DIALYSATE CYTOKINE LEVELS DO NOT PREDICT ENCAPSULATING PERITONEAL SCLEROSIS

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◆ **Background:** Encapsulating peritoneal sclerosis (EPS) is a rare but devastating complication of long-term peritoneal dialysis (PD). There is no well-validated method for predicting which patients will develop the condition, although known risk factors include long duration of PD, high glucose exposure and lack of residual renal function. We have investigated whether dialysate cytokines (MCP-1 (monocyte chemotactic protein-1), CCL18 (pulmonary and activation-regulated cytokine, PARC), IL-6 (interleukin-6), CCL15 (leukotactin) and angiogenin) could be used to predict the onset of EPS more effectively than known clinical risk factors.

◆ **Methods:** Samples of dialysate and clinical data were prospectively collected from 151 patients at the West London Renal center between 2003 and 2010. Dialysate cytokine levels were measured using the enzyme-linked immunoabsorbant assay (ELISA) technique. Encapsulating peritoneal sclerosis subsequently developed in 17 patients during a follow-up period of 27 – 113 months. Cytokines found at higher levels in dialysate of pre-EPS patients were investigated as candidate predictors of EPS using logistic regression analysis.

◆ **Results:** Dialysate IL-6, MCP-1 and CCL15 were significantly higher in patients who subsequently developed EPS; however, a logistic regression model using dialysate cytokines to predict EPS was no better than a model using well-recognized clinical markers (length of time on PD and membrane transport status).

◆ **Conclusions:** Although MCP-1, IL-6 and CCL15 were found at higher levels in the dialysate of patients who subsequently developed EPS, dialysate levels of these cytokines do not improve prediction of future EPS above a model using known clinical risk factors.

KEY WORDS: Cytokines; screening; encapsulating peritoneal sclerosis.

Many patients find peritoneal dialysis (PD) a convenient and effective mode of renal replacement therapy. However, the long-term use of PD is complicated by changes in the structure (1) and function (2) of the peritoneal membrane. With time, loss of the mesothelial cell layer and the development of submesothelial fibrosis creates a thickened and denuded peritoneum. This abnormal peritoneal membrane is the substrate for the later development of encapsulating peritoneal sclerosis (EPS).

Encapsulating peritoneal sclerosis is an uncommon but severe complication of PD. The bowel becomes cocooned in a thick layer of fibrous tissue and symptoms of bowel obstruction develop. It is diagnosed clinically, presenting with persistent vomiting, abdominal pain and distension, weight loss and ascites. There may be fever and raised inflammatory markers during the inflammatory phase (3). The diagnosis can be confirmed by CT scanning (4,5). Encapsulating peritoneal sclerosis commonly presents after transfer to hemodialysis (HD) or transplantation (6), although symptoms can occur while the patient is still using PD. The incidence and severity depends on the duration of PD (7–9); other risk markers include rapid membrane transport (10), reduced osmotic conductance of the peritoneal membrane (11), younger age (8) and ultrafiltration failure (12).

Encapsulating peritoneal sclerosis rarely develops when the duration of PD is less than four or five years and the incidence increases with longer PD duration. The question therefore arises as to whether PD patients should be advised to change to HD after four years. Assessing the risk of future EPS for an individual patient is challenging, making it difficult to give accurate guidance regarding an elective change in renal replacement modality. This decision can have a major impact on the

Perit Dial Int 2014; 34(6):594–604 www.PDIConnect.com
epub ahead of print: 01 Mar 2014 doi: 10.3747/pdi.2012.00305

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Received 10 November 2012; accepted 25 July 2013.

patient's quality of life, since HD will also entail its own set of complications for the patient.

Radiological screening of current PD patients for early signs of pathology is not satisfactory. Fluorodeoxyglucose positron emission tomography showed promise in detecting the early inflammatory phase of EPS (13) but is not widely available; cine magnetic resonance imaging (MRI) is abnormal in patients with EPS (14) but whether the abnormalities of bowel movement are present in pre-symptomatic patients is unknown. Computed tomography (CT) screening of asymptomatic patients is not reliable (15). Peritoneal biopsy is an invasive procedure that cannot always differentiate simple peritoneal sclerosis from the more marked changes of EPS (16).

The ability to quantify the risk of future EPS by measuring a biomarker in the effluent dialysis fluid would be extremely useful to PD patients. Dialysate cytokine levels are an attractive method of screening for EPS because the dialysate samples are readily available, the test is non-invasive and cytokines can easily be measured by commercially available enzyme-linked immunoabsorbant assay (ELISA) kits with acceptable inter-assay variability (6 – 11% in our laboratory).

The cytokines MCP-1 (monocyte chemotactic protein-1), IL-6 (interleukin-6), CCL15 (leukotactin), CCL18 (pulmonary and activation-regulated cytokine, PARC) and angiogenin were chosen for investigation because relatively high levels were found in dialysate samples examined using a multiplex antibody array, the RayBio Human Cytokine Antibody array (RayBiotech, Inc., Norcross, GA, USA) (17), which produces a semi-quantitative indication of the levels of 120 cytokines and related proteins. In addition, the known actions of these molecules suggest they might plausibly be involved in the pathogenesis of EPS. Peritoneal membrane changes are a combination of acute and chronic inflammation, angiogenesis and fibrosis. IL-6 mediates both acute inflammation and the switch to chronic inflammation (18). It is produced by mesothelial cells (19). CCL15 is a novel chemokine with the unusual property of inducing neutrophil chemotaxis in its 98 amino acid form. It is activated by matrix metallo-proteases (MMPs) (20) and neutrophil cathepsin (21) to become chemotactic for monocytes (22). CCL15 may be a link from an acute inflammatory response to sustained chronic inflammation and fibrosis. MCP-1 is produced by mesothelial cells (23) and the high glucose levels, polyol pathway metabolites and hyperosmolarity of dialysate promote MCP-1 production (24). MCP-1 is involved in fibrosis in the lung (25) and peritoneum (26) as well as attracting monocytes to establish a chronic inflammatory state. Angiogenin is essential for the angiogenic activity of

vascular endothelial growth factor (VEGF) (27), which is known to play a role in the vascular changes in the peritoneal membrane during PD (28). CCL18 is produced by alternatively activated macrophages in the peritoneum (29); the ability of these macrophages to stimulate fibroblast proliferation has been shown to correlate with CCL18 mRNA levels.

The aims of this study were firstly to establish whether dialysate levels of these cytokines were higher in samples from patients who subsequently developed EPS than in samples from patients who did not experience this complication. Logistic regression was then used to determine whether the levels of these cytokines in the effluent dialysate of stable PD patients could be used to predict future EPS with any more success than current well-recognized clinical risk markers.

SUBJECTS AND METHODS

Ethical approval was obtained from the local ethics committee (Riverside Research Ethics Committee, Charing Cross Hospital; REC number 08/H0706/20). We prospectively collected serum and dialysate samples and clinical data from our population of prevalent PD patients between 2003 and 2010. Samples were taken at the end of routine peritoneal equilibration tests (PET) to ensure a standardized method and a well-mixed representative sample of dialysate. All PD patients able to give informed consent were eligible for inclusion in the study. Dialysate samples were centrifuged at 4°C at 3,000 rpm (1,107 *g*) for 15 minutes then aliquoted and stored at –80°C. Dialysate levels of MCP-1, CCL18, angiogenin and CCL15 were measured using a sandwich ELISA technique (antibodies and standards from R&D systems, Minneapolis, MN, USA). IL-6 was measured using a high-sensitivity Quantikine ELISA kit (R&D systems). The ELISA results were analyzed using non-linear regression with GraphPad Prism software version 4 (GraphPad Software Inc, La Jolla, CA, USA). The coefficients of variation for dialysate cytokine measurements (calculated from a minimum of 10 pre-determined repeated measurements) are shown in Table 1. Intra-assay variation refers to the variation in results obtained when the same sample is repeated on a single ELISA plate; inter-assay variation describes the variability in results obtained when the same sample is analyzed on separate occasions.

To correct the concentration of cytokine measured in the dialysate for the varying volumes of dialysate drained at the end of the PET test, all dialysate cytokine levels are reported as the quantity present at the end of the four-hour dwell, expressed in nanograms per four hours, rather than the concentration of cytokine.

TABLE 1
Coefficients of Variation for Measurement of
Dialysate Cytokine Levels

	Inter-assay	Intra-assay
MCP-1	10.9%	6.3%
CCL18	6.2%	4.1%
CCL15	5.6%	5.1%
Angiogenin	11.0%	6.0%
IL-6	9.6%	5.6%

MCP-1 = monocyte chemotactic protein-1; CCL18 = pulmonary and activation-regulated cytokine, PARC; CCL15 = leukotactin; IL-6 interleukin-6.

Cytokine levels and clinical measures were compared between patients who later developed EPS and those who did not with non-parametric statistics (Mann-Whitney test) using GraphPad Prism version 4. Logistic regression was used to examine whether cytokine levels or clinical data could be used to predict whether EPS would later develop. Cytokine levels and duration of PD data required log transformation to satisfy model requirements. Regression analysis was performed using SPSS version 19 (IBM, New York, NY, USA) and the R project version 13.

RESULTS

Data and samples were available for 151 patients, 17 of whom (11%) subsequently developed EPS. The median final duration of PD was 44.3 months with nearly a third of the cohort remaining on PD for more than five years. During the follow-up period (until March 2012, 2 – 9 years from time of sample collection), 17 patients died while on PD, 48 patients transferred to HD and 49 were transplanted.

The clinical diagnosis of EPS, made when patients developed typical symptoms without an alternative diagnosis, was confirmed in all cases by radiology (CT scanning) by the criteria of Tarzi *et al.* (4). Two patients also had the diagnosis confirmed by direct inspection at surgery (one patient had surgery for a hernia repair and another had surgery for EPS). The time from PD cessation (transplantation or transfer to HD) until the development of EPS was short, with a median value of 3.1 months (range: 0.6 – 7.7 months); one patient was diagnosed with EPS while still on PD. The median time from baseline sampling to the development of EPS was 13.6 months (range: 4.8 – 46 months); all but one of the EPS patients developed the condition within 26 months of sample collection. Eight patients developed

EPS within one year of sample collection and seven more within two years.

Nine patients received parenteral nutrition; several more patients diagnosed early in the study period would certainly have been offered parenteral nutrition under the current guidelines for EPS management. Eight patients died of EPS; a further patient survived for several years but remained dependent on parenteral nutrition and eventually died from sepsis.

Details of clinical factors and cytokine levels in patients with and without EPS are shown in Table 2. We used the baseline samples taken at time of recruitment into the study for the analysis in both EPS and non-EPS patients. Compared to the remainder of the cohort, patients who developed EPS had been on PD for a longer period of time (median 76 vs 40 months, $p < 0.001$). Glucose exposure at time of sampling was higher (median 1,266 vs 796 mmol/24 h, $p < 0.001$) and both urine output (median 40 vs 764 mL/24 h, $p < 0.001$) and residual glomerular filtration rate (GFR) (median 0.4 vs 3.8 mL/min) were lower. Patients who later developed EPS had significantly higher dialysate levels of the cytokines MCP-1 ($p < 0.001$), IL-6 ($p < 0.001$) and CCL15 ($p = 0.0034$). Although levels of dialysate angiogenin and CCL18 appeared higher in patients who later developed EPS, dialysate levels of these cytokines were not significantly different from the patients who never developed EPS. Systemic levels of inflammation, as indicated by serum C-reactive protein levels, were no different in the pre-EPS group ($p = 0.28$), nor did CRP levels correlate with dialysate levels of MCP-1 ($p = 0.91$), IL-6 ($p = 0.13$), angiogenin ($p = 0.11$) or CCL15 ($p = 0.3$). There was a significant correlation between serum CRP and dialysate CCL18 levels only ($\rho = 0.34$, $p < 0.001$). Serum levels of cytokines were not higher in pre-EPS patients.

The median number of peritonitis episodes (1 vs 0, $p = 0.007$) was higher in the pre-EPS patients; similarly the pre-EPS group were more likely to have a history of peritonitis (76%, 95% confidence interval (CI) 56 – 97% vs 38%, 95% CI 30 – 46%; Fisher's exact test $p = 0.004$). The number of prior peritonitis episodes at the time of sampling was not significantly correlated with any of the measured dialysate cytokine levels, perhaps because in our population the peritonitis rate was low at one episode per 50 patient months and more than half of the sampled patients had never experienced peritonitis.

We found significant correlations between dialysate cytokine levels and other clinical risk factors for EPS. Figure 1 illustrates the relationship between dialysate MCP-1 and the dialysate to plasma creatinine ratio (D/P creatinine) (Spearman's $r = 0.485$, $p < 0.001$); very similar results were seen for all cytokines investigated: CCL18

TABLE 2
Clinical Features and Dialysate Cytokine Levels of Patients with EPS and Those Without EPS

	EPS (n=17) (median and IQ range)	Non-EPS (n=144) (median and IQ range)	p value (Mann-Whitney)
Age (years)	53 (49–66)	58 (48–69)	0.513
Total duration PD (months)	76 (46–94)	40 (8–45)	<0.001
Glucose exposure (mmol/24 h)	1,266 (1,032–2,076)	796 (573–1,205)	<0.001
Urine output (mL/24 h)	40 (0–364)	764 (431–1,285)	<0.001
GFR (mL/min)	0.4 (0–1.69)	3.8 (1.6–5.4)	<0.001
D/P creatinine	0.83 (0.74–0.9)	0.71 (0.63–0.78)	0.002
Peritonitis episodes	1 (0.5–1.5)	0 (0–1)	0.018
Peritonitis episodes/month on PD	0.012 (0.004–0.022)	0 (0–0.035)	0.181
	EPS (Percentage and CI)	Non-EPS (Percentage and CI)	p value (Fisher's exact)
Gender male	65 (42–88) %	61 (53–69) %	1
APD	82 (64–100) %	56 (48–64) %	0.04
Peritonitis ever	77 (56–97) %	38 (30–46) %	0.004
Ultrafiltration failure	47 (23–71) %	8 (4–13) %	<0.001
	EPS (ng/4 h) (median and IQ range)	Non-EPS (ng/4 h) (median and IQ range)	p value (Mann-Whitney)
MCP-1	658 (501–907)	396 (304–583)	<0.001
IL-6	92 (52–243)	40 (22–97)	<0.001
CCL15	8,017 (6,562–11,217)	4,014 (2,511–7,815)	0.0034
CCL18	8,437 (4,234–12,376)	6,440 (3,505–10,714)	0.25
Angiogenin	141,261 (76,319–169,751)	100,880 (69,293–140,860)	0.15

EPS = encapsulating peritoneal sclerosis; IQ = interquartile; PD = peritoneal dialysis; GFR = glomerular filtration rate; D/P creatinine = dialysate to plasma creatinine ratio; CI = confidence interval; APD = automated peritoneal dialysis; MCP-1 = monocyte chemotactic protein-1; IL-6 = interleukin-6; CCL15 = leukotactin; CCL18 = pulmonary and activation-regulated cytokine, PARC.

and D/P creatinine $r = 0.507$, IL-6 and D/P creatinine $r = 0.528$, CCL15 and D/P creatinine $r = 0.447$; angiogenin and D/P creatinine $r = 0.4889$; all $p < 0.001$. Dialysate levels of MCP-1, IL-6, CCL15 and CCL18 also correlated (less closely) with the duration of PD.

Because a relationship was seen between dialysate cytokine levels and the duration of PD, the association between cytokine levels and EPS could simply be a function of the duration of dialysis, the strongest risk factor for EPS. We performed a further analysis in which dialysate cytokine levels in the 17 EPS patients were compared with the levels found in 34 PD patients matched as closely as possible for duration of PD. The clinical characteristics of this group are shown in Table 3. There was no significant difference in duration of PD at time of sampling between these matched groups (median 64 months in the EPS group vs 60 in the non-EPS group, $p = 0.42$). We note that dialysate levels of MCP-1 (658 vs 451 ng/4 h, $p = 0.015$) remain significantly different even when

matched by duration of PD; the levels of IL-6 also remain significantly higher in the pre-EPS group (92.1 vs 59.9 ng/4 h, $p = 0.041$). CCL15 levels are higher in the pre-EPS group but the difference is not statistically significant (8,017 vs 5,235 ng/4 h, $p = 0.076$). CCL18 levels did not differ significantly between the two groups (8,124 vs 7,114 ng/4 h, $p = 0.803$), nor did angiogenin levels (141,261 vs 98,080 ng/4 h, $p = 0.154$). Figure 2 illustrates the key clinical features of the EPS and non-EPS matched cohort. Figure 3 shows the cytokine levels in the two groups. Of note is the lower urine output and GFR seen in the patients who went on to develop EPS, even when compared to a cohort matched for PD duration. Similarly glucose exposure and D/P creatinine are higher in the pre-EPS group than in the matched cohort. It is not possible to know from this data whether it is loss of residual function or the consequently greater glucose exposure, membrane damage and increase in D/P creatinine which is the most important marker for the development of EPS.

Figure 1 — The four-hour dialysate quantity of MCP-1 correlates with D/P creatinine. Similar correlations were seen for all cytokines studied. MCP-1 = monocyte chemotactic protein-1; D/P creatinine = dialysate to plasma creatinine ratio.

Although there were only a small number of patients with EPS, we investigated whether dialysate cytokine levels had any relationship with the outcome of EPS; we found no significant difference between cytokine levels in those who survived ($n = 9$) and those who died of EPS ($n = 8$). However, the number of results available for this analysis is low.

In addition to the cross-sectional data, follow-up samples were available at a median of 14 months from the original sample point in 50 patients. The change in median cytokine levels at one year was not a significant predictor of future EPS. This may be a reflection of the small number of patients in this cohort; also, this group of patients were all stable and did not require modality switch; they may not therefore be representative of the population as a whole.

The number of samples taken within twelve months of diagnosis of EPS is small (8 baseline samples and a further 3 samples from the follow-up samples collection), limiting further analysis. However, MCP-1 ($p = 0.008$), CCL15 ($p = 0.007$) and IL-6 ($p = 0.0008$) all remain significantly higher in pre-EPS patients when only samples taken within a year of diagnosis are used.

Due to the small number of events (17 EPS cases) in our cohort, it is not possible to analyze more than two or three candidate predictors by logistic regression analysis (30–32). We first analyzed the ability of clinical data to predict future EPS in our population, since any candidate biomarker would need to be an improvement on readily

available clinical data. Duration of PD is established as the chief risk factor for EPS. As a second predictor we selected D/P creatinine. We preferred this above other clinical predictors of EPS because GFR is an approximation in this population (usually estimated as the average of urea and creatinine clearance) and urine collections may be unreliable. Glucose prescription is dependent on the clinician. D/P creatinine is an objective, measurable factor and high values have been shown to be a risk factor for EPS (10).

Table 4 shows the details of the logistic regression analysis obtained using duration of PD and D/P creatinine as candidate predictors. The p values for both factors are significant. The value $\text{Exp}(B)$ is an odds ratio; high numerical values occur due to the use of the logarithm of PD duration in order to satisfy model requirements.

In order to assess the usefulness of this analysis in the prediction of EPS, a receiver operating characteristic (ROC) curve was constructed for the regression; this is shown in Figure 4.

The determination of the “best” cut-point for such a curve is a matter of debate, and depends on the clinical importance of sensitivity versus specificity. It could be argued that the risk of mortality associated with EPS exceeds the risks of changing dialysis modality; sensitivity should then be prioritized. The optimal cut-point can also be calculated as the shortest distance to the ideal test curve, that is, where $(100\% - \text{sensitivity})^2 + (100\% - \text{specificity})^2$ is at a minimum. A cut-point of > 0.1239 for the regression gives a sensitivity of 88.2% (95% CI 64 – 99%) and specificity of 76.6% (95% CI 68 – 84%) and 15 of 17 EPS cases are correctly predicted. The relatively low specificity, however, is not satisfactory. In addition, this model might be less successful in another PD population where EPS incidence differs.

We next investigated whether cytokines would be any more successful in the prediction of future EPS. A regression model was constructed with the cytokines MCP-1, IL-6 and CCL15 as the predictors, using a forward conditional model with a cut-off value for significance of < 0.05 to remain in the model. The only cytokine to remain significant in the logistic regression model was MCP-1, as shown in Table 5.

This model is likely to be less effective than the clinical model as the area under the ROC curve is smaller (0.78 for the cytokine model vs 0.886 for the clinical predictors). Figure 5 shows the ROC curve for the cytokine model.

The cut-point of > 0.1473 is the closest point on the observed ROC curve to the perfect curve (calculated as the point with the least sum of squares). At this point, sensitivity is 68.8% (95% CI 41 – 89%) and specificity is

TABLE 3

Clinical Characteristics of the EPS Patients Compared to a Group Matched for the Duration of PD at Time of Sampling

	EPS (n=17) (median and IQ range)	Non-EPS (n=34) Matched for PD duration at sample (median and IQ range)	p value (Mann-Whitney)
Age (years)	53 (49–66)	59 (51–72)	0.216
Total duration PD (months)	76 (46–94)	71 (51–87)	0.185
PD duration at sampling (months)	64 (46–99)	60 (45–75)	0.424
Glucose exposure (mmol/24 h)	1,266 (1,032–2,076)	857 (530–1,452)	0.0014
Urine output (mL/24 h)	40 (0–364)	602 (179–1,277)	0.0025
GFR (mL/min)	0.4 (0–1.69)	2.74 (0.85–4.75)	0.0026
D/P creatinine	0.83 (0.74–0.9)	0.71 (0.42–0.78)	0.0028
Peritonitis episodes	1 (0.5–1.5)	1 (0.5–1.5)	0.4556
Peritonitis episodes/month on PD	0.012 (0.004–0.022)	0.011 (0–0.023)	0.5736
	EPS (Percentage and CI)	Non-EPS (Percentage and CI)	p value (Fisher's exact)
Gender male	65 (42–88) %	65 (49–81) %	1
APD	82 (64–100) %	59 (42–75) %	0.1221
Peritonitis ever	77 (56–97) %	56 (39–73) %	0.2217
Ultrafiltration failure	47 (23–71) %	18 (5–30) %	0.0199
	EPS (ng/4 h) (median and IQ range)	Non-EPS (ng/4 h) (median and IQ range)	p value (Mann-Whitney)
MCP-1	658 (501–907)	451 (327–735)	0.0154
IL-6	92 (52–243)	60 (28–116)	0.0406
CCL15	8,017 (6,562–11,217)	5,235 (2,622–8,535)	0.0761
CCL18	8,437 (4,234–12,376)	7,114 (3,411–11,600)	0.8028
Angiogenin	141,261 (76,319–169,751)	98,080 (59,029–147,144)	0.1543

EPS = encapsulating peritoneal sclerosis; PD = peritoneal dialysis; IQ = interquartile; GFR = glomerular filtration rate; D/P creatinine = dialysate to plasma creatinine ratio; CI = confidence interval; APD = automated peritoneal dialysis; MCP-1 = monocyte chemotactic protein-1; IL-6 interleukin-6; CCL15 = leukotactin; CCL18 = pulmonary and activation-regulated cytokine, PARC.

77.3% (95% CI 69 – 84%), which is by no means adequate for use in clinical practice. If sensitivity is improved, specificity is poor: a cut-point of > 0.1062 gives a sensitivity of 75% (95% CI 48 – 93%) and specificity of only 69% (95% CI 60 – 77%).

Combining the duration of PD with D/P creatinine and MCP-1 is not an appropriate or useful analysis to perform because D/P creatinine and MCP-1 are closely correlated.

DISCUSSION

In keeping with the literature, the clinical features in our population associated with EPS after the cessation of PD were longer duration of PD, lack of residual renal function, high glucose requirements and high D/P creatinine ratio.

Several groups have investigated whether particular molecules are found at higher levels in the dialysate of patients who later developed the symptoms of EPS. Hirahara *et al.* (33) investigated matrix metallo-protease 2 as a marker of EPS; of fifteen patients with an MMP-2 level of more than 600 ng/mL, seven developed EPS. Only one patient with an MMP-2 level less than 600 ng/mL developed EPS. Sampimon *et al.* (34) found that a low level of CA 125 (appearance rate of < 33 U/min) and a high level of IL-6 (appearance rate > 350 pg/min) had a 70% sensitivity and 89% specificity for the diagnosis of later EPS. A recent proof-of-principle study demonstrates that the study of metabolomic profiles in dialysate might also predict future EPS (35).

In a smaller group of patients, our group had previously observed higher levels of CCL18 in the dialysate

Figure 2 — Clinical features of the EPS patients and of the cohort matched for PD duration. EPS = encapsulating peritoneal sclerosis; PD = peritoneal dialysis; D/P creatinine = dialysate to plasma creatinine ratio; GFR = glomerular filtration rate.

Figure 3 — Dialysate cytokine levels in the EPS patients and in the cohort matched for PD duration. EPS = encapsulating peritoneal sclerosis; MCP-1 = monocyte chemotactic protein-1; IL-6 = interleukin-6; CCL15 = leukotactin; CCL18 = pulmonary and activation-regulated cytokine, PARC; PD = peritoneal dialysis.

of patients developing EPS within one year of follow-up (17). We did not confirm this observation in this larger cohort, although the same assay and protocols were used. We have now followed our patients for a longer time period and documented further, later onset, cases of EPS. We note that dialysate levels of CCL18, a cytokine linked to fibrosis, were 35% higher in patients developing

EPS within one year of sampling as compared to those developing EPS at a later time. This may explain why, in our earlier study, patients with EPS within a year of sampling had higher levels of CCL18, whereas, in the current study (in which EPS often occurred after a longer period of follow-up), CCL18 levels were not significantly higher in the dialysate of the pre-EPS samples.

TABLE 4
Logistic Regression Analysis of Clinical Predictors of EPS

	B	S.E	Wald χ^2	df	Significance	Exp(B)
Log final duration PD	7.682	2.218	11.992	1	0.001	2,169.167
D/P creatinine	8.23	3.016	7.448	1	0.006	3,750.026
Constant	-22.165	4.894	20.509	1	<0.001	0

EPS = encapsulating peritoneal sclerosis; PD = peritoneal dialysis; D/P creatinine = dialysate to plasma creatinine ratio; B = constant; S.E = standard error around the constant; df = degrees of freedom; Exp(B) = exponentiation of the B coefficient.

Figure 4 — ROC (receiver operating characteristic) curve to illustrate the logistic regression analysis. The predictors of EPS are duration of PD and D/P creatinine. The area under the curve is 0.886. EPS = encapsulating peritoneal sclerosis; PD = peritoneal dialysis; D/P creatinine = dialysate to plasma creatinine ratio.

In this study, we found that dialysate MCP-1, IL-6 and CCL15 levels were significantly higher in patients who subsequently developed EPS during prospective follow-up. However, dialysate cytokine levels may be confounded by other clinical factors in the patients, including problems related to PD. Compared to the non-EPS cohort matched for duration of PD, dialysate IL-6 and MCP-1 remained significantly higher in the group which went on to develop EPS, but CCL15 levels were not significantly higher. Of note, residual function (as assessed by either urine output or GFR) was lower in the pre-EPS group even when compared to the PD duration-matched cohort. This, combined with a faster transport status, meant the pre-EPS patients also had greater glucose exposure. It is not possible from our data to determine

whether the higher dialysate cytokine levels are caused by these important clinical differences.

Using levels of the dialysate cytokines MCP-1, IL-6 and CCL15 as predictors of EPS in the logistic regression analysis did not have any benefits beyond that of a model using the basic clinical parameters, duration of PD and D/P creatinine. Clearly these cytokines may still be involved in the pathogenesis of EPS. Local levels of the cytokine, in and around the peritoneum, may be critical to any biological effects. Levels of the cytokine in dialysate may not adequately reflect these local effects of cytokines.

Cytokines found in dialysate might have been produced by peritoneal cells or alternatively enter the dialysate from the circulation (or both mechanisms may operate). Dialysate levels of MCP-1 and IL-6 may exceed serum levels, suggesting peritoneal production is substantial. The source of dialysate cytokines is an interesting question which we are investigating further. However, since D/P creatinine correlated with dialysate cytokine levels, combining D/P creatinine with dialysate cytokine levels in a predictive model was not appropriate. For example, the correlation between dialysate levels of MCP-1 and D/P creatinine is shown in Figure 1.

The combination of total duration of PD and D/P creatinine was at least as effective in the prediction of future EPS as the model using cytokine levels. Although this clinical model appeared to have some limited potential to predict EPS, investigation of model performance in different and larger dialysis populations would establish whether it has any real utility or not. Other potential predictors of EPS such as GFR or urine output could perhaps then be added into the model.

The main limiting factor in our study is the relatively small number of EPS cases, which impacts on the number of predictors that can be included in the analysis. The low incidence and hence the small number of cases in each center is a recurring problem in studies examining the pathogenesis of EPS and has prompted the development of an EPS Registry and a call for international cooperation (36) to investigate this disease.

TABLE 5
Logistic Regression Analysis of Cytokine Predictors of EPS

	B	S.E	Wald χ^2	df	Significance	Exp(B)
Log MCP-1	5.112	1.493	11.722	1	0.001	166.039
Constant	-16.041	4.194	14.632	1	<0.001	0.000

EPS = encapsulating peritoneal sclerosis; MCP-1 = monocyte chemotactic protein-1; B = constant; S.E = standard error around the constant; df = degrees of freedom; Exp(B) = exponentiation of the B coefficient.

Figure 5 — ROC (receiver operating characteristic) curve to illustrate the logistic regression analysis. The predictor of EPS is dialysate quantity of MCP-1. The area under the curve is 0.78. EPS = encapsulating peritoneal sclerosis; MCP-1 = monocyte chemotactic protein-1.

In 2009 the International Society for Peritoneal Dialysis (ISPD) issued a position paper discussing the length of time patients should spend on PD (37). This paper concluded that there was currently no evidence on which to base a rigid recommendation for the timing of elective transfer to HD; instead, the risks and benefits of remaining on PD versus transferring to HD must be evaluated for each patient in the context of their individual medical history and lifestyle wishes. This study confirms this recommendation. Clinical factors are useful in determining whether a patient is in a high- or low-risk group for EPS, but cannot give an accurate individual prediction. Measurement of dialysate MCP-1, IL-6, CCL15, angiogenin and CCL18 levels did not enhance the accuracy of predicting onset of EPS. While raised levels of these cytokines in the dialysate could perhaps help to confirm a clinical impression based on well-established

risk factors that the patient is at higher risk for EPS post-transfer to HD or transplantation, dialysate levels of these cytokines are not sufficient to use alone as a predictive tool.

ACKNOWLEDGMENTS

This project was supported by funding from the Renal Research Fund from the Imperial College Renal and Transplant Centre and Baxter Clinical Evidence Council. We are grateful for support from the National Institute for Health Research (NIHR) Biomedical Research Centre funding scheme. Frederick W.K. Tam is supported by the Diamond Fund from Imperial College Healthcare Charity.

DISCLOSURES

Frederick W.K. Tam has received research grants from AstraZeneca, Baxter Biosciences and Roche Palo Alto. Edwina A. Brown has received speaker fees and research funding from Baxter Healthcare.

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