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Original

Changes to Peritoneal Surrogate Markers in CAPD Patients Treated with Neutral pH, Low-GDP Peritoneal Dialysis Solution for Long Periods

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Introduction: Long-term peritoneal dialysis (PD) is known to injure the peritoneum. One cause of such damage is the bioincompatibility of conventional low-pH solutions containing glucose degradation products. New PD solutions with neutral pH and low levels of glucose degradation products have been available for peritoneal dialysis in Japan since 2000. We investigated changes to several peritoneal parameters in long-term PD-treated patients using the new solution. **Materials and Methods:** Participants were 78 patients who had undergone treatment with the new solution since starting PD between March 2001 and July 2012 and who continued PD for over 12 months. We measured the dialysate-to-plasma ratio of creatinine (D/Pcr) and several surrogate markers of peritoneal injury (cancer antigen (CA) 125, mesothelial cell area, hyaluronic acid (HA)) using overnight peritoneal effluent. We studied the relationships between these surrogate markers, and the influence of clinical factors on these peritoneal surrogate markers. **Results:** Concentrations of CA125 in peritoneal effluent tended to decrease, and mesothelial cell area in effluent tended to increase with increased duration of treatment. Using the MIXED procedure, these surrogate markers correlated significantly with duration of treatment. No changes in levels of HA in peritoneal effluent or in D/Pcr were observed during the treatment period. **Conclusion:** From these results, mesothelial cell injury may increase in patients on long-term treatment even with the use of new PD solutions.

Key Words: neutral pH, low-GDP peritoneal dialysis solution, peritoneum, CA125, mesothelial cell area

Introduction

Injury to the peritoneum occurs with long-term use of peritoneal dialysis (PD) in patients. Pathological changes to the peritoneum have been reported to include mesothelial detachment, fibrotic changes under the submesothelial compact zone and neoangiogenesis[1]. These morphological changes are considered to be the major causes of hyperpermeability and ultrafiltration failure, resulting in the technical failure of PD[2]. One of the causes of these peritoneal changes is the bioincompatibility of conventional solution, due to its low pH and the presence of glucose degradation products (GDP), among other factors[3]. In Japan, new PD solutions that are neutral pH and contain low levels of GDP have been available for PD treatment since 2000, and all PD patients have been changed from conventional solutions to these new PD solutions as of 2005[4]. Numerous reports have described the influence of the new PD solutions on the peritoneum in PD patients[5)~7)]. However, observation periods for these reports have ranged from several months to 2 years. The present study investigated changes to several peritoneal parameters in long-term PD-treated patients using the new solution in our hospital.

Materials and Methods

Patients

A total of 109 patients were treated with the new PD solution when starting PD treatment at Tokyo Women's Medical University Medical Center East between March 2001 and July 2012. Among these, participants in the present study were 78 patients (53 men, 25 women) who continued PD treatment for more than 12 months. Mean (± standard devia-

Table 1 Composition of each PD solution

Name of PD fluid	pH	Lactate (mEq/L)	Na (mEq/L)	Ca (mEq/L)	Mg (mEq/L)	Cl (mEq/L)	Glucose (g/dl)
PD solita®	7.0-7.5	40	132	3.5/2.0	0.5	96/94.5	1.55/2.27
Perisate N®	6.5-7.5	35	132	4.0/2.3	1	102/102	1.55/2.27
Midperic®	6.3-7.3	35/40	135	4.0/2.5	1.5/0.5	105.5/98	1.35/2.5
Dianeal N®	6.5-7.5	40	132	3.5/2.5	0.5	96/95	1.36/2.27

tion) age at the start of PD treatment was 56.7 ± 14.5 years (range, 24-89 years), and mean duration of PD treatment was 37.2 ± 22.2 months (range, 12-99 months). Etiology of end-stage renal disease (ESRD) was glomerulonephritis in 25 patients, diabetes in 24, nephrosclerosis in 8, polycystic kidney disease in 4, gout in 2, and other in 15. During PD treatment, the frequency of peritonitis was as follows: 0 episodes in 36 patients, 1 episode in 15, 2 episodes in 14, 3 episodes in 4, 4 episodes in 5, and 6 episodes in 2. Patient outcomes were as follows: 16 patients continued continuous ambulatory peritoneal dialysis (CAPD) treatment, 25 patients changed to hemodialysis (HD) treatment or PD + HD combined therapy, 22 patients moved to another hospital (because the physician in charge moved to another hospital or the patient moved to another area), 9 patients died, and 6 patients received renal transplantation. Patients were treated using several kinds of new PD solution (Table 1). Numbers of patients treated with each PD solution were: Perisate N®, 27 patients; Balance®, 16 patients; Midperic®, 3 patients; and Dianeal N®, 32 patients.

Study design

Effluent cancer antigen (CA) 125 levels have been considered likely to depend on mesothelial cell mass or turnover[8]. This study therefore used effluent CA125 level for mesothelial cell mass. We also studied the mesothelial cell area in overnight peritoneal effluent, as Yamamoto et al and Izumotani et al reported this as a useful marker of peritoneal mesothelial cell injury[9)10)].

Peritoneal inflammation occurs consequent to chronic exposure to bio-incompatible PD fluid and induces fibrotic changes under the submesothelial compact zone. Hyaluronic acid (HA) levels in peritoneal effluent are used as a marker of peritoneal inflammation[11)] in CAPD patients. This study therefore also analyzed HA levels in peritoneal effluent as a marker of peritoneal inflammation. Dialysate-to-plasma ratio of creatinine (D/Pcr) has been reported to increase during long-term PD treatment using conventional PD solution[12)]. In our patients, we measured peritoneal function during PD treatment using D/Pcr.

We measured CA125 and HA levels in overnight peritoneal effluent at several points during PD treatment. Samples of peritoneal effluent from the overnight bag were collected and frozen at −80°C for later analysis. Analysis of CA125 levels was performed by chemiluminescent enzyme immunoassay (Rumiparusu CA125II; Fujirebio, Tokyo, Japan). HA levels were measured by latex-enhanced immunoturbidimetric assay (Erupiae-su; Mitsubishi Chemical Medience, Tokyo, Japan). Analysis of mesothelial cell area in the present study was performed by Dr. Yamamoto. D/Pcr was determined at several time points during PD treatment.

Furthermore, we analyzed clinical data considered to influence peritoneal changes. We adopted clinical factors such as sex, age at start of PD, PD treatment duration, etiology of ESRD (diabetes mellitus (DM) or non-DM), frequency of peritonitis, residual renal function (urine volume), and the kind of PD solution for analysis (Table 2). The influences of clinical factors on the peritoneal surrogate markers were examined.

All subjects enrolled in this research provided informed consent prior to participation, and all study protocols were approved by our institutional committee on human research (registration number 2690).

Statistical analysis

To elucidate relationships between clinical fac-

Table 2 Study variables

Peritoneal surrogate markers
CA125
HA
Peritoneal mesothelial cel area
D/Pcr
Clinical factors
Sex
Age at the start of PD
Duration of PD treatment
Etiology of ESRD (DM or non-DM)
Frequency of peritonitis
Urine volume
Type of PD solution

tors and peritoneal surrogate markers, we performed multiple regression analysis using a stepwise method with JMP version 11.0 software (SAS Institute Japan, Tokyo).

In the stepwise method, peritoneal surrogate markers were applied to dependent variables and clinical factors were applied to independent variables. We used stepwise forward regression, with exclusion of covariates showing univariate p values greater than 0.2. Due to the retrospective nature of the study, the number of times measurements were taken differed between each patient. For surrogate markers identified as independent contributing factors by multiple regression analysis, we examined the relationship between variables using the MIXED procedure with SAS software (SAS Institute, Cary, NC, USA). Values of $p<0.05$ were considered significant.

Results

Distribution of surrogate markers of peritoneal injury in peritoneal effluent during PD treatment periods

CA125 levels in effluent have been considered a surrogate marker of mesothelial cell mass, and are known to decrease with increasing duration of treatment in patients treated using conventional dialysis solution[13]. In this study, the concentration of CA125 in peritoneal effluent tended to decrease during PD treatment (Fig. 1a).

Mesothelial cell area has been reported to increase during treatment in CAPD patients using conventional PD solution[14]. In our patients, mesothelial cell area in effluent also tended to increase with the duration of treatment (Fig. 1b).

Effluent levels in HA have been considered a surrogate marker of peritoneal inflammation and are reported to increase with the PD treatment period among patients using conventional PD solution[15]. However, HA concentration in this study was unchanged during the period of treatment with the new PD solution.

D/Pcr has been increasing with the PD treatment period in patients using conventional PD solution[12]. No changes in D/Pcr were observed during the PD treatment period in the present study (Fig. 2).

Influence of clinical factors on peritoneal surrogate markers

We analyzed the influence of clinical factors on peritoneal surrogate markers using multiple regression analysis.

Table 3 shows the results of multiple regression analysis. Duration of PD treatment was an independent factor on CA125 and mesothelial cell area in peritoneal effluent.

We studied the dependence of CA125 and mesothelial cell area on the duration of treatment by the MIXED procedure using SAS, and these surrogate markers showed a significant correlation with the duration of PD treatment ($p=0.0024$, $p=0.013$).

Discussion

A statistical study by the Japanese Society for Dialysis Therapy reported a mean duration for PD treatment in Japan of 3.18 ± 3.39 years as of the end of 2010[16]. Morphological changes to the peritoneal membrane have been observed with increasing duration of PD, including fibrotic changes to the submesothelial compact zone, loss of mesothelial cells and neoangiogenesis[1]. These changes have been reported to correlate with increasing small-solute permeability[2] and a reduction in peritoneal ultrafiltration capacity, and may be partially responsible for the shortened technical survival of PD. These deleterious changes occur under conditions of chronic exposure to conventional bioincompatible PD solution. In vitro and in vivo evidence suggests that these morphological changes are induced by low pH

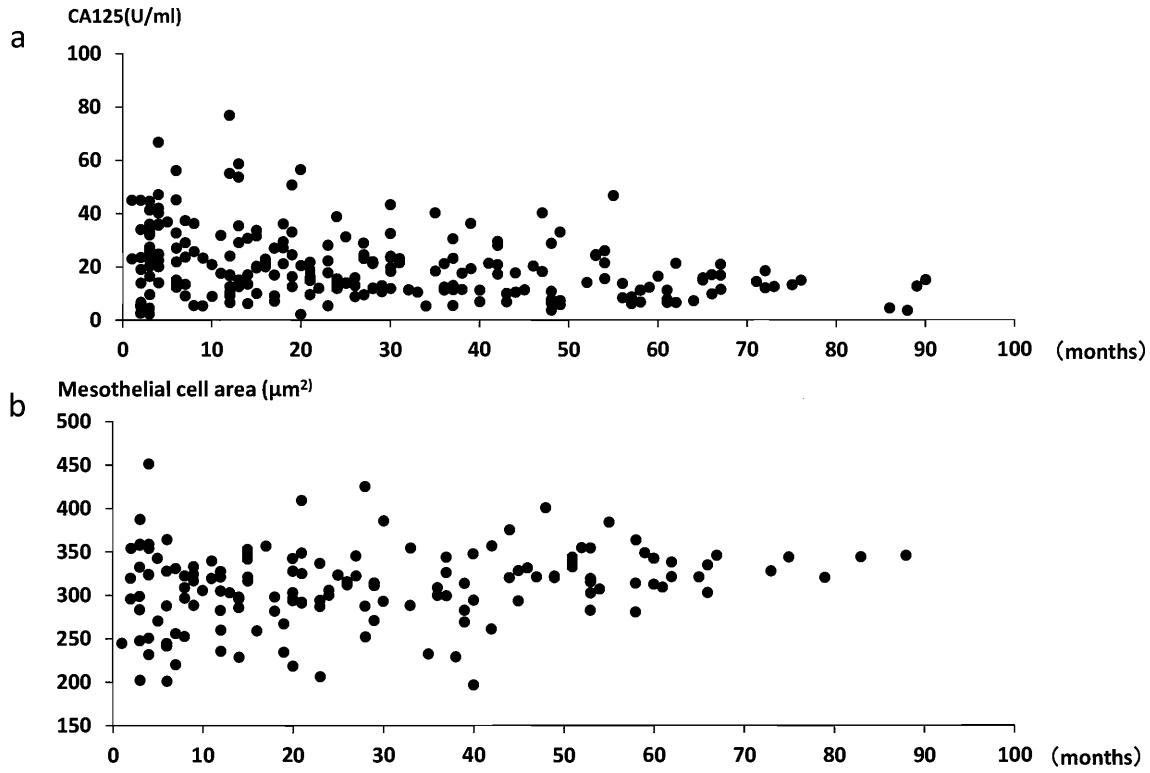

Fig. 1 Scattergram of CA125 concentrations and mesothelial cell surface area in peritoneal effluent in each patient during PD treatment
a) CA125 levels tended to decrease with increasing duration of PD treatment.
b) Mesothelial cell surface area tended to increase with increasing duration of PD treatment.

and the presence of glucose, GDP, and lactate[3]. Since 2000, new PD solutions have been available on the market, and all conventional solutions in Japan were changed to new PD solutions from 2005.

Various reports have described the impact of these new PD solutions on clinical outcomes, including peritoneal membrane function[5)~7)]. However, follow-up periods for these reports have generally been under 24 months. In the present study, we analyzed changes to several peritoneal parameters in long-term PD-treated patients using the new solution in our hospital.

In our study, the concentration of CA125 in peritoneal effluent decreased significantly during PD treatment. Longitudinal study of PD treatment with conventional dialysis solution has also demonstrated declining CA125 levels in effluent with increasing duration of treatment[13]. Several studies have found higher CA125 levels in effluent with the use of new PD solutions compared with conventional PD solutions[17)~19)]. In a crossover study, CA125 levels were increased in patients treated with new PD solution and decreased in patients with conventional solution[20]. Such results suggested the biocompatibility of new PD solutions, but study periods for these studies were only several months at best. No previous studies have reported CA125 levels in effluent with the use of new PD solutions for longer periods such as over 2 years. The present study revealed that CA125 levels in effluent declined with long-term PD therapy even when using new PD solutions. However, Breborowicz et al[21] had reported that the CA125 level in effluent was not correlated with number of cells in the monolayer of mesothelial cultures, and they concluded that CA125 is not a reflection of mesothelial cell mass. We considered that further research about the meaning of CA125 in effluent should be needed.

In this study, mesothelial cell area increased with time on PD. The increment in mesothelial cell area in peritoneal effluent reportedly correlates with the degree of peritoneal injury[9]. Maekawa et al showed

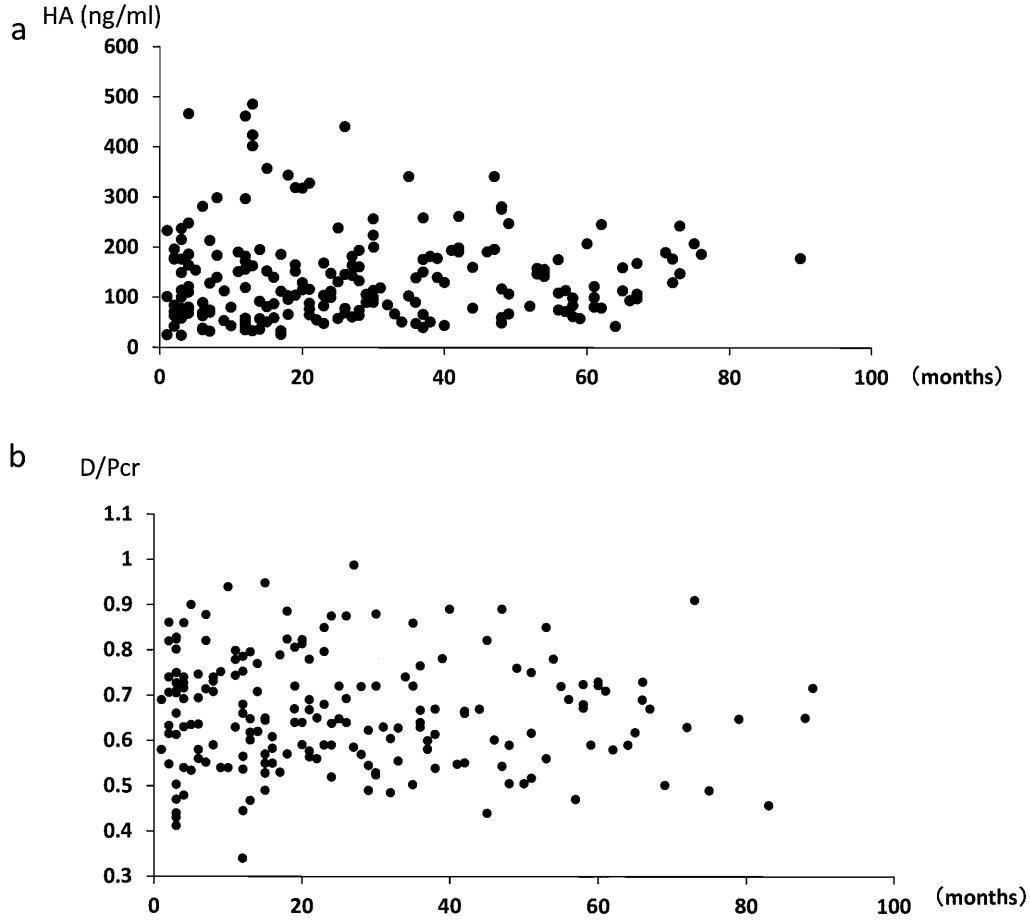

Fig. 2 Scattergram of HA levels in PD effluent and D/Pcr during PD treatment
a) HA; b) D/Pcr.
HA levels in PD effluent and D/Pcr remained unchanged with increasing duration of PD treatment.

Table 3 Results of regression coefficient and P values between peritoneal surrogate markers and clinical factors

		CA125	HA	Mesothelial cell area	D/Pcr
Sex	regression coefficient	−0.027	−0.309	0.063	−0.174
	p value	0.926	0.731	0.817	0.492
Age at start of PD	regression coefficient	0.046	−0.016	−0.308	−0.001
	p value	0.641	0.982	0.374	0.866
Duration of PD treatment	regression coefficient	−0.173	−0.202	0.535	−0.001
	p value	0.013*	0.663	0.011*	0.188
DM/non DM	regression coefficient	0.400	−0.309	0.322	−0.303
	p value	0.161	0.227	0.234	0.240
Frequency f peritonitis	regression coefficient	−0.965	3.517	−0.470	0.011
	p value	0.279	0.603	0.882	0.277
Urin volume	regression coefficient	0.006	−0.039	0.004	0.001
	p value	0.078	0.068	0.711	0.641
Type of PD solution	regression coefficient	−3.192	0.146	0.313	0.05
	p value	0.995	0.558	0.234	0.839

Duration of PD treatment was independently associated with CA125 and mesothelial cell area in peritoneal effluent.
*: $p<0.05$.

that mesothelial cell area increased during treatment in CAPD patients using conventional PD solution[14]. Mesothelial cell area was lower with new PD solutions than with conventional solution according to several reports[14)22)]. In the Japan Balance study, the effect of the new PD solution in lowering mesothelial cell area was recognized only within the first 9 months of the 27-month follow-up period. In the present study, mesothelial cell area increased during prolonged PD treatment. Given these results of increased mesothelial cell area and decreased CA 125 levels in effluent during PD treatment, mesothelial cell injury may occur during long-term PD treatment even with the use of new PD solutions.

HA levels in peritoneal effluent are considered as a marker of peritoneal inflammation[11] in CAPD patients. Effluent levels of HA reportedly show significant positive correlations with time on CAPD among patients using conventional PD solution[15]. In the present study, effluent HA levels were unchanged during the PD treatment period. Based on these results, we considered that peritoneal inflammation in patients using the new PD solution may be milder than that with conventional PD solutions. However, this study did not conduct direct comparisons with parameter from patients using conventional PD solution, and further research in this area is warranted.

In the present study, duration of PD treatment was not independently associated with D/Pcr. D/Pcr has been reported to increase during long-term (84-month) PD treatment using conventional PD solution[12]. Several studies using new PD solutions have suggested no differences in D/Pcr compared to conventional PD solution[23)24)]. However, follow-up periods for those reports were under 2 years. In the present study, D/Pcr among patients treated with new PD solutions was unchanged during long-term PD treatment. Our results suggest that the effect of new PD solutions on changes in solute transport with long-term PD treatment may differ from that of conventional PD solution.

Conclusion

In PD patients treated with new PD solutions for long periods, concentrations of CA125 in PD effluent decreased, and mesothelial cell area in peritoneal effluent increased with treatment period. Given these results, mesothelial cell injury may increase in patients receiving long-term treatment, even with the use of new PD solutions.

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The authors have no conflicts of interest to declare.

References

1) **Williams JD, Craig KJ, Topley N et al**: Morphological changes in the peritoneal membrane of patients with renal disease. J Am Soc Nephrol **13**: 470–479, 2002
2) **Krediet RT, Lindholm B, Rippe B**: Pathophysiology of peritoneal membrane failure. Perit Dial Int **20** (Suppl 4): S22–S42, 2000
3) **Chaudhary K, Khanna R**: Biocompatible peritoneal dialysis solutions; do we have one? Clin J Am Soc Nephrol **5**: 723–732, 2010
4) **Higuchi C, Nishimura H, Sanaka T**: Biocompatibility of peritoneal dialysis fluid and influence of compositions on peritoneal fibrosis. Ther Apher Dial **10**: 372–379, 2006
5) **Fusshoeller A, Plail M, Grabensee B et al**: Biocompatibility pattern of a bicarbonate / lactate-buffered peritoneal dialysis fluid in APD: a prospective, randomized study. Nephrol Dial transplant **19**: 2101–2106, 2004
6) **Kim S, Oh J, Kim S et al**: Benefits of biocompatible PD fluid for preservation of residual renal function in incident CAPD patients: a 1-year study. Nephrol Dial Transplant **24**: 2899–2908, 2009
7) **Rippe B, Simonsen O, Heimbürger O et al**: Long-term clinical effects of a peritoneal dialysis fluid with less glucose degradation products. Kidney Int **59**: 348–357, 2001
8) **Krediet RT**: Dialysate cancer antigen 125 concentration as marker of peritoneal membrane status in patients treated with chronic peritoeneal dialysis. Perit Dial Int **21**: 560–567, 2001
9) **Yamamoto T, Izumotani T, Otoshi T et al**: Morphological studies of mesothelial cells in CAPD effluent and their clinical significance. Am J Kidney Dis **32**: 946–952, 1998
10) **Izumotani T, Ishimura E, Yamamoto T et al**: Correlation between peritoneal mesothelial cell cytology and peritoneal histopathology with respect to prognosis in patients on continuous ambulatory peritoneal dialysis. Nephron **89**: 43–49, 2001
11) **Yung S, Chan TM**: Hyaluronan-regulator and initiator of peritoneal inflammation and remodeling. Int J Artif Organs **30**: 477–483, 2007

12) **Davies SJ**: Longitudinal relationship between solute transport and ultrafiltration capacity in peritoneal dialysis patients. Kidney Int **66**: 2437–2445, 2004
13) **Ho-Dac-Pannekeet MM, Hiralall JK, Struijk DG et al**: Longitudinal follow-up of CA125 in peritoneal effluent. Kidney Int **51**: 888–893, 1997
14) **Maekawa K, Yamamoto T, Hino H et al**: Relationship between duration of peritoneal dialysis and mesothelial cell area in elderly patients—Comparison of neutralized dialysate with conventional dialysate. Kidney and Dialysis **61** (Suppl): 237–239, 2006
15) **Wakabayashi Y, Yamada K, Miura Y et al**: Type III procollagen N-peptide and hyaluronate in serum and dialysate of CAPD patients. Nihon Jinzo Gakkai Shi **39**: 408–413, 1997
16) **Nakai S, Iseki K, Itami N et al**: An overview of regular dialysis treatment in Japan (As of 31 December 2010). Ther Apher Dial **45**: 1–47, 2012
17) **Jones S, Holmes CJ, Krediet RT et al**: Bicarbonate/lactate-based peritoneal dialysis solution increases cancer antigen 125 and decreases hyaluronic acid levels. Kidney Int **59**: 1529–1538, 2001
18) **Choi HY, Kim DK, Lee TH et al**: The clinical usefulness of peritoneal dialysis fluids with neutral pH and low glucose degradation product concentration: An open randomized prospective trial. Perit Dial Int **28**: 174–182, 2008
19) **Haag-Weber M, Kramer R, Haake R et al**: Low-GDP fluid (Gambrosol trio®) attenuates decline of residual renal function in PD patients: a prospective randomized study. Nephrol Dial Transplant **25**: 2288–2296, 2010
20) **Williams JD, Topley N, Craig KJ et al**: The eurobalance trial: The effect of a new biocompatible peritoneal dialysis fluid (balance) on the peritoneal membrane. Kidney Int **66**: 408–418, 2004
21) **Breborowicz A, Breborowicz M, Pyda M et al**: Limitations of CA 125 as an index of peritoneal mesothelial cell mass. Nephron Clin Pract **100**: c46–c51, 2005
22) **Yamamoto T, Higuchi C, Nakamoto H et al**: The Japan Balance study: Evaluation of peritoneal dialysis fluid with neutral pH and low levels of glucose degradation products in peritoneal dialysis patients. J Jpn Soc Dial Ther **42**: 835–846, 2009
23) **Tranaeus A**: A long-term study of bicarbonate/lactate-based peritoneal dialysis solution—clinical benefits. Perit Dial Int **20**: 516–523, 2000
24) **Fan SLS, Pile T, Punzalan T et al**: Randomized controlled study of biocompatible peritoneal dialysis solutions; effect on residual renal function. Kidney Int **73**: 200–206, 2008

中性・低 GDP 透析液長期使用による CAPD 患者の腹膜パラメーターの変化

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〔緒言〕長期腹膜透析では腹膜が障害されるが，その原因のひとつとして従来透析液の低い PH（酸性）およびグルコース分解産物（GDP）があげられていた．これに対し本邦では中性・低 GDP の新しい透析液が 2000 年より使用されるようになった．今回，中性・低 GDP 透析液の長期使用による腹膜パラメーターの変化について検討した．〔対象，方法〕2001 年 3 月から 2012 年 7 月の間に新しい透析液を用いて腹膜透析を開始し，12 ヵ月以上腹膜透析を継続した 78 名の患者を対象とした．透析施行期間中の透析排液/血清のクレアチニン比（D/Pcr）および夜間貯留排液中の種々の腹膜障害パラメーター（腫瘍抗原 125：CA125，中皮細胞面積，ヒアルロン酸：HA）を測定し，これらのパラメーター間の関連および臨床データとの関連について検討した．〔結果〕透析期間に伴い排液中 CA125 濃度は減少し，中皮細胞面積は増加を認め，MIXED 検討にてこれらの変化は統計上有意であった．排液中 HA 濃度や D/Pcr は治療期間中変化はみられなかった．〔結論〕今回の結果より，長期腹膜透析では新しい中性・低 GDP 透析液使用においても中皮細胞は障害されると考えられた．