# A prospective, proteomics study identified potential biomarkers of encapsulating peritoneal sclerosis in peritoneal effluent

Vasileios Zavvos[1,2], Anthony T. Buxton[2], Caroline Evans[3], Mark Lambie[4], Simon J. Davies[4], Nicholas Topley[5], Martin Wilkie[2], Angela Summers[6], Paul Brenchley[6], Dimitrios S. Goumenos[1] and Timothy S. Johnson[2]

*[1]Department of Nephrology, University Hospital of Patras, Patras, Greece; [2]Academic Nephrology Unit and Sheffield Kidney Institute, University of Sheffield, Sheffield, UK; [3]Proteomics Unit, Chemical Engineering, University of Sheffield, Sheffield, UK; [4]Institute of Applied Clinical Sciences, Keele University, Keele, UK; [5]Wales Kidney Research Unit, Division of Infection and Immunity, Cardiff University School of Medicine, Cardiff, UK; and [6]Kidney Research Laboratories, Manchester Royal Infirmary, Manchester, UK*

**Encapsulating peritoneal sclerosis (EPS) is a potentially devastating complication of peritoneal dialysis (PD). Diagnosis is often delayed due to the lack of effective and accurate diagnostic tools. We therefore examined peritoneal effluent for potential biomarkers that could predict or confirm the diagnosis of EPS and would be valuable in stratifying at-risk patients and driving appropriate interventions. Using prospectively collected samples from the Global Fluid Study and a cohort of Greek PD patients, we utilized 2D SDSPAGE/ MS and iTRAQ to identify changes in the peritoneal effluent proteome from patients diagnosed with EPS and controls matched for treatment exposure. We employed a combinatorial peptide ligand library to compress the dynamic range of protein concentrations to aid identification of low-abundance proteins. In patients with stable membrane function, fibrinogen γ-chain and heparan sulphate proteoglycan core protein progressively increased over time on PD. In patients who developed EPS, collagen-α1(I), γ-actin and Complement factors B and I were elevated up to five years prior to diagnosis. Orosomucoid-1 and a2-HS-glycoprotein chain-B were elevated about one year before diagnosis, while apolipoprotein A-IV and α1-antitrypsin were decreased compared to controls. Dynamic range compression resulted in an increased number of proteins detected with improved resolution of protein spots, compared to the full fluid proteome. Intelectin-1, dermatopontin, gelsolin, and retinol binding protein-4 were elevated in proteome-mined samples from patients with EPS compared to patients that had just commenced peritoneal dialysis. Thus, prospective analysis of peritoneal effluent uncovered proteins indicative of inflammatory and pro-fibrotic injury worthy of further evaluation as diagnostic/prognostic markers.**

**Correspondence:** *Timothy S. Johnson, Academic Nephrology Unit and Sheffield Kidney Institute, Room K120, Department of Infection and Immunity, The Medical School, University of Sheffield, Beech Hill Road, Sheffield S10 2RZ, UK. E-mail: t.johnson@sheffield.ac.uk*

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Encapsulating peritoneal sclerosis (EPS) is a rare complication of peritoneal dialysis (PD), resulting in significant morbidity and mortality.[1] Overall reported life-time risk ranges from 0.54%[2] to 3.3%.[3] Incidence increases with the duration of PD treatment,[4–6] ranging from 0.7%[4] to 8.1%[5] at 5 years and from 2.1%[4] to 19.4%[6] at 8 years. The development of EPS has been associated with numerous factors including glucose load, rapid solute transport status and i.p. inflammation, requirement for icodextrin use, ultrafiltration failure associated with reduced free water transport, modality change, young age, and genetic predisposition.[7–14]

The pathogenesis of EPS remains poorly defined, but multiple "hits" appear to be required in patients predisposed to peritoneal injury by uremia and dialysis solution exposure.[15] Although episodic infection, epithelial-to-mesenchymal transition,[16] and the involvement of uncontrolled inflammatory cascades (e.g., transforming growth factor β, vascular endothelial growth factor)[17–20] may be contributory factors, to date no single definitive pathway has been identified.

Clinical signs of EPS include persistent or recurrent bowel dysfunction and nutritional impairment.[1] Diagnosis is confirmed at laparotomy or on positron emission tomographic/computed tomographic imaging,[21] but the rarity of the disorder, variable presentation, and lack of screening tools frequently lead to delayed diagnosis. Peritoneal dialysis effluent (PDE) biomarkers that could predict the onset or confirm the diagnosis of EPS would allow the development of prognostic tools and, potentially, the identification of future therapeutic targets. Proteomics technologies provide us with powerful tools for an in-depth exploration of the PDE

proteome, yet relevant studies have been few and, to date, preliminary.[22–38]

In the present study, we have used an optimized proteomics approach to detect prospective changes in the PDE proteome of clinically well-described patients from the GLOBAL Fluid Study (GFS) cohort who subsequently developed EPS. Control subjects were patients from the same cohort with stable membrane function who did not subsequently develop EPS. EPS and stable patients were matched for time on treatment or time to developing EPS or stopping PD. The aim of this study was to identify proteins in the drained PD effluent whose measurement might predict the onset of EPS. Further analysis, using a cohort of Greek PD patients, used methods to allow detection of lower abundance proteins by using a combinatorial peptide ligand library. On the basis of these experiments, our data identifies proteins whose expression changes in EPS patients and in some cases are expressed in PDE significantly in advance of clinical diagnosis. These data support growing evidence that EPS is preceded by inflammatory and fibrotic peritoneal injury and would seem worthy of further exploration as candidate markers of EPS diagnosis and monitoring.

## RESULTS

### Determination of protein changes with time on PD in patients with stable peritoneal membrane function

To ascertain the time course of changes in the levels of proteins in PDE, samples from 5 patients with ≥10 sequentially collected effluent samples spanning a period of >4 years on PD, were analyzed by 2-dimensional (2D) sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). Over 800 protein spots were consistently detectable in all samples when analysed by PDQuest (Bio-Rad Laboratories Ltd., Hercules, CA). The majority of these proteins were relatively constant in expression, providing confidence of the validity and fidelity of the methodology selected (Figure 1). PDQuest identified approximately 25 spots that either increased (×2) or were reduced (×2) in intensity in a progressive manner with dialysis duration. Of these, however, only 2 spots were consistently changed in ≥4 of the 5 patient samples evaluated. Mass spectrometry (MS) identified these proteins as fibrinogen γ-chain (Figure 2a) and heparan sulfate-proteoglycan (Figure 2b). Based on spot intensities, fibrinogen γ-chain began to be elevated after approximately 30% of total dialysis duration before plateauing, whereas heparan sulfate-proteoglycan levels showed a progressive increase over the entire duration of PD treatment.

### Determination of protein changes in patients that developed EPS

***2D sodium dodecylsulfate–PAGE MS proteomics.*** To determine which proteins were changing in patients who were ultimately diagnosed with EPS, 6 UK patients from the GFS, who had EPS diagnosis confirmed by computed tomographic scan, and who had ≥4 sequential samples prior to this diagnosis were selected. Each of these patients was matched to

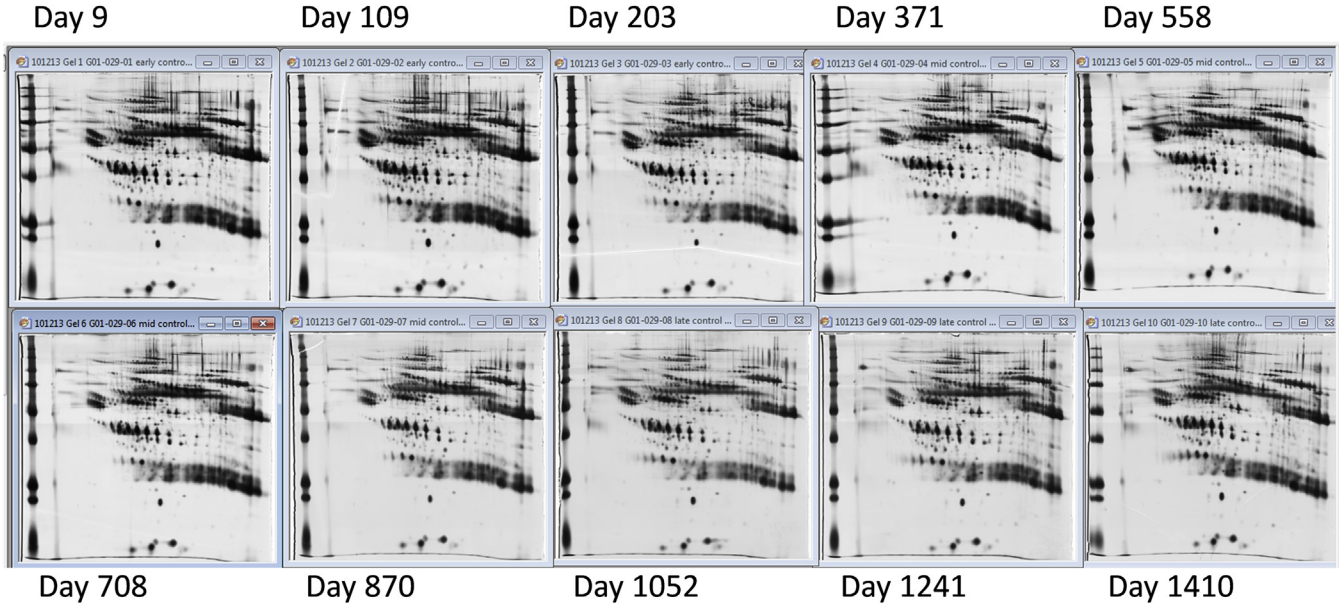


**Figure 1 | Exemplar images showing the changes in the peritoneal dialysis fluid proteome over 4 years on dialysis in a patient with stable membrane function using 2-dimensional sodium dodecylsulfate polyacrylamide gel electrophoresis.** Ten peritoneal dialysis effluent samples spanning days 9 to 1410 of peritoneal dialysis of approximately 2 ml volume were precipitated by adding trichloroacetic acid to 10% (w/v) and 3.5 volumes of acetone. The pellet was resolubilized in buffer. A volume equivalent to 60 μg of protein was subjected to isoelectric focusing on a pH 4 to 7 immobilized pH gradient strip (Bio-Rad) using passive rehydration with proteins further separated on a Bio-Rad Dodeca gel system and fractionated overnight. Each gel was recovered and silver stained using the Blum method. Gels were scanned on a Gene Tools densitometer (transmission) and entered into PDQuest software and spot patterns were compared. Exemplar images are from GLOBAL Fluid Study patient G01-029 (stable membrane function). To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.