CLINICAL VIGNETTE

Inflammatory Biomarker Pairs as Outcome Measures in Peritoneal Dialysis: A Pilot Study

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Introduction

Peritoneal dialysis (PD) is a replacement therapy used by end-stage renal disease (ESRD) patients worldwide. The global annual growth rate of ESRD patients undergoing PD in 2013 was approximately 8%,[1] and the number of patients treated with PD has especially increased in developing countries.[2] Although PD is more cost-effective than hemodialysis,[3-6] there are limitations in using PD as a long-term treatment. Structural and functional alterations of the peritoneal membrane can occur in long-term PD patients. The inflammatory foreign-body response to the catheter,[7,8] continuous exposure of the peritoneal membrane to bioincompatible dialysis solutions (low pH, hyperosmolarity, the presence of lactate, high concentration of dextrose, glucose degradation products (GDPs) and advanced glycation end-products (AGEs)), peritonitis, uremia, and chronic inflammation during PD induce structural and functional changes and limit the long-term viability of the technique. Phenotypic changes in peritoneal mesothelial cells may induce peritoneal sclerosis characterized by the denudation of mesothelial cells from the basement membrane, progressive thickening of the submesothelial compact zone by developing fibrosis, and vascular alterations including vasculopathy and neoangiogenesis.[9-12] As a result, these structural changes alter peritoneal membrane function.

Structural peritoneal membrane changes are believed to induce functional impairment. Increased effective peritoneal surface area and impaired free water transport are the main causes of peritoneal functional changes.[13,14] These changes increase over time on PD.[14,15] In a recent review, the four-year technique survival for automated as well as for continuous ambulatory peritoneal dialysis was approximately 45% with better technique survival for automated than ambulatory PD, particularly in the first year, but with the difference narrowing over time.[16] Ultrafiltration failure is a major contributor to technique failure, occurring in 36% of patients treated with PD for more than 4 years in one report.[13]

Peritoneal membrane injury results from and reflects the elaboration of proinflammatory cytokines, chemokines, and growth factors.[11] Biomarkers have been studied to predict peritoneal membrane changes, but so far, no single marker has emerged as validated and robust. Due to the association between changes in peritoneal membrane solute transport rate and the risk for mortality and technique failure,[17] the ability to predict functional changes early is important. While systemic inflammation, as evidenced by elevated levels of inflammatory cytokines, is a feature of advanced renal failure and predicts worse survival, the linkage between intraperitoneal inflammation and a patient's outcome remains controversial.[18] We previously reported that long-term PD patients had higher mean levels of the inflammatory and fibrotic biomarkers monocyte chemoattractant protein-1 (MCP-1) and periostin than new PD patients. High MCP-1 and periostin levels were observed in a subset but not all long-term patients.[19]

We studied the same cohort to see if combining PD effluent inflammation and injury biomarkers might predict peritoneal membrane function and/or patient survival better than individual biomarkers. Now with longer follow-up, we sought to identify pairs of biomarkers that might predict the clinically significant outcomes of peritoneal membrane failure or death.

Methods

Patients

This study was a cross-sectional, prospective, long-term follow-up study. We enrolled a total of sixteen patients over the age of 18 who were treated with PD. New patients had been initiated on PD within 2 weeks of study entry. Long-term patients had been on dialysis at least 6 months without evidence of peritonitis. Twenty PD effluents were obtained from eight new and eight long-term PD patients from October 2011 to April 2012. The protocol was approved by the Institutional Review Board of Los Angeles Biomedical Research Institute. Informed written consent was obtained from all participants.

Data Collection and Analysis

Baseline PD effluent biomarkers were measured by electrochemiluminescence (ECL, measurements kindly performed by Meso Scale Discovery, Gaithersburg, MD). We tested 83 analytes; 28 analytes could be reliably measured by ECL. Biomarkers were expressed corrected for CA125 (Table 2) and as simple concentrations (pg/ml) uncorrected for CA125 in (Figure 1). Pairs of baseline PD effluent inflammatory biomarkers in the long-term PD patient group (n=8) and the new PD patient group (n=8) were analyzed to identify those that separated long-term patients with higher levels of inflammation/injury biomarkers from new and long-term patients with lower values.

Patients were followed up for 29±2 months in order to evaluate the composite outcome of death or PD membrane failure (defined by the subsequent need for icodextrin or transfer to hemodialysis due to insufficient adequacy and/or ultrafiltration failure).

Statistical Analysis

Initial differences between PD effluent biomarkers in new and long-term PD patients were assessed using the Mann Whitney U-test. Paired PD effluent inflammatory biomarkers were analyzed by Chi-square analysis for their predictive value for the composite outcome of death or PD membrane failure. *P* values less than 0.05 were considered to be statistically significant.

Results

Patient Characteristics

The clinical characteristics of the sixteen patients undergoing PD who participated in this study are shown in Table 1. The average PD vintage in the long-term and new PD patient groups were 36.6 months and 4 days, respectively. The major cause of ESRD was diabetic nephropathy in both groups.

Biomarkers in PD Effluent

We analyzed the baseline levels of 83 biomarkers in PD effluent from long-term and new patients, 28 of which were present at a concentration sufficient to yield reliable results by electrochemiluminescence. Of these, 18 showed mean values that were significantly different between new and long-term patients when corrected for CA125 (Table 2). Only MCP-1 was statistically significant without correction for CA125 ($p < 0.001$).[19]

Apart from MCP-1, osteonectin (secreted protein acidic and rich in cysteine, SPARC) in PD effluent was one of the best biomarkers for distinguishing long-term patients from new patients (Figure 1a). Plotting SPARC against P-cadherin (CDH3), CA125, or TNF-alpha nearly completely separated long-term from new patients, while plotting SPARC against ICAM1 and MCP-1 completely separated long-term from new patients. When combining SPARC with other biomarkers in pairs, there were five pairs of biomarkers in PD effluent that separated long-term patients with higher levels of inflammation/injury from new and long-term patients with lower values (Figure 1b-f). However in each case of pairs, only MCP-1 added significantly to SPARC in separating long-term patients with high values from new patients and long-term patients with lower values.

Composite Outcome

During the follow-up period, the composite outcome of death and/or peritoneal membrane failure occurred in four patients from the long-term group who had consistently high biomarker pair levels (shown in orange in Figure 1b-f; two of the four patients had two values separated in time by 7 days). Three patients died, and one patient required icodextrin before being transferred to hemodialysis for membrane failure. Although in five biomarker pairs, high levels predicted a bad composite outcome ($\chi^2=4.8$ $P=0.028$), the significance of this relationship was driven by the SPARC level. Only SPARC and MCP-1 emerged as the best pair to predict the composite outcome (death and/or PD membrane failure) (Chi square $p < 0.05$).

Discussion

We showed that the combination of high levels of both SPARC and MCP-1 in the peritoneal effluent of PD patients dialyzing for longer than 6 months was associated with a higher risk of the composite outcome of peritoneal membrane failure and/or death compared to long-term PD patients who did not develop high levels and to new patients.

SPARC (also known as osteonectin) is an extracellular matrix (ECM)-associated glycoprotein synthesized by a variety of cells and expressed at sites of tissue remodeling. SPARC expression is increased by a variety of cytokines and growth factors, including TGF-beta1 and IL-1, and binds to collagen, implicating it in ECM deposition and turnover.[20] In animal models of fibrotic disease and in human fibrotic tissues, SPARC is expressed in many tissues including heart, lungs, kidneys, liver, dermis, intestine, and eyes.[21] SPARC is implicated in the progression of many types of cancer and has been advocated as a prognostic marker in cancer patients.[22-24] Given its known role in ECM turnover, SPARC is a biologically plausible biomarker of peritoneal membrane failure and/or fibrosis. To our knowledge, this is the first study to implicate peritoneal effluent SPARC as a biomarker for peritoneal membrane failure or death in PD patients.

We previously reported that peritoneal effluent MCP-1 levels distinguished long-term from new PD patients.[19] In this study, MCP-1 values, when taken into consideration along with SPARC, added significantly to the determination of risk for the composite outcome death and/or membrane failure. MCP-1 is one of the key chemokines that regulate migration and infiltration of monocytes/macrophages, memory T lymphocytes, neutrophils and natural killer (NK) cells to sites of inflammation and tissue injury. After induction by oxidative stress, cytokines, or growth factors, MCP-1 is produced by many cell types, including monocyte/macrophages, fibroblasts, and endothelial, epithelial, smooth muscle, mesangial, and peritoneal mesothelial cells.[25] Peritoneal mesothelial cells have been shown to produce MCP-1 in

response to proinflammatory mediators that are synthesized during exposure to a high concentration of dextrose in PD fluid and in the setting of peritonitis.[26-28] One study found that MCP-1 was involved in peritoneal mesothelial cell transdifferentiation and ECM accumulation via the TGF-beta1 pathway in addition to its role as a mediator of monocyte recruitment.[29] Clinically, MCP-1 values in PD effluent were shown to be related to past episodes of peritonitis and serum MCP-1 but not related to change in membrane function parameters over time on multivariate analysis.[30] Another recent study showed effluent MCP-1 levels were closely correlated with systemic inflammatory markers and an increased MCP-1 level was associated with higher all-cause and cardiovascular mortality in PD patients.[31] The findings we report are consistent with the latter observations.

Our study identified a pair of PD effluent biomarkers, SPARC and MCP-1, which, when both were increased, was associated with the occurrence of the important outcomes of peritoneal membrane failure and/or death. This observation is subject to several limitations. First, our sample size was small. However, this observation is valuable for assessing outcomes in a larger validation set. Second, the composite outcome was comprised of only one peritoneal membrane failure event and three deaths. This was unanticipated, given our prior assumption that the inflammation and fibrosis observed in the peritoneal compartment would predominantly reflect local events in the peritoneum. As part of the Global Fluid Study, Lambie, et al[18] also found that after adjustment for multiple covariates, systemic inflammation was associated with age and comorbidity and independently predicted patient survival in both incident and prevalent PD patients. Intraperitoneal inflammation was the most important determinant of peritoneal small solute transport rate but did not affect survival. In contrast, in our small study, the more frequent event of death suggests that peritoneal inflammation, perhaps through a shared biology with systemic inflammation, may also indicate risk for serious systemic outcomes. Pecoits-Filho, et al[32] observed that dialysate IL-6 concentrations was associated with variability in peritoneal small solute transport rate and also linked to patient survival. Another recent study also showed increased effluent MCP-1 levels were associated with higher all-cause and cardiovascular mortality in PD patients.[29] An association between peripheral inflammation, inflammatory biomarkers, and cardiovascular death has also been described in rheumatoid arthritis.[33]

In conclusion, our data suggest that pairs of the PD effluent inflammatory and fibrosis biomarkers SPARC and MCP-1 may be useful in predicting membrane failure and/or death in long-term PD patients.

Tables and Figures

Table 1. Clinical Characteristics of Long-term and New Patients

Group	Gender	ESRD Etiology	PD Vintage	CAPD/ CCPD	Dextrose Conc.
Long Term	Female	Nail Patella/ FSGS	39 Mos.	CCPD	1.5%
	Female	DN	7 mos.	CAPD	2.5%
	Female	DN	10 mos.	CAPD	1.5%
	Female	DN	56 mos.	CCPD	1.5%
	Female	DN+HTN	80 mos.	CAPD	1.5%
	Male	HTN	63.5 mos.	CAPD	2.5%
	Male	Unclear Etiology	16.5 mos.	CAPD	1.5%
	Male	DN	13 mos.	CAPD	2.5%
New	Male	DN	2 weeks	CAPD	2.5%
	Female	HTN	1st exchange	CAPD	2.5%
	Female	DN	1 week	CAPD	1.5%
	Female	DN	1st exchange	CAPD	4.25%
	Male	HTN	2 days	CAPD	2.5%
	Male	DN	3 days	CAPD	2.5%
	Male	HTN	1st exchange	CAPD	2.5%
	Male	DN	6 days	CAPD	1.5%

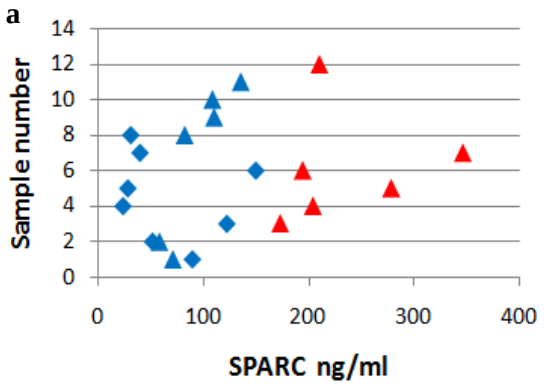
FSGS = focal segmental glomerulosclerosis; DN = diabetic nephropathy; HTN = hypertension; CAPD = continuous ambulatory peritoneal dialysis; CCPD = continuous cycling peritoneal dialysis.

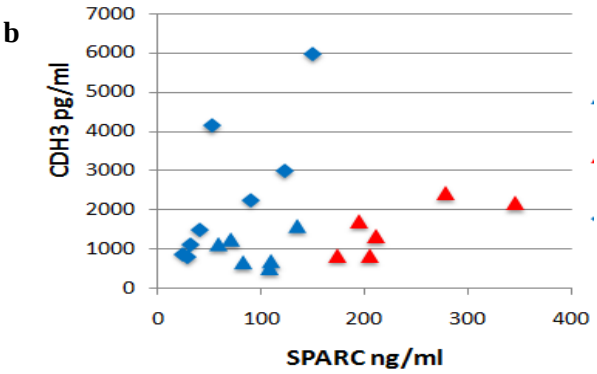
Table 2. PD Effluent Inflammation and Injury Biomarkers after Correction for CA125 Level between Long Term and New PD Patients

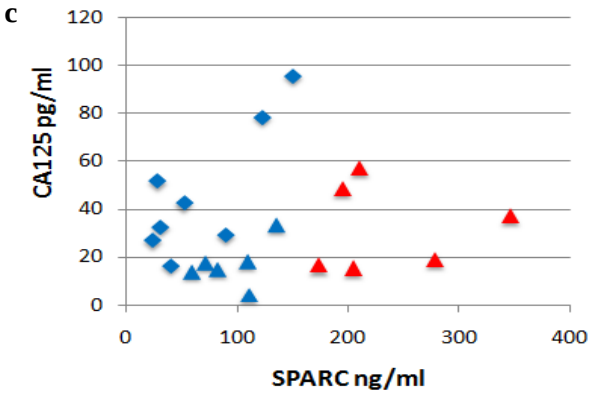
Assay	Long-term Patients	New Patients	*P* Value
AKR1B1	11.8	4.59	0.014
ALP	3.7	1.2	0.0004
β_2-microglobulin	222,176	94,781	0.01
Cystatin C	24,497	13,222	0.009
IL 6	1.8	0.6487	0.012
IL8	0.8	0.275	0.02
IL 15	0.04	0.02	0.014
MCP 1	21.27	5.19	0.00052
MCP 4	2.7	1.5	0.06
NGAL	4045	2241	0.04
NME 2	9.31	3.36	0.02
Osteocalcin	6393	2077	0.004
SPARC	8569	2240	0.0016
PSAT 1	2.31	0.48	0.002
S100A6	564	202	0.028
TGF-β	6	2.76	0.004
Trefoil factor 3	35.79	8.25	0.03
VEGF	3.94	1.98	0.02

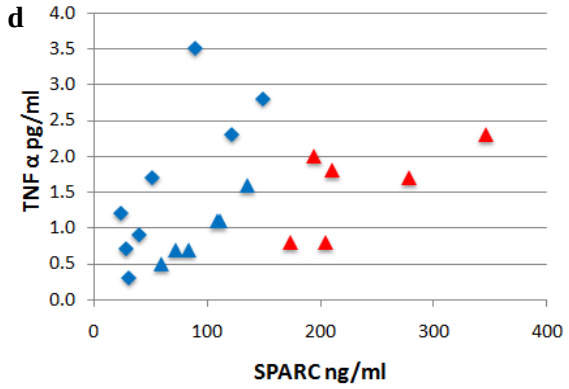
AKR1B1 = aldo-keto reductase family 1 member B1; ALP = alkaline phosphatase; IL = interleukin; MCP = monocyte chemoattractant protein; NGAL = neutrophil gelatinase-associated lipocalin; NME 2= Non-metastatic 2; SPARC = Secreted protein acidic and rich in cysteine; PSAT = phosphoserine Aminotransferase; TGF = transforming growth factor; VEGF = vascular endothelial growth factor

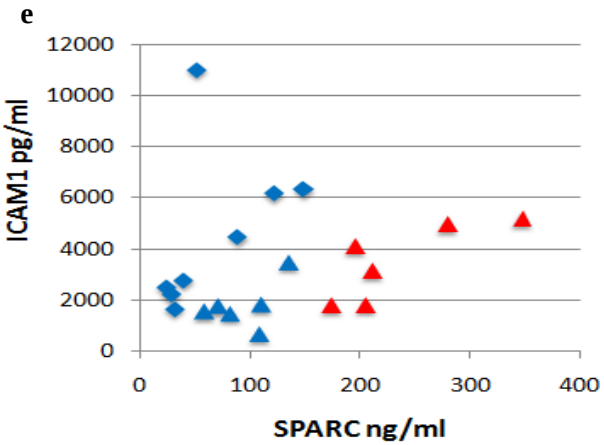
Figure 1. Plotting SPARC levels in long-term and new PD patients' effluent (**a**) and plotting pairs of biomarkers in PD effluent of long-term and new PD patients (**b-f**). SPARC = Secreted protein acidic and rich in cysteine; CDH3= P-cadherin; CA125= Cancer antigen 125; TNF-α= Tumor necrosis factor-α; ICAM1=Intercellular cell adhesion molecule 1; and MCP-1 =Monocyte chemoattractant protein-1. ▲ Long term patients without composite outcome. ▲ Long term patients with composite outcome. ◆ New patients.

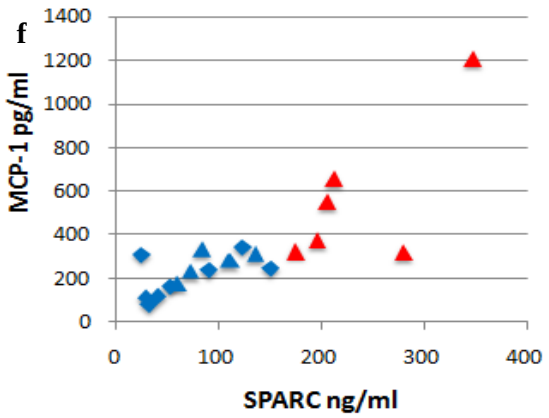












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Submitted March 2, 2016