Peritoneal Inflammation Precedes Encapsulating Peritoneal Sclerosis: Results from the GLOBAL Fluid Study

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**Abstract**

**Background:** Encapsulating Peritoneal Sclerosis (EPS) is an uncommon condition, strongly associated with a long duration of peritoneal dialysis (PD) itself associated with increased fibrosis in the peritoneal membrane. The peritoneal membrane is inflamed during PD, and inflammation is often associated with fibrosis. We hypothesised that patients who subsequently develop EPS might have a more inflamed peritoneal membrane during PD.

**Methods:** We performed a nested, case control study, identifying all EPS cases in the UK arm of the Global Fluid Study and matching them by centre and duration of PD with 2 to 3 controls. Dialysate and plasma samples taken during repeated peritoneal equilibration tests prior to cessation of PD from cases and controls. Samples were assayed by electrochemiluminescence immunoassay for IL-1β, TNF-α, IFN-γ and IL-6. Results were analysed by linear mixed models adjusted for age and time on PD.

**Results:** 11 EPS cases were matched with 26 controls. Dialysate TNF-α, (0.64, 95% CI 0.23, 1.05), and IL-6 (0.79, 95% CI 0.03, 1.56), were significantly higher in EPS cases, whilst IL-1β (1.06, 95% CI -0.11, 2.23), and IFN-γ (0.62, 95% CI -0.06, 1.29), showed a similar trend. Only IL-6 was significantly higher in the plasma, (0.42, 95% CI 0.07, 0.78). Solute transport was not significantly different between cases and controls but did increase in both groups with duration of PD.

**Conclusions:** The peritoneal cavity has higher levels of inflammatory cytokines during PD in patients who subsequently develop EPS, but neither inflammatory cytokines nor peritoneal solute transport clearly discriminate EPS cases. Increased systemic inflammation is also evident and is probably driven by increased peritoneal inflammation.

**Keywords**

Peritoneal Dialysis

Peritoneal Membrane

Chronic Inflammation

Encapsulating Peritoneal Sclerosis

**Short Summary**

Using a nested, case-control study of the GLOBAL Fluid Study, we studied inflammatory cytokines in dialysate effluent and plasma in EPS and controls. EPS cases had higher levels of effluent cytokines (both IL-6 and TNF-α with a similar trend for IFN-γ and IL-1β) and plasma IL-6. This suggests that peritoneal inflammation drives membrane fibrosis and subsequent risk of EPS.

**Introduction**

EPS is an uncommon but serious condition, primarily associated with a prolonged duration of PD. It is characterised by marked fibrosis ‘cocooning’ the gut, leading to functional impairment with malnutrition and obstruction. Histological studies have confirmed that after diagnosis there is a significant intra-peritoneal inflammatory component in EPS patients. 1 PD induces intra-peritoneal inflammation during routine PD 2–5 but it is not certain if this inflammatory response is greater in those patients who subsequently develop EPS.

Increased fibrosis manifesting as a decreased osmotic conductance to glucose is an established risk marker for EPS 20–22. There is strong evidence that inflammation drives fibrosis in a wide variety of pathologies, and animal models of PD suggest that IL-6 signalling in recurrent inflammation is key. 23 This suggests that the risk of EPS is likely associated with an inflammation-induced fibrotic response. Our recent data from proteomic analysis of EPS prone patients supports the notion that ongoing inflammation and extracellular matrix turnover are critical processes in its development (data not shown).

It is also now clear that increased intra-peritoneal inflammation is a strong predictor of PSTR as evidenced by higher levels of IL-6. 2,6–8 Faster solute transport in patients who develop EPS is a consistent finding in most case series published 9–11 which suggests increased inflammation but neoangiogenesis could also contribute to this difference. Two case-control studies have shown that dialysate effluent IL-6 was significantly higher in the EPS group, one at two years prior to EPS diagnosis but not at other time points measured, the other at one year prior. 12,13

EPS is also associated with an increase in systemic inflammatory markers both before and after diagnosis 14 but how this relates to intra-peritoneal inflammation is unclear. As the Global Fluid Study collected dialysate and plasma samples on large numbers of routine PD patients, including prevalent patients with a prolonged duration of PD, we sought to explore the roles of intra-peritoneal and systemic inflammation prior to the onset of EPS. We specifically examined IL-6, TNF-α, IFN-γ and IL-1β as these are cytokines that play a dominant role in the acute inflammatory response.

**Methods and Materials**

*Study design*

This is a matched, nested, case-control study. The parent study has been described in detail elsewhere 2but in brief, the Global Fluid Study is an international, multicentre, prospective cohort study of incident and prevalent patients commenced in 2002. Eligible patients were any PD patients over the age of 16 providing informed consent. Incident patients were defined as first data collection time point within the first 90 days of PD. Follow up was censored in December 2011. Ten centres were included in the primary analysis, and for this analysis the 5 UK centres were selected for identification of all patients who developed EPS diagnosed according to the International Society of Peritoneal Dialysis diagnostic recommendations (symptoms of gastro-intestinal dysfunction with radiological and/or surgical evidence). 15These cases were then assigned 2 to 3 controls who had finished PD and not developed EPS, matched by centre and duration of completed PD episode.

*Data collection*

All clinical data was recorded on a custom built database (PDDB). Demography was recorded and comorbidity was assessed with the validated Stoke comorbidity index.16 Routine blood tests, including albumin, were performed locally and, if necessary, converted into the same units. All samples of dialysate and plasma from the cases and controls were assayed for TNF-α, IFN-γ, IL-1β and IL-6 by electrochemiluminescence immunoassay (Pro-Inflammatory I 4-plex from Meso-Scale Discovery, Gaithersburg, MD).

PD related measurements included residual renal function, dialysis regime and dose, and peritoneal membrane function using the peritoneal equilibration test (solute transport rate: dialysate to plasma creatinine ratio (PSTR) and net ultrafiltration (UF) capacity at 4 hours with 2.27% or 3.86% glucose).

*Dialysate results*

All dialysate samples were 4 hour samples so no correction was made for length of dwell. A previous study found a slight benefit in using concentrations over appearance rates when assessing the effect of dialysate cytokines so concentrations were used. 2 No correction was made for possible changes in hydraulic permeability as a significant effect of solute drag would cause non-linear changes over time, but this has not been found, 17 plus any effect would be trivial, in particular for IL-6. For predicted cytokine concentrations based on molecular radius as calculated from molecular weight, and assuming diffusion only we used the 3 pore model. 18

*Statistical analysis*

Missing data, ranging from 0 to 4.8% for different variables, were considered missing at random and complete case analysis was used. Descriptive data was compared with chi-squared tests, t-tests or Mann-Whitney U tests depending on whether variables were categorical or continuous variables, and if continuous, whether they were normally distributed or not.

Multivariable, 3-level, random intercept linear mixed models, accounting for measurements (level 1) clustering within person (level 2) and clustering within case-control groups (level 3), were used to explore determinants of log-transformed cytokine levels. Normal probability distributions were checked for level 1 residuals. Because IL-1β had a highly skewed distribution, a logistic model was used with IL-1β concentrations as a binary variable (detectable/undetectable). In addition to development of EPS, models included age, as it is known to affect inflammatory cytokine concentrations and time from sample acquisition to the end of the PD episode to account for temporal changes. A sensitivity analysis using time from PD start instead was performed but this made no difference to interpretation of the results. To avoid over-fitting, further covariates were not added as models included 3 residuals and 3 covariates with a limited number of samples, although 2-way interactions were tested for. Significance testing was by the Wald test and the Iterative Generalised Least Squares method was used for coefficient estimation. All values on the graph for dialysate TNF concentration are +0.1 to allow a logarithmic scale (i.e. 0.1 = 0). Intra-class correlation coefficients (ICC) were post-estimation commands from random-intercept/no covariate models.

MLWin 2.26 19 was used via runmlwin for multilevel regression and StataIC 12 (StataCorp LP, College Station, TX) for the other calculations.

**Results**

*Patient Characteristics*

Demographic and basic clinical details are shown in Table 1. 3 EPS patients were diagnosed during PD, and the remaining 8 cases were diagnosed a median of 7.9 months (range 1.2 to 18.4 months) after stopping PD. The EPS group had 41 samples and the control group had 106 samples. There were potentially clinically relevant differences that were not statistically different in age, residual renal function and PSTR at the time of first sample acquisition in the EPS group. 34.6% of controls and 18.2% of cases had diabetes, with similar overall co-morbidity levels and number of peritonitis episodes. More EPS cases stopped PD due to UF failure than controls, consistent with previous findings. 20,21

*Differences between EPS and controls*

Determinants of dialysate and plasma cytokine concentrations are shown in Table 2. All dialysate cytokine concentrations tended to be higher in EPS cases but the difference was only statistically significant for IL-6 and TNF-α. Of the plasma cytokines, only IL-6 was significantly higher in EPS cases although the average difference was only 0.42 log10 concentrations, compared to 0.79 log10 concentrations in dialysate IL-6. PSTR did not differ between cases and controls, even if just the D/P Cr at the time of the last sample was compared (EPS 0.820, Control 0.818, p=0.98). In a sensitivity analysis, cytokine models were run with PSTR as a covariate but this made no significant difference to the results.

*Other determinants of cytokines and solute transport*

Age was associated with higher plasma cytokines except for IL-1β but out of the dialysate cytokines it was only associated with TNF-α. PSTR rose with time (Figure 1), as did dialysate and plasma IL-6 as well as plasma IFN-γ. A sensitivity analysis substituting time from PD start to sample acquisition for time from sample acquisition to stopping PD made no tangible difference to the results. There were no significant interactions between EPS status, age and time to PD finish although the power to detect them would have been weak. Dialysate cytokines had ICC’s between 0.039 and 0.046, whilst plasma cytokines had ICC’s between 0.095 and 0.41 and PSTR had a value of 0.59.

*Dialysate/plasma interactions*

In view of the possible link between dialysate and plasma IL-6 over time, we plotted the dialysate/plasma ratio (Figure 3), which showed no apparent change. A univariable, multilevel regression model for dialysate to plasma IL-6 ratios confirmed that time had no effect (coefficient 0.98, 95% CI -2.65 to 4.60, p=0.6) on this. 34.4% of dialysate samples had a concentration of TNF-α greater than predicted by diffusion according to the 3 pore model, assuming plasma TNF-α exists as a homotrimer. If plasma TNF-α was assumed to be monomeric the proportion was 31.9%. The dialysate TNF-α results are demonstrated in Figure 4.

**Discussion**

We have demonstrated that intra-peritoneal inflammation, compared to controls who do not develop EPS, appears to be increased for several years prior to EPS and this difference in intra-peritoneal inflammation is more readily apparent than the associated difference in PSTR. However the considerable overlap between the groups precludes use as a prognostic discriminator. There is also an increase in plasma IL-6 levels, although the magnitude of this difference is less marked, and none of the other plasma cytokines are elevated.

We have replicated two previous findings of raised dialysate effluent IL-6 in patients who subsequently develop EPS. 12,13 These previous studies found a difference at two and one year prior to EPS/PD end respectively, but our study has extended this by looking at samples up to 6 years prior to EPS/PD. Whether IL-6 in isolation represents inflammation is unclear but the finding of greater dialysate TNF-α strengthens the notion of ongoing inflammatory activity. IFN-γ and IL-1β levels, whilst not meeting the pre-defined threshold for significance, showed a tendency to higher levels in patients who subsequently developed EPS. Goodlad et al also found dialysate MCP-1 to be significantly higher in EPS patients strengthening the link with ongoing inflammation. The peritoneum is known to be inflamed during peritoneal dialysis, 2–5 and with this consistent pattern of higher dialysate inflammatory cytokines in patients who develop EPS, increased local inflammation appears to contribute to the risk of EPS development. The EPS patients may also differ from the control group in residual renal function, dialysate glucose exposure and use of Icodextrin, all potential pathways driving increased inflammation.

The clearest difference in cytokine levels was for dialysate TNF-α. Any biomarker predictive of EPS should reflect local pathophysiology and therefore will be produced locally however previous studies of dialysate TNF-α have found low levels compatible with diffusion from the systemic circulation. 2,24 We found over 30% of dialysate samples had levels higher than compatible with diffusion, whether monomeric or heterotrimeric forms were used in the calculation, strongly suggesting local production. Dialysate TNF-α has previously been shown to correlate with other locally produced cytokine levels.2 These data would be compatible with intra-peritoneal TNF-α being part of a pro-fibrotic milieu but whether TNF-α is associated with fibrosis in the peritoneum is not clear in the existing literature. 25,23,26

One of the unexpected findings was the lack of difference in solute transport which is frequently reported as a risk for EPS. A rise in solute transport with long term PD, as we have replicated in this study, is well recognised 27 so fully controlling for time is crucial in EPS studies but most reports have not quite managed this. 9,10,21 Our study has fully adjusted for time on PD, and found no clinically significant difference in solute transport between EPS patients and controls, suggesting that this is not a good method for identifying patients at high risk of EPS. This is similar to a previous study where no clinically significant difference in solute transport was found until the last year of PD, although that study of 9 EPS patients included 6 from this study. 20 The analysis presented here modelled all measures over 6 years prior to stopping PD, and with this strategy, testing for a divergence shortly before stopping PD would require a significantly larger sample to detect a difference. One recent study that matched accurately for time on PD also found a difference one year prior to stopping PD, 13 raising the possibility that clinically significant differences may occur close to the development of EPS.

Solute transport is closely linked with inflammation through local IL-6 2 so a difference in solute transport might have been expected given the dialysate IL-6 findings. That we didn’t find a clear separation probably reflects two issues. Firstly, there is marked overlap in dialysate IL-6 values, with no clear boundary distinguishing EPS from non-EPS patients. Secondly, whilst dialysate IL-6 is the strongest known predictor of PSTR, it explains only a modest amount of the variance. Using the analysis from Lambie et al 2 the predicted difference in D/P Cr for the difference in dialysate IL-6 observed in this study is only 0.07, a value clearly within the confidence intervals for the estimated D/P Cr difference here. Although there appeared to be a slight discrepancy, a striking finding was the increase with time in both dialysate IL-6 and PSTR, suggesting that dialysate IL-6 is a major determinant of long term increases in PSTR.

We also showed no difference in plasma inflammatory cytokines with the exception of plasma IL-6. Previous studies of systemic inflammatory markers have only examined CRP, showing either no difference 28 or a difference one year prior to diagnosis 14. We have therefore extended this by showing changes evident systemically well before the diagnosis of EPS is made. One of the striking findings was a change with time in inflammatory cytokines but this was only evident for dialysate and plasma IL-6 and IFN-γ. The simplest explanation would be that the same change is driving these changes, suggesting that either local processes affect systemic inflammation or vice versa. There is a sharp diffusion gradient from dialysate to plasma for IL-6, not present for most inflammatory cytokines. Furthermore, the diffusion gradient does not change with time, strongly suggesting a coupling of the rise in dialysate and plasma IL-6. The most likely explanation of these findings is that dialysate IL-6 diffuses into the circulation causing an increase in systemic inflammation, hence the more inflamed peritoneum of EPS patients, both before and after diagnosis, drives systemic inflammation.

One of the primary reasons for this study was to seek biomarkers which might be able to identify patients likely to develop EPS before it becomes inevitable. None of the markers examined here showed significant promise in that regard. The dialysate cytokines (Figures 1, 2 and 4) show marked overlap in values between cases and controls, high intra-individual variability and little between-person variance (i.e. ICC’s), similarly to previously results. 17 This suggests that any cut-off value would likely lead to different conclusions regarding EPS risk after repeated sampling of the same patient. Indeed, close inspection of the spaghetti plots (actual values in figures 2 and 4) suggests that the difference in mean values between cases and controls may be because almost none of the EPS cases had low levels of inflammatory cytokines rather than EPS cases having abnormally high levels. The length of PD exposure has been shown to be a major risk factor for EPS, so it is likely that any minor differences in PSTR and dialysate IL-6 will add little extra predictive power to the duration of PD. As PSTR rises with the duration of PD, some of the larger differences in PSTR found in previous studies are likely to be due to failing to fully adjust for the duration of PD.

Limitations of this study include a relatively small number of EPS cases, although it is an uncommon condition so this still represents the largest collection of dialysate effluent samples pre-diagnosis allowing us to adjust for confounding by time more robustly than previous studies. As an observational study, cause and effect cannot be proven so it is not definite that inflammation is pathophysiologically involved in driving EPS. As with all EPS studies, another limitation is the potential variability in interpretation of diagnostic guidelines. To conclusively demonstrate increased inflammation in the peritoneal membrane other evidence such as histology would be necessary although previous studies of dialysate cytokines have correlated well with published histology reports. 1,2,5

In conclusion, peritoneal membranes are more inflamed in patients with subsequent EPS, whilst any difference in peritoneal transport rate was too small to detect. There is probable ‘spillover’ of inflammation into the systemic circulation with increased plasma IL-6 in EPS patients but none of these differences are sufficient to be used as prognostic tools in isolation.

**Disclosures**

M. Lambie currently receives research funding from Baxter Healthcare.

A.J. Williams, A. Summers, P. Williams and J. Chess have no competing interests to declare.

S.J.Davies currently receives research funding and honoraria from Baxter Healthcare and Fresenius AG.

N. Topley has in the past received honoraria and speaker fees from Baxter Healthcare and Fresenius AG.

**Declaration**

The results presented in this paper have not been published previously in whole or part, except in abstract format.

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**Tables**

Table 1: Descriptive Data for Cases and Controls

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | **EPS (11)** | **Control (26)** |
| **Time Invariant** | **Age (years)** | 53.6 (15.5) | 63.0 (14.8) |
| **Male Gender** | 50% | 57.5% |
| **Comorbidity (Low/Intermediate/High)** | 45.5/54.5/0% | 32.0/48.0/20.0% |
| **Completed PD Episode (months)** | 69.0 (35.4) | 69.9 (34.4) |
| **Number of Samples**  **Total peritonitis episodes** | 4 (1-7)  3 (1-4) | 4 (2-6)  3 (1-7) |
| **Reason for stopping PD**   * **Transplant** * **Peritonitis** * **Other technique failure** * **Death** * **Exit site infection** * **EPS** * **UF Failure** * **Patient choice** | 18%  0  18%  0  0  36%  18%  9% | 35%  30%  17%  13%  4%  0  0  0 |
| **At First Sample** | **Months Until PD End** | 33.2 (21.8) | 31.7 (18.9) |
| **Months Since PD Start** | 35.7 (40.1) | 36.9 (40.3) |
| **Urine volume (mls)** | 501 (0-987) | 838 (169-1432) |
| **Icodextrin Usage** | 45.5% | 28.0% |
| **Dialysate Glucose Exposure (g/day)** | 165.0 (74.9) | 138.9 (42.6) |
| **APD usage** | 18.2% | 20.0% |
| **Dialysate IL-6 (pg/ml)** | 10.67 (8.04-21.53) | 6.05 (1.54-14.89) |
| **Plasma IL-6 (pg/ml)** | 0.95 (0.85-1.76) | 1.30 (0.71-2.63) |
| **Serum Albumin (g/l)** | 37.3 (5.0) | 36.8 (5.8) |
| **D/P Cr** | 0.81 (0.15) | 0.72 (0.16) |
| **Blood Pressure (mmHg)** | 142/83 (24/16) | 146/83 (26/11) |

Figures are percentages, mean (SD) or median (IQR) depending on variable type and skewness.

Table 2: Determinants of Inflammatory Cytokine Levels by EPS Status

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Dependent Variable** | | **EPS** | | **Age** | | **Time until PD End** | |
| **Coefficient (95% CI)** | **p value** | **Coefficient**  **(95% CI)** | **p value** | **Coefficient**  **(95% CI)** | **p value** |
| **Dialysate** | **IL-6** | 0.79 (0.03, 1.56)\* | 0.043 | 0.009 (-0.014, 0.033) | 0.43 | 0.27 (0.13, 0.42)\* | <0.001 |
| **IL-1β** | 1.06 (-0.11, 2.23) | 0.075 | 0.022 (-0.012, 0.056) | 0.20 | 0.19 (-0.08, 0.47) | 0.17 |
| **IFN-γ** | 0.62 (-0.06, 1.29) | 0.073 | 0.016 (-0.005, 0.036) | 0.14 | 0.085 (-0.045, 0.215) | 0.20 |
| **TNF-α** | 0.64 (0.23, 1.05)\* | 0.002 | 0.019 (0.007, 0.031)\* | 0.001 | 0.048 (-0.026, 0.123) | 0.20 |
| **Plasma** | **IL-6** | 0.42 (0.07, 0.78)\* | 0.020 | 0.016 (0.005, 0.026)\* | 0.003 | 0.13 (0.05, 0.21)\* | 0.001 |
| **IL-1β** | 0.66 (-0.65, 1.97) | 0.33 | -0.023 (-0.064, 0.017) | 0.26 | -0.21 (-0.55, 0.13) | 0.23 |
| **IFN-γ** | -0.30 (-0.69, 0.09) | 0.14 | 0.014 (0.001, 0.027)\* | 0.036 | 0.12 (0.02, 0.22)\* | 0.017 |
| **TNF-α** | 0.13 (-0.13, 0.39) | 0.31 | 0.010 (0.002, 0.017)\* | 0.011 | 0.45 (-0.007, 0.098) | 0.090 |
| **Solute Transport** | **D/P Cr** | 0.024  (-0.054, 0.102) | 0.55 | -0.0017  (-0.0039, 0.0006) | 0.14 | 0.035  (0.023, 0.047) \* | <0.001 |

\*p<0.05. Results from models with continuous dependent variables (log transformed if cytokine) except for dialysate and plasma IL-1 models which were logistic models for detectable vs undetectable. Values for all cytokines other than IL-1 were pg/ml.

**Figures and Legends**

Figure 1: Peritoneal Solute Transport Rate With Time to PD Finish By EPS Status

The top row of graphs represent the predicted values of D/P Cr for EPS cases and controls from the multilevel model with a mean value of age. The bottom row shows spaghetti plots of the actual values of D/P Cr with lines denoting values from individual patients.

**Figure 2: Dialysate IL-6 With Time to PD Finish By EPS Status**

The top row of graphs represent the predicted values of dialysate IL-6 concentration for EPS cases and controls from the multilevel model with a mean value of age. The bottom row shows spaghetti plots of the actual values of dialysate IL-6 with lines denoting values from individual patients. All graphs have a natural log scale on the y-axis.

Figure 3: Dialysate to Plasma IL-6 Ratio With Time to PD Finish By EPS Status

Spaghetti plots of the actual values of the ratio of dialysate to plasma IL-6 concentrations with lines denoting values from individual patients.

Figure 4: Dialysate TNF-α With Time to PD Finish By EPS Status

The top row of graphs represent the predicted values of dialysate TNF-α concentration for EPS cases and controls from the multilevel model with a mean value of age. The bottom row shows spaghetti plots of the actual values of dialysate TNF-α with lines denoting values from individual patients. All graphs have a natural log scale on the y-axis.

Figure 1



Figure 2



Figure 3



Figure 4

