapsulating peritoneal sclerosis (4,5). Of the functional changes, increasing peritoneal solute transport rate (PSTR) and a reduction in osmotic conductance (reduced ultrafiltration capacity independent of osmotic gradient) are well established (6,7). Longitudinal changes in protein clearance, predominantly a reflection of hydrostatic pressure–driven leak of plasma proteins through the large-pore pathway, are less clear. Cross-sectional and longitudinal studies (7–10) have suggested that this does not necessarily increase with time on treatment, but it is not clear whether this represents a real difference from the observed changes in the PSTR. This is further confused by the observation that peritoneal protein clearance (Pcl) is associated with increased age and comorbidity at the start of treatment. Moreover, in some (8,11–14) but not all (15) studies it is an independent predictor of survival, resulting in the potential confounding of longitudinal data due to earlier dropout of patients with high Pcl (informative censoring).

Pcl, representing the large-pore pathway, and PSTR, proportional to the total membrane small-pore area, are known to be correlated at the start of PD treatment. There are at least two reasons for this. First, given that both pore systems are located in the capillary vessel wall, there will probably be considerable anatomic coupling. Second, because intraperitoneal production of the proinflammatory cytokine IL-6 is correlated with, and is probably a key determinant of, PSTR (16), local inflammation would be expected to cause increased numbers of large pores per unit of capillary length. If the increase in PSTR occurring with time on treatment is a function of increasing intraperitoneal inflammation, then a parallel increase in Pcl would be anticipated. The purpose of this analysis was to test this hypothesis in a cohort of patients

treated continuously over 4 years in order to avoid the influence of informative censoring caused by patients with increased Pcl discontinuing treatment early, leading to nonrandom missing data.

Materials and Methods

Study Design and Patient Population

The Stoke PD study is a long-term, single-center, longitudinal, prospective observational cohort of consecutive new patients commencing PD. Since April 2000, peritoneal losses were measured every 6 months as part of routinely collected such data as demographic characteristics, comorbidity, peritonitis record, membrane function (using a standard peritoneal equilibration test that includes PSTR and ultrafiltration capacity), residual renal function, dialysis prescription, clearances, achieved ultrafiltration, and sodium removal. Data are collected and stored on a validated database (Peritoneal Dialysis Database), and since 2002 all patients have given their informed consent for one of the recruiting centers for the Global Fluid Study (Multi-Center Research Ethics Committee: 02/9/14). The database was interrogated in July 2011 to identify all patients for whom there were at least 4 years' worth of continuous data for the purpose of this analysis; additional analyses were also performed for patients completing 2 and 3 years of treatment. During this period, most patients (>95%) used conventional glucose fluids (Dianeal), with an increasing proportion using icodextrin (Extraneal).

Measurement of Membrane Function and Pcl

Peritoneal equilibration tests were used to measure PSTR using the dialysate-to-plasma creatinine ratio and the ultrafiltration capacity at 4 hours, as previously described. The peritoneal dialysate protein loss was measured by the biuret method from the collection of 24-hour peritoneal dialysate effluent.

Plasma albumin levels were measured using the bromcresol green method before August 22, 2007, and measurement was switched to bromcresol purple method afterward. An average difference of 0.55 g/dl between the results of the two methods had been established (17) and this conversion factor was applied so that all values are expressed as if measured by the bromcresol purple method. Dialysate protein was measured using a modified dye-binding method (Siemens Healthcare Diagnostics), analytic range of 10–1250 mg/L (coefficient of variation, 2.0%). Pcl was calculated using a validated formula: 24-hour dialysate protein loss/(serum albumin/0.4783); it was expressed as ml/d (17).

Average Glucose Exposure

Peritoneal average glucose exposure was calculated by summing the total glucose exposure in each exchange and dividing by total volume (4). For example, for a patient using 2×2 L exchanges of 1.36% (1.5%) glucose, 1×2 L exchanges of 2.27% (2.5%) glucose, and icodextrin 2 L overnight, the average glucose exposure would be as follows:

$$(1.36\%\times4 + 2.27\%\times2)/8 = 1.25\%.$$

Comorbidity and Demographic Characteristics

Demographic characteristics, primary renal disease, and comorbidity were recorded at the start of PD. Comorbidity was defined as described previously. Briefly, seven comorbid domains were considered: noncutaneous malignancy, ischemic heart disease, peripheral vascular disease, left ventricular dysfunction, diabetes mellitus, systemic collagen vascular disease, and any other condition thought to reduce life expectancy. The comorbidity score for each patient was defined as the number of these domains affected. The comorbidity grade was then derived from the comorbidity score. Grade 0 (low risk) was a score of 0, grade 1 (medium risk) was a score of 1–2, and grade 2 (high risk) was a cumulative score of ≥3.

Statistical Analyses

Continuous data are expressed as means±SD or medians (interquartile range) according to the distribution. For multivariate models, nonparametric data were log or square root transformed to fit a normal distribution. Between-group comparisons used the two-tailed unpaired *t* test, the Mann-Whitney *U* test, one-way ANOVA, independent samples Kruskal-Wallis test, or the chi-square test depending on the data type, the number of groups, and the distribution. For single-level analysis, data were annualized such that if more than one set of measurements was available, the mean was taken for further analysis to limit bias.

A multilevel mixed linear model was applied to determine associations with Pcl and was performed using MLwin software, version 2.22 (Centre for Multilevel Modeling, University of Bristol). Repeat observations (*e.g.*, membrane characteristics) (level 1) were nested within individuals (level 2). The dependent variable was the daily protein Pcl, and the independent covariates were included in the multivariate model if they were significantly related to the dependent variable on the unilevel bivariate correlation or were plausible explanatory variables according to theoretical modeling or other studies. Constant covariates, such as sex and comorbidity at baseline, were level 2 variables. The intercept was set as random in level 2 to allow for between-patient differences. The continuous variables were centered on the mean to facilitate the clinical interpretation of the model.

Results

Patient Demographic Characteristics and Baseline Membrane Function

Combined measurements of membrane function tests and Pcl were obtained at 1176 time points in 280 patients (Figure 1); 335 time points were from 49 patients treated continuously for 4 years. Of these, 175 were obtained during treatment with automated PD and 190 of the prescriptions included icodextrin. Additional sensitivity analyses were performed on the 123 and 80 patients who completed 2 and 3 years, respectively (Figure 1).

The demographic details of the cohort selected for this study are displayed in Table 1; compared with all patients they were significantly younger. When patients were split according to ever or never having peritonitis during the observation period, demographic characteristics

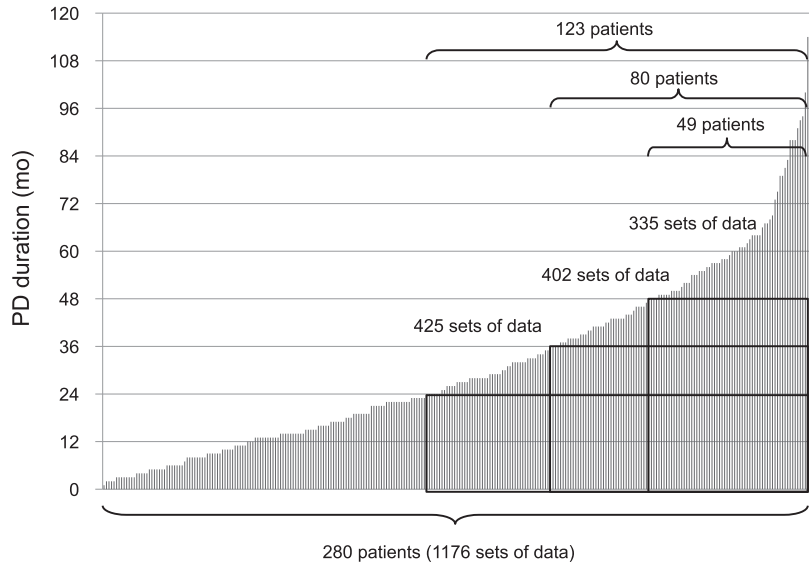

Figure 1. | Data selection process: 1176 sets of membrane function test and peritoneal protein clearance (Pcl) measurement in 280 patients were available for analysis. To elucidate the long-term change of Pcl, data from the first 4 years among patients who were receiving peritoneal dialysis for more than 4 years were included in the analysis; this was done to eliminate the bias of information censoring (335 sets of data in 49 patients). Sensitivity analyses were performed with the 2- and 3-year datasets; see Supplemental Table 2.

Table 1. Demographic characteristics of selected cohort compared with all incident patients during study period

Characteristic	Selected Longitudinal Subcohort (*n*=49)				Whole Cohort (*n*=280)
	Without Peritonitis (*n*=24)	With Peritonitis (*n*=25)	*P* Value	Combined (*n*=49)	
Women/men (*n*/*n*)	11/13	10/15	0.68	21/28	120/160
Diabetes (%)	29.2	32	0.83	30.6	29.6
Ischemic heart disease (%)	12. 5	16	0.73	14.3	21.1
Left ventricular dysfunction (%)	4.2	4	0.98	4.1	7.5
Comorbidity grade (low/medium/high) (*n*/*n*/*n*)	11/12/1	9/14/2	0.71	20/26/3	107/146/27
Age (yr)	52±17	49±19	0.51	51±18[a]	55.3±16.5
Total time on PD (mo)	67±17	66±14	0.82	67±15[b]	30.9±22
Patient survival (mo)	85±21	80±21	0.39	83±21[b]	56.2±35.1
Mean baseline PSTR (4-h dialysate/plasma creatinine ratio)	0.64±0.15	0.69±0.11	0.21	0.66±0.13	0.68±0.13
Baseline plasma albumin (g/dl)	3.22±0.35	3.09±0.45	0.28	3.15±0.41	3.07±0.48
Peritoneal protein clearance (ml/d)	78.4 (71.4–104.1)	78.9 (67.9–110.2)	0.77	78.4 (68.9–104.1)	84.8 (67.0–109.5)

Values expressed with a plus/minus sign are mean±SD. PD, peritoneal dialysis; PSTR, peritoneal solute transport rate.
[a]$P<0.05$ compared with patients treated less than 4 years.
[b]$P<0.01$ compared with patients treated less than 4 years.

and membrane function did not significantly differ between the two subgroups. Of the 25 patients who had peritonitis, 11 of them had one episode, 8 of them had two episodes, and 6 of them had three or more episodes of peritonitis.

Longitudinal Relationship between Pcl and PSTR

The changes in PSTR and Pcl with time on treatment are shown in Figure 2; PSTR increased significantly over time (baseline, 0.66±0.13; year 4, 0.74±0.12; $P<0.01$), whereas Pcl tended to decrease, indicating a progressive

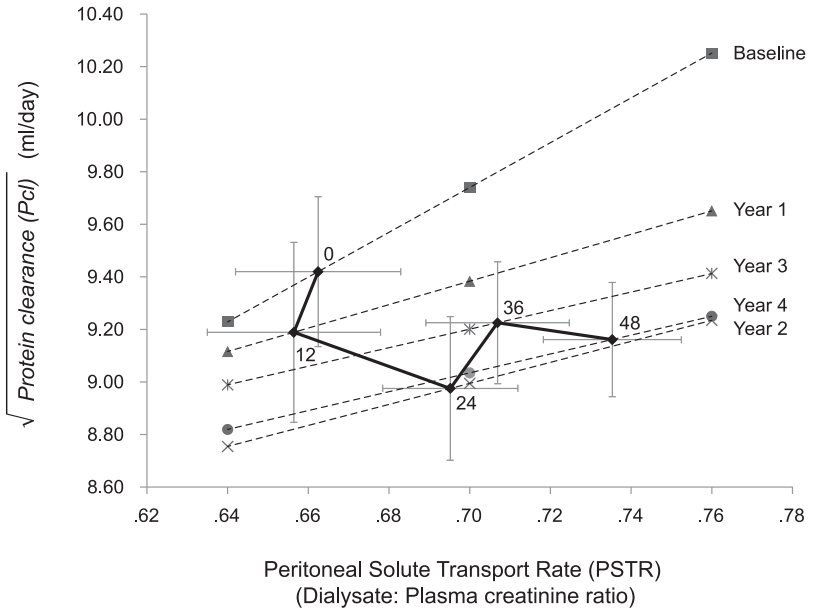

Figure 2. | **Relation of peritoneal Pcl to peritoneal solute transport rate (PSTR) (4-hour dialysate/plasma creatinine ratio).** The relationship was initially positive, but it changed with time on PD, as indicated by the broken linear regression lines for each year of treatment. The actual mean values±SEM for Pcl and PSTR at each time point are shown superimposed. The increase in PSTR over time was significant ($P<0.01$). Mean PSTRs±SD: baseline, 0.66±0.13; year 1, 0.66±0.13; year 2, 0.70±0.11; year 3, 0.71±0.12; year 4, 0.74±0.12. √Pcl values±SD: baseline, 9.4±1.8; year 1, 9.2±2.0; year 2, 8.9±1.8; year 3, 9.2±1.6; year 4, 9.2±1.5.

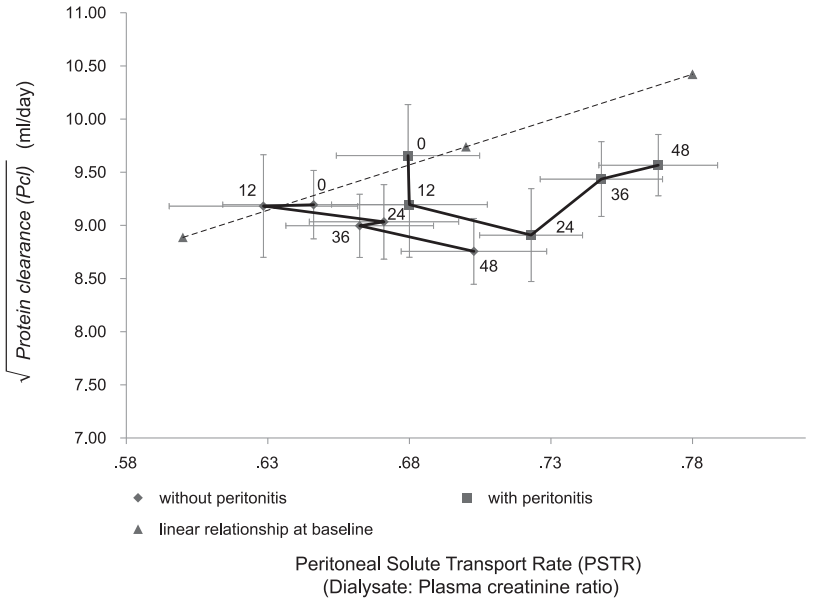

Figure 3. | **Progressive uncoupling for patients with and without peritonitis.** With peritonitis: peritoneal solute transport rates±SD: baseline, 0.68±0.11; year 1, 0.68±0.12; year 2, 0.72±0.08; year 3, 0.75±0.11; year 4, 0.77±0.10. √Pcl values±SD: baseline, 9.66±2.15; year 1, 9.2±2.2; year 2, 8.9±1.9; year 3, 9.4±1.7; year 4, 9.6±1.4. Without peritonitis: PSTRs±SD: baseline, 0.65±0.15; year 1, 0.63±0.13; year 2, 0.67±0.13; year 3, 0.66±0.13; year 4, 0.70±0.13. √Pcl values±SD: baseline, 9.2±1.5; year 1, 9.2±1.9; year 2, 9.0±1.7; year 3, 9.0±1.4; year 4, 8.8±1.5.

dissociation of these components of membrane function. The mean values of Pcl and dialysate/plasma creatinine are shown in Figure 3. The plasma albumin was stable throughout the observation period: baseline, 3.17±0.4 g/dl; year 1, 3.1±0.4 g/dl; year 2, 3.17±0.3 g/dl; year 3, 3.19±0.3 g/dl; year 4, 3.21±0.3 g/dl.

The strength of the relationship between PSTR and Pcl also weakened over time, as shown by the changing slope

Table 2. Multilevel mixed linear model predicting daily peritoneal protein clearance (square root transformed): base model and model adding dialysate glucose effect

Variable	Base Model			Model with Glucose Exposure		
	Coefficient	SEM	*P* Value	Coefficient	SEM	*P* Value
Constant	9.57	0.3	<0.01	9.44	0.34	<0.01
PSTR (for each 0.1 increase)	0.39	0.11	<0.01	0.43	0.11	<0.01
Comorbidity score (for each 1 unit increase)	−0.13	0.2	0.51	−0.18	0.21	0.39
Sex (if female)	−0.52	0.38	0.17	−0.37	0.4	0.35
Age (year)	0.016	0.011	0.14	0.019	0.011	0.1
UF capacity on PET (for each 100-ml increase)	0.04	0.03	0.24	0.04	0.04	0.22
Urine volume (for each 1000-ml increase)	0.2	0.1	0.2	0.2	0.2	0.15
PSTR×PD duration (year 2 versus year 1 for each 0.1 increase)	−0.23	0.14	0.11	−0.25	0.14	0.08
PSTR×PD duration (year 3 versus year 1 for each 0.1 increase)	−0.35	0.14	<0.01	−0.37	0.14	<0.01
PSTR×PD duration (year 4 versus year 1 for each 0.1 increase)	−0.30	0.14	<0.05	−0.27	0.15	0.07
Icodextrin use (versus not used)				0.007	0.019	0.7
Medium glucose exposure (daily average, 1.36% (1.5%)–2.27% (2.5%) (versus low glucose exposure) (*n*=51)				0.02	0.02	0.41
High glucose exposure (daily average≥2.27%) (versus low glucose exposure) (*n*=13)				0.03	0.03	0.45

See Supplemental Table 1 for calculation of the progressive effect over time on the PSTR β coefficient. UF, ultrafiltration; PET, peritoneal equilibration test; PD, peritoneal dialysis.

and statistical significance of the regression line, especially after baseline (Figure 2). The Pearson correlation coefficients (R) were as follows: baseline, 0.61 ($P<0.01$); year 1, 0.28 ($P=0.10$); year 2, 0.25 ($P=0.11$); year 3, 0.27 ($P=0.06$); year 4, 0.28 ($P=0.05$).

Multivariate Analysis of Pcl Determinants

Table 2 summarizes the initial multilevel mixed linear models constructed to investigate the relationship between Pcl and PSTR. Their positive correlation remained after adjustment for comorbidity score, sex, age, urine volume, and membrane ultrafiltration capacity. However, the time on therapy interaction shows that this correlation weakened, as demonstrated by the significant negative β coefficient (Supplemental Table 1 shows how the β coefficient linking PSTR to Pcl changed over time). Addition of glucose exposure and use of icodextrin to the model (Table 2) had little overall effect. Because only a few patients in this cohort were on a dry-day regimen (11 of 335 data sets), no subanalysis on this group was undertaken. Sensitivity analyses comparing the observations for the 4-year cohort with the 2- and 3-year cohorts are shown in Supplemental Table 2. These show that these observations can be generalized to the 3-year but not the 2-year cohort, indicating that time on treatment is required to demonstrate uncoupling, whereas at an earlier phase of treatment there is a correlation between age and Pcl that disappears because of selective loss of older patients from the program (Table 1).

Effect of Accounting for Ultrafiltration and Sodium Removal

One potential confounder of this analysis could be the increased requirement over time to obtain more ultrafiltration as a consequence of loss in residual renal function. Because albumin is able to pass through small pores, increased ultrafiltration rates have the potential to increase the convective removal of albumin and thus increase Pcl. Furthermore, although peritoneal ultrafiltration and sodium removal are two highly correlated variables ($r=0.88$; $P<0.01$), by introducing them separately or together in the model it is possible to explore the effect of ultrafiltration *via* the small pores (sodium-coupled) or aquaporins (uncoupled). Table 3 summarizes this by showing that when ultrafiltration or sodium was included separately, Pcl was positively associated with increased fluid or sodium removal. Included together, the relationship remained positive with sodium removal (small pore–coupled ultrafiltration) but was negatively associated with ultrafiltration. For all these more complex models, the time-dependent relationship between Pcl and PSTR remained unaffected. In separate models, continuous ambulatory PD and automated PD were treated independently, but no differences were observed (data not shown).

Effect of Peritonitis

The longitudinal relationship between Pcl and PSTR in patients who did or did not have peritonitis is shown in Figure 3. The pattern of dissociation was seen in both

Table 3. Multilevel mixed linear models predicting daily peritoneal protein clearance (square root transformed) demonstrating associations with ultrafiltration and sodium removal separately and together

Variable	Model with Daily Sodium Loss			Model with Daily UF			Model with UF and Sodium Loss		
	Coefficient	SEM	*P* Value	Coefficient	SEM	*P* Value	Coefficient	SEM	*P* Value
Constant	9.56	0.33	<0.01	9.54	0.34	<0.01	9.29	0.32	<0.01
PSTR (for each 0.1 increase)	0.47	0.11	<0.01	0.46	0.11	<0.01	0.40	0.10	<0.01
Comorbidity score (for each 1 unit increase)	−0.13	0.2	0.5	−0.16	0.21	0.43	−0.14	0.19	0.46
Sex (if female)	−0.18	0.38	0.63	−0.31	0.4	0.43	−0.14	0.37	0.71
Age (year)	0.02	0.01	0.09	0.02	0.01	0.11	0.02	0.01	<0.05
UF capacity on PET (for each 100-ml increase)	0.04	0.04	0.23	0.04	0.04	0.24	0.05	0.03	0.12
Urine volume (for each 1000-ml increase)	0.4	0.2	<0.05	0.32	0.16	<0.05	0.2	0.2	0.23
PSTR×PD duration (year 2 versus year 1 for each 0.1 increase)	−0.31	0.14	<0.05	−0.28	0.14	0.05	−0.28	0.13	<0.05
PSTR×PD duration (year 3 versus year 1 for each 0.1 increase)	−0.41	0.13	<0.01	−0.40	0.14	<0.01	−0.37	0.13	<0.01
PSTR×PD duration (year 4 versus year 1 for each 0.1 increase)	−0.31	0.15	<0.05	−0.28	0.15	0.06	−0.32	0.14	<0.05
Icodextrin use (versus not used)	−0.03	0.02	0.11	−0.01	0.02	0.54	−0.03	0.21	0.9
Medium glucose exposure (daily average, 1.36% (1.5%)–2.27% (2.5%) (versus low glucose exposure) (*n*=51)	0.02	0.02	0.25	0.02	0.02	0.43	0.36	0.19	0.05
High glucose exposure (daily average≥2.27%) (versus low glucose exposure) (*n*=13)	−0.01	0.03	0.78	0.009	0.04	0.81	0.1	0.33	0.75
Daily UF (for each 100-ml increase)				0.032	0.016	<0.05	−0.15	0.03	<0.01
Daily sodium loss from dialysate (mmol)	0.006	0.001	<0.01				0.02	0.00	<0.01

UF, ultrafiltration; PET, peritoneal equilibration test.

patient groups, but PSTR tended to be increased at each time point in those who had peritonitis, especially at year 4 ($P<0.05$). Multilevel modeling by peritonitis subgroup (see Table 4) did not alter the previously described associations except for revealing a significant relationship between Pcl and peritoneal ultrafiltration capacity in the peritonitis-positive group.

Discussion

The intention of this analysis was to determine as cleanly as possible whether longitudinal peritoneal Pcl is coupled to changes in PSTR by studying a continuously treated cohort of patients, uncontaminated by dropout and adjusted for known clinical and theoretical factors that could influence their association. We demonstrated unequivocally, for the first time to our knowledge, that these two measures of membrane function dissociate over time and that this is independent of other predictors of Pcl (age, sodium coupled/small pore ultrafiltration) or known drivers of membrane change, such as glucose exposure and peritonitis.

The progressive uncoupling of Pcl and PSTR was due to a rise in the PSTR that was not matched by an increase in Pcl in line with the relationship seen between these two measures at baseline. Increasing PSTR with time on PD is well established (2–5) and from a theoretical perspective could be due to an increase in the anatomic membrane in contact with dialysate, an increase in the density of perfused capillaries (to include neoangiogenesis), or an increase in capillary perfusion rate or any combination of the above. There are good reasons to believe that the

Table 4. Multilevel mixed linear model of daily peritoneal protein clearance (square root transformed) subgroup analysis to investigate peritonitis effect

Variable	Model without Peritonitis (n=171)			Model with Peritonitis (n=164)		
	Coefficient	SEM	P Value	Coefficient	SEM	P Value
Constant	9.36	0.38	<0.01	9.35	0.51	<0.01
PSTR (for each 0.1 increase)	0.38	0.14	<0.01	0.43	0.17	<0.01
Comorbidity score (for each 1 unit increase)	−0.12	0.28	0.68	−0.28	0.28	0.31
Sex (if female)	−0.54	0.43	0.21	0.3	0.6	0.62
Age (year)	0.016	0.012	0.21	0.031	0.016	0.06
UF capacity on PET (for each 100-ml increase)	0.01	0.05	0.88	0.12	0.05	<0.01
Urine volume (for each 1000-ml increase)	0.3	0.2	0.08	−0.2	0.2	0.43
PSTR×PD duration (year 2 versus year 1 for each 0.1 increase)	−0.19	0.16	0.24	−0.46	0.23	<0.05
PSTR×PD duration (year 3 versus year 1 for each 0.1 increase)	−0.27	0.16	0.1	−0.54	0.21	<0.05
PSTR×PD duration (year 4 versus year 1 for each 0.1 increase)	−0.44	0.19	<0.01	−0.29	0.22	0.19
Icodextrin use (versus not used)	−0.14	0.31	0.65	0.1	0.27	0.73
Medium glucose exposure (daily average, 1.36% (1.5%)–2.27% (2.5%) (versus low glucose exposure) (n=51)	0.38	0.28	0.17	0.42	0.25	0.09
High glucose exposure (daily average ≥2.27% (2.5%) compared with low glucose exposure) (n=13)	−0.08	0.54	0.89	0.26	0.42	0.54
Daily UF (for each 100-ml increase)	−0.11	0.04	<0.01	−0.21	0.05	<0.01
Daily sodium loss from dialysate (mmol)	0.01	0.00	<0.01	0.02	0.00	<0.01

UF, ultrafiltration; PET, peritoneal equilibration test.

clinical variability in PSTR at the start of PD is in large part due to local membrane inflammation (16), and the strong relationship with Pcl at baseline observed in this study supports this because inflamed capillaries will be leaky. If this had been purely an anatomic coupling, this should have remained the case throughout the study; in fact, however, an increase in small-pore area with a relative decline in the large-pore area occurred. This relative reduction in large-pore area could reflect many processes, including a resolution of the early inflammatory state of the membrane after the start of PD. Several studies have also shown that depending on when the initial membrane function tests are done, PSTR also decreases in the months after PD is established before the longer-term increase (10,18–21). Alternatively, it could mean that large pores are underrepresented in newly formed vessels (surprising because new vessel formation in diabetic nephropathy is associated with increased protein extravasation) or that interstitial and perivascular fibrosis, known to occur over time (1), is impeding large-pore leak through the interstitial structures. Distinguishing these mechanisms is beyond the capacity of this study. However, it is possible that further development of the three-pore membrane/fiber-matrix model, which may explain the observed "uncoupling" of small solute transport and ultrafiltration coefficient seen in long-term PD (22), might provide mechanistic insights. Measuring Pcl in the clinic is relatively easy and may provide an additional method of identifying membrane injury.

Known drivers of membrane injury, namely glucose and/or glucose degradation products (>95% of patients in this study were treated with conventional glucose solutions), and peritonitis did not appear to have a marked effect on the progressive uncoupling of PSTR and Pcl. There is more than a suggestion that in the peritonitis group membrane changes were overall more severe over time, especially the increase in PSTR. In addition, in the multivariate analysis of the peritonitis subgroups there was an association between membrane ultrafiltration capacity as well as PSTR in patients who had infections. Cause and effect cannot be inferred from this type of analysis, but this would be in line with more severe membrane injury causing reduced osmotic conductance and Pcl through more severe fibrosis (3,20–22).

This study's failure to detect a relationship between Pcl and comorbidity on multivariate analysis deserves comment. There are several possible explanations. First, a relationship with increasing age, as described previously (8–10), was seen in the patients treated for 2 and 3 years (see Supplemental Table 2), and this could account for some hidden association with comorbid disease. Second, this is a deliberately selected patient cohort and patients with more serious comorbidity—or specific types, such as peripheral vascular disease—were under-represented. Finally, only

49 patients were included, which may have led to type 2 statistical error in the level 2 component of the mixed-linear model. It is also in keeping with recent observations that it is the leak of albumin from the systemic circulation, reflecting endothelial dysfunction, rather than peritoneal Pcl, that is the link to comorbid phenotype (23).

It was important to correct for other known theoretical determinants of Pcl that might change with time on PD. Albumin, by far the most abundant protein lost in PD effluent, is of a size that can potentially pass through small as well as large pores. Small-pore albumin flux is driven by convection and hydrostatic pressure. To determine the contribution of achieved ultrafiltration (small-pore convection) to Pcl, we included this in the model, where it was associated with Pcl independent of PSTR and without affecting the time on treatment interaction. By including sodium removal, which is removed predominantly *via* small-pore convection, the prediction by the three-pore model that Pcl (the albumin component) is partly determined by this pathway is confirmed.

As already alluded to, this study has several limitations. Although our primary hypothesis was refuted, the study was observational and generates more questions than it answers, especially with respect to cause and effect. In dealing with the concern related to informative censoring, we had to define a selected patient group, so generalizability should be considered carefully. Our supplementary analyses of 2- and 3-year data support our approach because there is clearly a relationship between Pcl and age that disappears over time and is independent of the increasing dissociation between the membrane changes that take time to develop. Selection is a problem that affects all long-term studies of patients undergoing PD, and in this sense the present study is no exception and the characteristics of the population studied are typical. Thus, there is no reason not to conclude that our observations cannot be generalized to "longer-term" PD patients. For some of the covariates measured (*e.g.*, glucose exposure), variability in the population may have been insufficient to allow adequate power to detect an effect (only 13 observations used a high glucose regimen [mean concentration >2.27%]) in this cohort, in which the clinical strategy since 2000 has been to avoid such prescriptions. The subgroup analysis by peritonitis is of interest but potentially underpowered so should be viewed with caution. Finally, the method we used to estimate Pcl is inversely dependent on plasma albumin, which in turn covaries with comorbidity. This partly explains the relationship observed in cohort studies between Pcl and survival. This is unlikely to be a confounder in the present analysis because of lack of dropout and the stability of plasma albumin throughout the study.

In conclusion, we have shown that the small- and large-pore pathways are initially linked at the start of PD but become progressively uncoupled over time. The chief implication of this observation is what it adds to our understanding of the progressive changes to the membrane that occur over time. It supports a view that two processes are occurring—increased vascular surface area and progressive fibrosis—and that research should be done to identify biomarkers of this process (*e.g.*, using the bioresource established for the Global Fluid Study [24]) and develop antifibrotic treatment strategies. There is potential to use this uncoupling as an easily-obtained clinical measurement to identify membrane change and monitor treatment interventions in the future.

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Disclosures

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