HIGHER DIALYSATE MATRIX METALLOPROTEINASE-2 LEVELS ARE ASSOCIATED WITH PERITONEAL MEMBRANE DYSFUNCTION

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◆ **Background:** Peritoneal dialysis (PD) patients develop progressive and cumulative peritoneal injury with longer time spent on PD. The present study aimed to a) describe the trend of peritoneal injury biomarkers, matrix metalloproteinase-2 (MMP-2) and tissue inhibitor of metalloproteinase-1 (TIMP-1), in incident PD patients, b) to explore the capacity of dialysate MMP-2 to predict peritoneal solute transport rate (PSTR) and peritonitis, and c) to evaluate the influence of neutral pH, low glucose degradation product (GDP) PD solution on these outcomes.

◆ **Methods:** The study included 178 participants from the balANZ trial who had at least 1 stored dialysate sample. Changes in PSTR and peritonitis were primary outcome measures, and the utility of MMP-2 in predicting these outcomes was analyzed using multilevel linear regression and multilevel Poisson regression, respectively.

◆ **Results:** Significant linear increases in dialysate MMP-2 and TIMP-1 concentrations were observed ($p < 0.001$), but neither was affected by the type of PD solutions received (MMP-2: $p = 0.07$; TIMP-1: $p = 0.63$). An increase in PSTR from baseline was associated with higher levels of MMP-2 ($p = 0.02$), and the use of standard solutions over longer PD duration ($p = 0.001$). The risk of peritonitis was independently predicted by higher dialysate MMP-2 levels (incidence rate ratio [IRR] per ng/mL 1.01, 95% confidence interval [CI] 1.005 – 1.02, $p = 0.002$) and use of standard solutions (Biocompatible solution: IRR 0.45, 95% CI 0.24 – 0.85, $p = 0.01$).

◆ **Conclusion:** Dialysate MMP-2 and TIMP-1 concentrations increased with longer PD duration. Higher MMP-2 levels were associated with faster PSTR and future peritonitis risk. Administration of biocompatible solutions exerted no significant effect on dialysate levels of MMP-2 or TIMP-1,

but did counteract the increase in PSTR and the risk of peritonitis associated with the use of standard PD solutions. This is the first longitudinal study to examine the clinical utility of MMP-2 as a predictor of patient-level outcomes.

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Technique survival on peritoneal dialysis (PD) is frequently limited by peritoneal membrane injury, which clinically manifests as impaired solute clearance, inadequate ultrafiltration from an increase in peritoneal solute transport rate (PSTR), and impaired peritoneal defence against infection (1–6). Peritoneal injury is progressive and cumulative with longer time spent on PD, and poses a risk of developing encapsulating peritoneal sclerosis (EPS) (7,8).

Matrix metalloproteinases (MMPs) play a central role in mediating extracellular matrix turnover during peritoneal membrane homeostasis, injury, and fibrosis. Their actions are counterbalanced by those of tissue inhibitors of metalloproteinase (TIMPs). There has been a growing interest in MMP-2 and TIMP-1, produced by activated cells in the peritoneum (9–11), as biomarkers of peritoneal injury in PD patients. Studies have reported their significant associations with PSTR (12) and peritoneal injury, including EPS (9,10). However, the vast majority of evidence pertaining to these markers stems from animal models (10,13), and clinical evidence is limited to observational studies that are restricted by cross-sectional design (9,12) and small sample size (14).

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Furthermore, only 1 small observational study ($n = 13$) has assessed and reported a decrease in dialysate MMP-2 concentrations with use of biocompatible PD solutions (i.e. neutral pH, low glucose degradation product [GDP] PD solution) (14).

The aims of the present study were to describe the trend in PD effluent MMP-2 and TIMP-1 concentrations in incident PD patients, explore the utility of these biomarkers as predictors of PSTR and peritonitis in this patient group, and to evaluate the impact of neutral pH, low GDP PD solutions, on these outcomes.

SUBJECTS AND METHODS

STUDY POPULATION

Data were obtained from the participants of the balANZ trial (ACTRN12606000044527) (15). Detailed description of the study design and methodology has been previously published (16), as have the results of the main primary and secondary analyses (15,17,18). Briefly, incident, adult PD patients who had both a residual measured glomerular filtration rate (GFR) ≥ 5 mL/min/1.73m² and a measured urine volume ≥ 400 mL/day at enrolment were included in the study. Pregnant or breastfeeding patients, individuals expected to die within 12 months, patients participating in trials targeting residual renal function in PD or those with a significant cancer history in the past 5 years, acute infection at enrolment, contra-indications to PD, any physical or mental disorder that appreciably hampered study protocol compliance or known or suspected allergy to trial product or related products were excluded. Of the 185 participants of the balANZ trial, 178 participants (Balance [Fresenius Medical Care North America, Waltham, MA, USA] $n = 89$; Stay.Safe [Fresenius Medical Care North America, Waltham, MA, USA] $n = 89$) with at least 1 PD effluent (PDE) sample stored during trial participation were included in the present investigation.

MMP-2 AND TIMP-1 MEASUREMENTS

Peritoneal dialysis effluent samples were collected at baseline, 6-, 12-, 18-, and 24-month visits. Peritoneal dialysis effluent was immediately stored in a -20°C or -80°C freezer locally, and then transported frozen to a central storage facility and kept in a -80°C freezer. Samples were thawed once only during the aliquoting process prior to analysis. Matrix metalloproteinase-2 (total) and TIMP-1 (total) were measured by an electrochemiluminescence immunoassay technique using the manufacturer's protocols. The 96-well plates measuring

MMP-2 and TIMP-1 were analyzed on a Sector Imager 6000 (Mesoscale Discovery [MSD], Gaithersburg, MD, USA). Samples and standards were analyzed in duplicates with a maximum tolerated coefficient of variation (CV) of 20%. No inter-assay CV was determined as all samples from an individual were run at the same time to minimize between-assay variability.

CLINICAL OUTCOME MEASUREMENTS

The clinical outcome measures were: 1) PSTR defined as 4-hour dialysate:plasma creatinine ratio ($D:P_{Cr4h}$) measured during a peritoneal equilibration test (PET), and 2) episodes of PD-related peritonitis during study participation.

CALCULATIONS

To account for individual variation in baseline PSTR, the change in PSTR was calculated for months 6 (month 6 – baseline), 12 (month 12 – baseline), 18 (month 18 – baseline), and month 24 (month 24 – baseline). Baseline values of PSTR were measured at month 1.

STATISTICAL ANALYSIS

Results were expressed as frequencies (percentages) for categorical variables, mean $\pm$ standard deviation (SD) for continuous normally distributed variables, and median (interquartile range) for continuous non-normally distributed variables. Differences between groups on baseline characteristics were analyzed by χ^2 test for categorical data, and t -test or Mann-Whitney U test for continuous data, as appropriate. Trends in MMP-2 and TIMP-1 concentrations over the follow-up period were analyzed using multilevel linear regression models. Continuous time was included as a predictor variable. Random intercepts and slopes were added to allow for the repeated measurements over time. To determine whether there was a significant linear trend beyond 6 months, analyses were repeated excluding the baseline data. To evaluate the differences between the 2 treatment groups on these markers, PD solution type (Biocompatible vs Control) and a variable representing the interaction between solution type and time were subsequently added to the model as predictor variables. If the interaction was not statistically significant, it was dropped from the model. The MMP-2 and TIMP-1 data were log transformed due to their non-normal distributions.

The relationship between MMP-2 and TIMP-1 was explored using Pearson's correlation. As there was a strong positive linear relationship between these

biomarkers ($r > 0.7$), only MMP-2 was pre-specified to be explored as a predictor of primary outcome measures. To determine whether MMP-2 was associated with changes in PSTR, a multilevel linear regression model was fitted. Clinically recognized risk factors that affect PSTR (such as time on PD, racial origin, age, body mass index, gender) (19,20) and randomly assigned type of PD solutions (17) received were included as predictor variables in an initial full model. Only variables with statistically significant effects were retained in the final model. The fit of the final model was checked against the full model using the likelihood ratio test. To evaluate the relationship between MMP-2 and peritonitis risk, counts of peritonitis episodes were analyzed using a multilevel Poisson regression model with MMP-2 as a time varying covariate. Other covariates added to the model were PD solution type, age, sex, racial origin, body mass index, diabetes mellitus, PD modality (automated PD/continuous ambulatory PD) as well as all 2-way interactions. Patients who experienced peritonitis ($n = 12$) prior to the first dialysate collection were excluded from the analysis. The event counts of peritonitis were recorded at 6-monthly intervals from baseline to 24 months; therefore each participant had up to 4 values for peritonitis. To examine the robustness of the results, time to first peritonitis was analyzed by a multivariable Cox proportional hazards model. Baseline MMP-2 or averages of MMP-2 for each subject, the aforementioned variables, and all 2-way interactions were explored as covariates. Data were analyzed using the software package Stata/SE12.1 (College Station, TX, USA). $P < 0.05$ was considered to represent statistically significant differences.

RESULTS

PATIENT CHARACTERISTICS

The patients (biocompatible $n = 89$; control $n = 89$) were well matched for all baseline characteristics other than significantly higher peritoneal ultrafiltration ($p < 0.001$) at 3 months and lower PSTR ($p = 0.003$) at 1 month in the control group (Table 1). The baseline characteristics of this subgroup were comparable to those of the original balANZ trial cohort (15). The median number of samples analyzed for patients during the study period was 4 (interquartile range: 2 – 5).

THE TREND OF DIALYSATE PERITONEAL INJURY MARKERS

Matrix metalloproteinase-2 and TIMP-1 concentrations increased linearly with longer PD duration ($p < 0.001$, Figure 1). The median MMP-2 and TIMP-1

concentrations were 34.2 ng/mL and 152.1 ng/mL at baseline, and 58.4 ng/mL and 199.5 ng/mL at month 24, respectively (Table 2, Supplementary Figure 1). While there was a significant linear trend for both variables, the majority of the increment occurred in the first 6 months and the linear trends beyond 6 months for MMP-2 (coefficient 0.002; $p = 0.45$) or TIMP-1 (coefficient 0.006; $p = 0.11$) were not statistically significant. Similar trajectories were observed for biocompatible and control groups (MMP-2: $p = 0.07$; TIMP-1: $p = 0.63$; Figure 2). Excluding outliers did not affect the results (MMP-2: $p = 0.09$; TIMP-1: $p = 0.68$). There were no significant interactions between the type of PD solutions received and time on PD (MMP-2: $p = 0.40$; TIMP-1: $p = 0.11$). Peritoneal dialysis duration was a significant and independent predictor of MMP-2 and TIMP-1 after the type of PD solutions received was added to the model (Table 3).

RELATIONSHIP BETWEEN MMP-2 AND TIMP-1

There was a consistently strong positive linear correlation between dialysate MMP-2 and TIMP-1 levels across all visits from baseline ($r = 0.73$, $p < 0.001$) to 24 months ($r = 0.80$, $p < 0.001$; Supplementary Table 1; Figure 3).

DIALYSATE MATRIX METALLOPROTEINASE-2 AS A PREDICTOR OF PERITONEAL SOLUTE TRANSPORT RATE

Changes in PSTR from baseline were significantly greater in those with higher levels of MMP-2 in the dialysate ($p = 0.02$). The 2-way interaction between PD duration and PD solution type was significant ($p = 0.001$), such that patients who had received standard solutions experienced a greater increase in PSTR with longer PD duration whilst those who received biocompatible solutions maintained a relatively stable PSTR over time (Table 4; Supplementary Figure 2). This relationship was not evident at month 6 ($p = 0.16$) and only became apparent with longer PD duration. Substituting TIMP-1 for MMP-2 in the analysis yielded virtually identical results (not shown).

MATRIX METALLOPROTEINASE-2 AS A PREDICTOR OF PERITONITIS

Sixty patients (biocompatible $n = 20$; control $n = 40$) experienced 88 episodes of peritonitis amongst the analyzed participants. There were 37 (42%), 18 (20.5%), 20 (22.7%), and 13 (14.8%) peritonitis episodes recorded at 6, 12, 18 and 24 months, respectively. Mean peritonitis rates, expressed as episodes per patient-year were 0.35 (95% confidence interval [95% CI] 0.28 – 0.44) overall,

TABLE 1
Baseline Characteristics

Characteristic	Total (n=178)	Biocompatible (n=89)	Control (n=89)	p values
Age (yr)	61.5 (50–69)	64 (50–69)	60 (51–68)	0.48
Female	80 (44.9)	39 (43.8)	41 (46.1)	0.76
Ethnicity				0.43
Caucasian	142 (79.8)	75 (84.3)	67 (75.3)	
ATSI	23 (12.9)	10 (11.2)	13 (14.6)	
Asian	3 (1.7)	1 (1.1)	2 (2.3)	
MPI	10 (5.6)	3 (3.4)	7 (7.9)	
Diabetes mellitus	60 (33.7)	29 (32.6)	31 (34.8)	0.75
Body mass index (kg/m ²)				0.26
<20	8 (4.5)	2 (2.3)	6 (6.7)	
20–24.9	49 (27.5)	26 (29.2)	23 (25.8)	
25–30	68 (38.2)	38 (42.7)	30 (33.7)	
>30	53 (29.8)	23 (25.8)	30 (33.7)	
Initial PD modality				0.81
CAPD	159 (89.3)	79 (88.7)	80 (89.9)	
APD	19 (10.7)	10 (11.2)	9 (10.1)	
GFR (mL/min per 1.73m ²)	6.85 (5.36–8.9)	6.85 (5.9–9)	6.84 (5–8.6)	0.34
Urine volume (mL/day)	1,367 (982–1,870)	1,380 (981–1,869)	1360 (1,010–1,870)	0.70
Ultrafiltration ^a (mL/day)	900 (495–1,320)	700 (300–1,183)	1100 (750–1,423)	<0.001
Normalized ultrafiltration corrected for total glucose exposure ^a	3.6 (1.9–5.5)	2.7 (1.3–4.2)	4.6 (2.9–6.6)	<0.001
D:P _{Cr4h} ^b	0.65±0.11	0.67±0.10	0.63±0.10	0.003
MMP-2 (ng/mL)	34.2 (19.8–57.6)	34.9 (22.1–59.2)	31.9 (16.3–56.9)	0.23
TIMP-1 (ng/mL)	152.1 (101.5–273.4)	160.3 (101.1–263.1)	144.2 (102.2–293.6)	0.82

ATSI = Aboriginal and Torres Strait Islanders; MPI = Maori and Pacific Islanders; PD = peritoneal dialysis; CAPD = continuous ambulatory peritoneal dialysis; APD = automated peritoneal dialysis; GFR = glomerular filtration rate; D:P_{Cr4h} = dialysate:plasma creatinine at 4 hours; MMP-2 = matrix metalloproteinase-2; TIMP-1 = tissue inhibitor of metalloproteinase-1.

^a Measured at month 3.

^b Measured at month 1.

0.24 (95% CI 0.16 – 0.35) in the biocompatible group, and 0.46 (95% CI 0.35 – 0.59) in the control group. Peritonitis episodes were significantly and independently predicted by dialysate MMP-2 levels (incidence rate ratio [IRR] per ng/mL 1.01, 95% CI 1.005 – 1.02, $p = 0.002$) and standard PD solutions use (Biocompatible solution: IRR 0.45, 95% CI 0.24 – 0.85, $p = 0.01$). There was no significant interaction between diabetic status and MMP-2 concentrations ($p = 0.23$). Biocompatible solution use was also associated with a longer time to first peritonitis (adjusted hazard ratio [AHR] 0.44, 95% CI 0.25 – 0.77, $p = 0.004$). A significant interaction between baseline MMP-2 levels and diabetic status was observed in the

time to first peritonitis ($p = 0.02$). Even after accounting for the significant interaction, there was a statistically significant association between baseline MMP-2 levels with a shorter time to first peritonitis (AHR 1.02, 95% CI 1.003 – 1.03, $p = 0.02$). Subgroup analyses revealed a significant association between higher baseline MMP-2 levels and a shorter time to first peritonitis in non-diabetic patients only (AHR 1.01 per ng/mL, 95% CI 1.003 – 1.03; $p = 0.02$; diabetic patients: AHR 1.00 per ng/mL, 95% CI 0.98 – 1.01; $p = 0.57$). Higher average MMP-2 levels were associated with a significantly shorter time to first peritonitis (AHR 1.01 per ng/mL, 95% CI 1.002 – 1.02; $p = 0.02$), independent of diabetic status.

Figure 1 — Log-transformed dialysate: (A) Matrix metalloproteinase-2 (MMP-2) and (B) Tissue inhibitor of metalloproteinase-1 (TIMP-1) concentrations over time in incident peritoneal dialysis patients with fitted values from multilevel linear regression.

TABLE 2
Trend in Dialysate Matrix Metalloproteinase-2 and
Tissue Inhibitor of Metalloproteinase-1 Levels^a

Month	Matrix metalloproteinase-2 (ng/mL)	Tissue inhibitor of metalloproteinase-1 (ng/mL)
0 (n=172)	34.2 (19.8–57.6)	152.1 (101.5–273.4)
6 (n=144)	59.0 (43.6–77.5)	184.8 (135.9–323.4)
12 (n=122)	57.7 (43.9–77.8)	209.1 (151.9–333.0)
18 (n=111)	60.9 (43.1–82.7)	235.3 (142.8–356.4)
24 (n=86)	58.4 (45.6–72.3)	199.5 (134.9–277.4)

^a Median (interquartile range).

DISCUSSION

The present investigation is the first study to examine the utility of MMP-2 as a predictor of PSTR and peritonitis in incident PD patients, and one of the largest studies examining the longitudinal trends in dialysate MMP-2 and TIMP-1 levels. Significant linear increases in MMP-2 and TIMP-1 concentrations with longer time on PD were observed whilst the type of PD solution (biocompatible vs standard) received had no impact on these levels. A strongly positive linear correlation between dialysate MMP-2 and TIMP-1 concentrations was observed. Higher

Figure 2 — Log-transformed dialysate: (A) Matrix metalloproteinase-2 (MMP-2) and (B) Tissue inhibitor of metalloproteinase-1 (TIMP-1) concentrations over time by type of peritoneal dialysis solutions received (biocompatible n=89; control n=89) with fitted values from multilevel linear regression.

TABLE 3
Multilevel Linear Regression to Evaluate the Effect of Peritoneal Dialysis Solution Type on Dialysate Matrix Metalloproteinase-2 and Tissue Inhibitor of Metalloproteinase-1 Concentrations

Variable	Matrix metalloproteinase-2 (n=178)			Tissue inhibitor of proteinase-1 (n=178)		
	Regression coefficient	95% confidence interval	p values	Regression coefficient	95% confidence interval	p values
PD solution						
Control	Reference	Reference	Reference	Reference	Reference	Reference
Biocompatible	0.12	-0.008-0.25	0.07	0.04	-0.12-0.20	0.63
PD duration			<0.001			0.0001
Month 0	Reference	Reference	Reference	Reference	Reference	Reference
Month 6	0.60	0.50-0.71	<0.001	0.18	0.05-0.32	0.008
Month 12	0.59	0.48-0.70	<0.001	0.27	0.12-0.41	<0.001
Month 18	0.65	0.54-0.77	<0.001	0.34	0.19-0.49	<0.001
Month 24	0.61	0.49-0.73	<0.001	0.25	0.09-0.41	0.003

Figure 3 — Scatter plot demonstrating a relationship between dialysate matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-1 by time and overall.

TABLE 4
Multilevel Linear Regression of Change in Peritoneal Solute Transport Rate in Incident Peritoneal Dialysis Patients

Variable	Full model			Final model		
	Coefficient	95% confidence interval	<i>p</i> values	Coefficient	95% confidence interval	<i>p</i> values
Log ₁₀ MMP-2	0.02	0.004–0.04	0.01	0.02	0.003–0.04	0.02
PD solution						
Control	Reference	Reference	Reference	Reference	Reference	Reference
Biocompatible	–0.02	–0.05–0.02	0.31	–0.02	–0.06–0.01	0.17
PD duration			<0.001			<0.001
6 month	Reference	Reference	Reference	Reference	Reference	Reference
12 month	0.02	0.004–0.05	0.02	0.02	0.004–0.05	0.02
18 month	0.05	0.02–0.07	<0.001	0.05	0.02–0.07	<0.001
24 month	0.07	0.05–0.09	<0.001	0.07	0.05–0.09	<0.001
Age	–0.001	–0.002– –0.00003	0.04	–0.001	–0.002 – –0.00001	0.05
Male	–0.01	–0.04–0.02	0.52			
BMI (kg/m ²)			0.17			
<20	Reference	Reference	Reference			
20–24.9	–0.07	–0.15–0.01	0.11			
25–30	–0.07	–0.16–0.006	0.07			
>30	–0.05	–0.13–0.04	0.28			
Ethnicity			0.58			
Caucasian	Reference	Reference	Reference			
Asian	–0.02	–0.07–0.02	0.29			
ATSI	–0.05	–0.15–0.05	0.36			
MPI	0.002	–0.06–0.07	0.95			
Two-way interaction (PD duration*PD solution):			0.001			0.001
Biocompatible*6 month	Reference	Reference	Reference	Reference	Reference	Reference
Biocompatible*12 month	–0.02	–0.05–0.01	0.20	–0.02	–0.05–0.01	0.19
Biocompatible*18 month	–0.05	–0.08– –0.02	0.002	–0.05	–0.08– –0.02	0.002
Biocompatible*24 month	–0.06	–0.09 – –0.03	0.001	–0.06	–0.09 – –0.03	0.001

MMP-2 = matrix metalloproteinase-2; PD = peritoneal dialysis; BMI = body mass index; ATSI = Aboriginal and Torres Strait Islanders; MPI = Maori and Pacific Islanders.

levels of MMP-2 were predictive of an increase in PSTR and peritonitis occurrence.

There is limited information available on the impact of PD duration on dialysate MMP-2 and TIMP-1 concentrations. A large, observational study of prevalent PD patients (*n* = 215) previously reported a weak correlation between PD duration and dialysate MMP-2 (*r* = 0.21) and TIMP-1 (*r* = 0.22) levels (12). Although the study was strengthened by its relatively large sample size recruited from 20 centers, it was limited by its cross-sectional study design and exclusion

of incident PD patients. More recently, a single-center, prospective longitudinal observational study of incident PD patients receiving treatment using biocompatible solutions reported no significant difference in the appearance rates of MMP-2 with longer PD duration (*n* = 79; *p* = 0.37) (21). The dialysate samples were collected as part of an annual standard peritoneal permeability analysis and the baseline values were obtained at some point during the first year of PD treatment. In contrast to their findings, the present investigation observed significant linear increases in dialysate MMP-2 and

TIMP-1 levels with longer PD duration. The discrepancy in results might have stemmed from differences in the timing of dialysate collection. As aforementioned, the majority of increases in biomarker levels were seen during the first 6 months of PD therapy in the present study. For instance, the median dialysate MMP-2 levels were 34.2 ng/mL, 59.0 ng/mL, 57.7 ng/mL, 60.9 ng/mL and 58.4 ng/mL at baseline, 6, 12, 18, and 24 months, respectively. Therefore, if the first dialysate collection occurred at month 6 or later, the dialysate MMP-2 levels would have been described as being relatively stable over time. However, it is equally conceivable that the relative plateau observed with longer follow-up duration might have resulted from patient drop-out over time (i.e. 50% of patients analyzed at 24 months compared to baseline, Table 2).

Unlike PD duration, the type of PD solutions received exerted no significant effect on the trend of dialysate MMP-2 or TIMP-1 concentrations. This was an unexpected outcome because the use of biocompatible solutions has been associated with an improvement in peritoneal membrane morphology (22). Similarly, an observational study reported a significant decrease in both PD effluent MMP-2 levels (141.4 ± 52.5 to 91.3 ± 15.1 ng/mL; $p < 0.05$) and PSTR ($D:P_{Cr4h}$ 0.72 ± 0.09 to 0.60 ± 0.06 ; $p < 0.03$) in prevalent PD patients (mean PD duration 44.6 months) when their treatments were switched from standard solutions to biocompatible solutions (14). The apparent disparity in results with those of the present investigation may have been explained by differences in patient populations (prevalent vs incident) or by the shorter duration of exposure to PD in our study (median PD duration 23.3 months), such that the extent of accumulated peritoneal membrane damage was not severe enough to be able to demonstrate a significant difference in biomarker levels between the different types of PD solutions received.

Matrix metalloproteinase-2 has a variety of roles in the peritoneal cavity, which include degradation of the endothelial basal lamina (23) and type IV collagen, and promotion of angiogenesis and basement membrane injury (24). These changes functionally manifest as fast solute transport rate. Therefore, any correlation between dialysate MMP-2 levels and PSTR is conceivable and has previously been described (12,21). However, the findings from these studies were restricted by cross-sectional study design (12), absence of a comparison group (i.e. biocompatible vs standard) (12,21), and a relatively small sample size ($n = 86$) (21). Although the study by Lopes Barreto *et al.* was a longitudinal study, the relationship between PSTR and dialysate MMP-2 levels was explored in a cross-sectional manner by

only analyzing results obtained from the last dialysate collection for each participant (21). The present investigation is the first longitudinal study to examine the relationship between PSTR and dialysate MMP-2 levels using models to analyze longitudinal data. Significant associations between increasing PSTR and higher dialysate MMP-2 levels were observed, which conclusively support the findings from previous studies. Similarly, higher dialysate levels of TIMP-1 were associated with changes in PSTR. These results are not surprising given the inhibitory effect mediated by TIMP-1 on MMP-2 activities.

The breach in the peritoneal membrane integrity mediated by MMP-2 (24) might not only manifest with higher levels of PSTR but also increase the risk of PD-related peritonitis, as intact mesothelial cells provide the first line of defence against any insult (25). Fukudome *et al.* (26) have previously reported a lack of peritonitis-related effect on dialysate MMP-2 levels, and therefore its utility as a predictor of peritonitis was explored in the present investigation using a baseline, an average for each subject and a time varying covariate in 3 separate models. Regardless of analysis approach, higher dialysate MMP-2 levels were consistently associated with higher peritonitis risk. However, when baseline MMP-2 level was examined, a significantly shorter time to first peritonitis was only observed in the non-diabetic patients. In the absence of a biologically plausible explanation as well as contribution from possibly small numbers of diabetic patients ($n = 56$; 33.7%), the interaction observed in the first model is likely a consequence of a statistical aberration rather than a true association.

The present study is strengthened by its sample size in comprehensively described cohorts who were participants of a well-controlled randomized controlled trial. It is one of the largest studies with the longest follow-up duration and the first study to truly explore the role of MMP-2 and TIMP-1 from a longitudinal study. However, the conclusions that can be drawn from this study are challenged by several limitations. First, the balANZ trial was a randomized controlled trial with the primary outcome measure of residual renal function decline, rather than a biomarker study. Therefore, the study design did not account for potential biological and pre-analytic sources of variations of MMP-2 or TIMP-1, which could have affected the measured results. Second, the baseline PSTRs were not true baseline values as they were obtained at 1 month after study commencement. Third, although it is one of the largest studies to date, the size is relatively small. Furthermore, for the peritonitis-related analyses, patients who experienced peritonitis prior to the month 1 visit were excluded ($n = 12$)

introducing the risk of selection bias. Fourth, inclusion of incident PD patients minimized the risk of Neyman bias; however, the duration of PD might have been too short to develop peritoneal membrane changes severe enough to detect difference in effect mediated by the use of biocompatible solutions. Fifth, dialysate interleukin-6 (IL-6), which has been recognized as the best predictor of PSTR to date from several studies including the GLOBAL study (27–29), has not been measured in the current cohort. Therefore, it is plausible that some of the results pertaining to MMP-2's ability to predict PSTR may in fact be driven by the presence of IL-6. Certainly, a recent pre-clinical study has suggested an association between MMP and TIMP activities in PD models with inflammatory cytokines including IL-6 and interferon- γ (30). Although these findings require further substantiation in humans, a sub-study of the balANZ trial has also previously demonstrated the association between dialysate IL-6 and PSTR (31). However, due to differences in the characteristics of the included patient cohorts between the dialysate IL-6 and the present study (i.e. dialysate IL-6 study was conducted in 88 patients with IL-6 measured at months 0, 12, and 24), analyses could not be combined to evaluate an independent effect of dialysate MMP-2. Finally, total MMP-2 levels rather than their activities were measured; therefore a proportion of measured MMP-2 would be in a latent form with no biological activity.

In conclusion, dialysate MMP-2 and TIMP-1 concentrations increased with longer time on PD, with greater increment observed in the first 6 months after PD commencement. The type of PD solution received did not have a statistically significant effect on dialysate MMP-2 or TIMP-1 concentrations, which may have resulted from analyzed participant characteristics (i.e. relatively short PD exposure compared to previous literature). Higher dialysate MMP-2 levels were associated with faster PSTR and future peritonitis risk. Future studies should aim to identify the causes of higher dialysate MMP-2 levels and to validate its use as a potential tool to help risk stratifying PD patients at risk of adverse peritoneal membrane outcomes.

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DISCLOSURES

David Johnson is a consultant for Baxter Healthcare Pty Ltd and has previously received research funds from this company. He has also received speakers' honoraria and research grants from Fresenius Medical Care. He has previously been a consultant to Gambro Pty Ltd. He is an International Society of Peritoneal Dialysis Councillor and is a current recipient of a Queensland Government Health Research Fellowship. Yeoungjee Cho is a current recipient of Australian Postgraduate Award and is a recipient of 2012 Jacquot Research Entry Scholarship. Carmel Hawley has received research grants from Baxter Healthcare Pty Ltd and Gambro Pty Ltd, and has been a consultant to Fresenius Medical care. Margaret Clarke is an employee of Fresenius Medical Care. Nicholas Topley and David Vesey have no conflicts of interest.

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

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