

# Impact of Uremia, Diabetes, and Peritoneal Dialysis Itself on the Pathogenesis of Peritoneal Sclerosis: A Quantitative Study of Peritoneal Membrane Morphology

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**Background and objectives:** Peritoneal interstitial fibrosis and hyalinizing vasculopathy were induced by peritoneal dialysis and other associated conditions (e.g., uremia). A quantitative method for peritoneal biopsy evaluation is required to investigate possible causative factors and severity of the peritoneal dialysis-related peritoneal alterations.

**Design, setting, participants, & measurements:** Peritoneal biopsy specimens from 173 uremic (before peritoneal dialysis) and 80 peritoneal dialysis patients with or without impaired ultrafiltration capacity were evaluated by average peritoneal thickness of submesothelial compact zone measured at five randomly selected points of peritoneum and by lumen/vessel diameter ratio at postcapillary venule.

**Results:** The average peritoneal thickness was increased in uremic patients and progressively thickened as the duration of peritoneal dialysis prolonged. The lumen/vessel diameter ratio was lower in uremia than normal and progressively decreased as the duration of peritoneal dialysis prolonged. In pre-peritoneal dialysis peritoneum, patients with diabetes showed significant decrease in lumen/vessel diameter ratio compared with patients without diabetes. The average peritoneal thickness was significantly higher in patients with impaired ultrafiltration capacity than in patients with maintained ultrafiltration capacity; however, no significant difference was observed in the postcapillary venule thickness and lumen/vessel diameter ratio between the two groups.

**Conclusions:** The average peritoneal thickness and lumen/vessel diameter ratio were useful morphologic parameters to quantify the severity of the peritoneal alterations in uremic and peritoneal dialysis patients. Uremia and diabetes had an impact on the pathogenesis of peritoneal sclerosis in pre-peritoneal dialysis peritoneum. Peritoneal dialysis treatment itself had a much stronger impact on the progression of peritoneal sclerosis.

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**T**herapeutic advances in peritoneal dialysis (PD) contributed to prolongation of this therapy; however, several problems derived from this therapy have been recognized. Major complications are ultrafiltration failure and solute transport abnormality, which are associated with morphologic deteriorations of peritoneal membrane, interstitial fibrosis, and hyalinizing vasculopathy (1–6). These morphologic alterations of peritoneal membrane were categorized as “peritoneal sclerosis.”

The cause of peritoneal sclerosis has been considered as bioincompatible effects of dialysate to peritoneal membrane, such as high osmolarity, low pH, high concentration of glucose, advanced glycation end product (AGE), and glucose degradation product (GDP), through various biologic mechanisms including degenerative damage on the tissue components and abnormal biologic responses (7). In addition, there is some evidence showing that some factors other than PD treatment (e.g., uremia) played roles in the pathogenesis of peritoneal sclerosis in pre-PD patients (6,8).

In these circumstances, histologic evaluation of peritoneal membrane has become important to determine the extent of peritoneal damage as a result of PD treatment and to decide whether the PD treatment can be continued. The histologic evaluation of peritoneal membrane also provides us some clues to understanding the pathogenesis of peritoneal sclerosis and

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establish clinical strategies for the prevention of PD-related complications.

To evaluate peritoneal biopsy appropriately, we need a standardized and quantitative method for histologic evaluation. Williams *et al.* (6) reported the morphologic changes of peritoneal membrane of patients with renal disease using a quantitative and semiquantitative method for histologic evaluation. They measured thickness of submesothelial compact zone that increased with the duration of PD therapy. They also evaluated the vasculopathy using a semiquantitative grading system to assess the thickness of subendothelial hyaline material and the features of luminal distortion, narrowing, and obliteration.

Considering their method, we designed a new procedure for quantitative evaluation of peritoneal membrane morphology using average peritoneal thickness (APT) measured at five randomly selected points of peritoneum and luminal/vessel diameter ratio (L/V) of affected postcapillary venule (PCV). We applied this quantitative method to 253 peritoneal biopsy specimens registered in the Peritoneal Biopsy Program in Japan to confirm its utility for the evaluation of peritoneal sclerosis in uremic and PD patients. The impacts of uremia and diabetes on the pathogenesis of peritoneal sclerosis were also evaluated in pre-PD and PD peritoneum. Morphologic and functional relationship was evaluated by comparison of the peritoneal alterations between the patients with impaired and maintained ultrafiltration capacity (UFC).

## Materials and Methods

### Patients

Peritoneal biopsy specimens were obtained from 253 patients who participated in the Peritoneal Biopsy Program (directed by Dr. H. Hirano from 1994 to 2005). The biopsies were performed at the time of initial PD catheter insertion (pre-PD peritoneum,  $n = 173$ ) or removal of PD catheter as a result of PD-related problems (*e.g.*, ultrafiltration failure) or patient's intention to transfer to hemodialysis or transplantation. (PD peritoneum,  $n = 80$ ; average PD duration  $62.5 \pm 43.3$  mo). The patients who had a history of peritonitis during their PD therapy were excluded from this study; therefore, any influences by peritonitis on the peritoneal histology were eliminated in this study. Among the 80 PD patients, 16 were regarded as having impaired UFC, which was defined by use of more than four hypertonic bags (2.5% glucose, 4.25% glucose or icodextrin) in each 24 h to maintain their fluid balance. The existence of diabetes was identified from clinical records. Approval for the study was obtained from the local ethics committees, and all patients gave written informed consent. Normal peritoneal biopsy samples ( $n = 9$ ) from kidney donors were provided by Prof. J.D. Williams (University of Wales College of Medicine, Cardiff, UK), which were the same specimens of his previous study (6).

### Processing of Biopsy Samples

Samples of parietal peritoneum were biopsied in the usual manner. Briefly, the peritoneal tissue was cut by scalpel at approximately 1 cm in size and up to 5 mm in depth. The tissue sample was placed on a small board or filter paper with mesothelial surface uppermost, extending it as the same size as *in situ*, and fixed with 20% buffered formalin. After overnight fixation at room temperature, the samples were routinely processed for light microscopy and embedded in paraffin. The 4- $\mu$ m sections were cut routinely and stained with hematoxylin and eosin and Masson trichrome.

### Sample Analysis Methods

The samples were assessed by microscopy using a standardized method described next. Two experienced examiners, one pathologist (K.H.) and one nephrologist (C.H.), who were unaware of patients' clinical backgrounds, evaluated the samples independently.

**Adequacy of Specimen for Histologic Evaluation.** The adequacy of specimen for histologic evaluation regarding peritoneal thickness and vasculopathy was determined independently. For peritoneal thickness measurement, each specimen was assessed in terms of size, site, and direction of the specimen and classified into adequate or inadequate specimen. The adequate specimen had enough size of sampling and contained several layers of peritoneum (mesothelial, submesothelial, and adipose tissue layers). The direction of embedding was almost vertical, so the thickness of the submesothelial layer could be measured properly. For the evaluation of vasculopathy, adequate specimen must contain PCV at the level of 25 to 50  $\mu$ m in external diameter. Figure 1 shows the proportion of adequate and inadequate specimens for the evaluation of peritoneal thickness (Figure 1A) and vasculopathy (Figure 1B).

**Evaluation of Peritoneal Fibrosis.** The extent of peritoneal fibrosis was determined by thickness of submesothelial interstitial layer (submesothelial compact zone) between basal border of surface mesothelial cells and upper border of peritoneal adipose tissue. The thick-

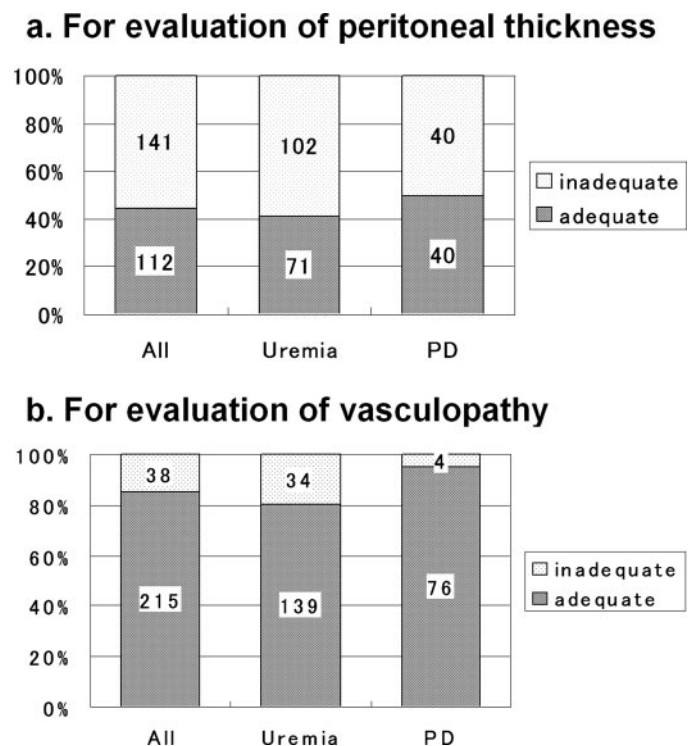


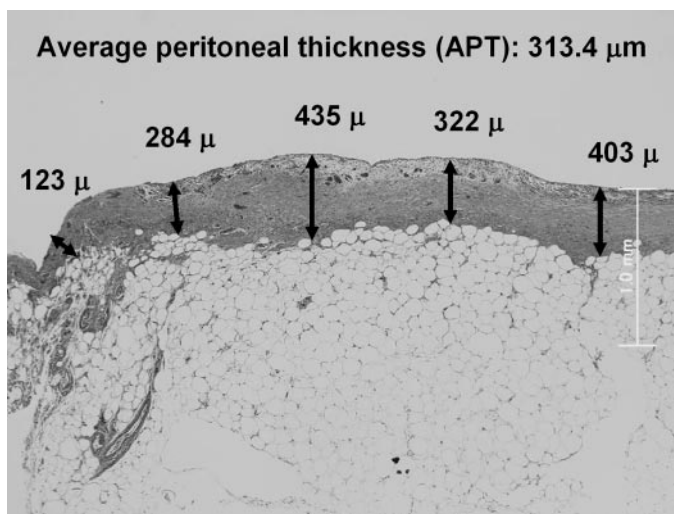
Figure 1. Adequacy of the samples for evaluation of peritoneal thickness (A) and vasculopathy (B). (A) By observation of 253 peritoneal samples, 71 samples from the 173 pre-peritoneal dialysis (pre-PD) patients and 40 samples from the 80 PD patients were adequate for the evaluation of peritoneal thickness. (B) For the evaluation of vasculopathy, adequate specimens must contain postcapillary venules (PCV) at the level of 25 to 50  $\mu$ m in external diameter. By observation of the 253 peritoneal samples, 139 samples from the 173 pre-PD patients and 76 samples from the 80 PD patients were adequate for the evaluation of vasculopathy.

ness of the mesothelial cell layer was excluded from the measurement, when it was present on the peritoneal surface. When the submesothelial interstitium was continuous to underlying dense connective tissue (abdominal fascia) without peritoneal adipose tissue, the peritoneal thickness could not be measured. Five portions were randomly selected for the measurement of submesothelial thickness. The thickness was measured by micrometer on microscopic lens or by image analyzer, and then the APT was calculated (Figure 2). The portion where the peritoneum looked severely fibrotic as a result of tangential embedding or miscellaneous inflammatory reactions was excluded from the measurement. The mean value of two APT determined by the two examiners was taken as a representative APT of that case.

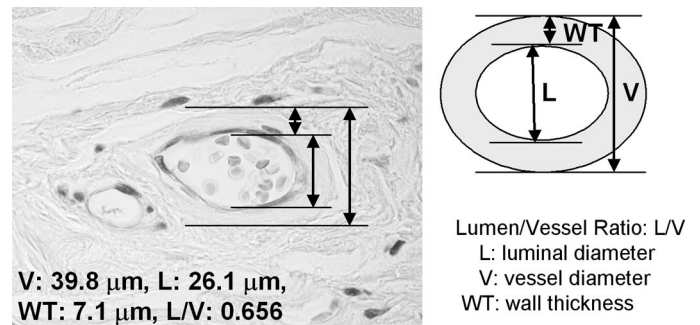
**Evaluation of Vasculopathy.** The extent of vasculopathy was determined by presence of hyalinosis, thickness of vascular wall, and severity of luminal narrowing at the level of PCV. For evaluation of the severity of luminal narrowing, a ratio of luminal diameter to vessel external diameter (L/V) was determined, which represents an extent of patency of the blood vessel (Figure 3). In general, hyalinizing vasculopathy in PD patients is usually observed at the PCV or capillary level; therefore, we selected the PCV whose diameter ranged from 25 to 50  $\mu\text{m}$  for the morphologic measurement, because the L/V was influenced by the level of blood vessel examined. The measurement was done in short axis to avoid the artificial effect of elongated distance as a result of tangential cutting of the vessel during the histologic preparation. When different vessels showed different severities of the vasculopathy, the most severely affected vessel of each specimen was chosen for the measurement. The average of two thicknesses of vascular wall and L/V measured by two examiners was taken as the representative value of that case.

#### Statistical Analyses

Data are expressed as means  $\pm$  SD. Parametric comparison was performed by *t* test, and nonparametric comparison was done by Mann-Whitney *U* test or Kruskal-Wallis test to examine statistical sig-



**Figure 2.** Average peritoneal thickness (APT) by five-point measurement method. The peritoneal thicknesses at randomly selected five points were measured, and then the APT was calculated. In this PD patient, the peritoneal thicknesses ranged from 123 to 435  $\mu\text{m}$ , and the APT was 313.4  $\mu\text{m}$ . The average of two APT values determined by two examiners was taken as a representative APT of that case.



**Figure 3.** Quantitative evaluation of vasculopathy at PCV. For evaluation of the severity of luminal narrowing, a ratio of luminal diameter to vessel diameter (L/V) was determined, representing the extent of patency of the blood vessel. The PCV whose diameter ranged from 25 to 50  $\mu\text{m}$  was selected for the measurement. The distance is measured in short axis. The most severely affected vessel was chosen for the measurement. The average of two L/V values determined by two examiners was taken as the representative L/V of that case.

nificance. Categorized data were analyzed by  $\chi^2$  test.  $P < 0.05$  was considered to be significant.

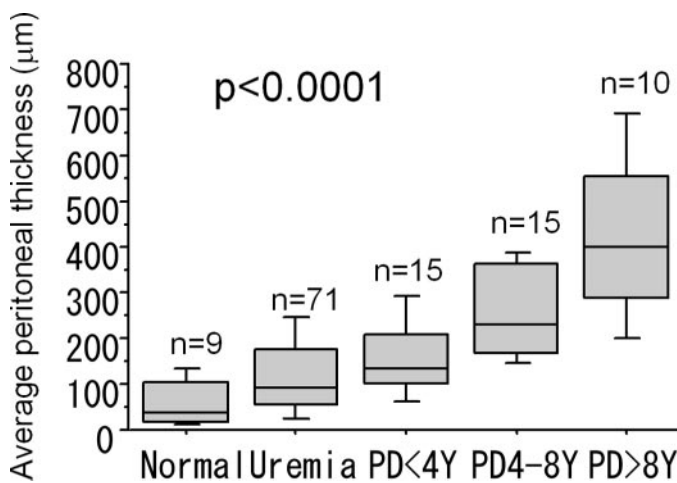
## Results

### Peritoneal Fibrosis of Normal, Uremic (Pre-PD), and PD Peritoneum

The average peritoneal thickness of each category was  $62.4 \pm 52.0$   $\mu\text{m}$  in normal peritoneums ( $n = 9$ ),  $120.0 \pm 84.4$   $\mu\text{m}$  in uremic peritoneums ( $n = 71$ ),  $166.3 \pm 101.7$   $\mu\text{m}$  in peritoneums of PD  $< 4$  yr ( $n = 15$ ),  $261.9 \pm 97.0$  in peritoneums of PD 4 to 8 yr ( $n = 15$ ), and  $466.6 \pm 190.1$   $\mu\text{m}$  in peritoneums of PD  $> 8$  yr ( $n = 10$ ), showing significant difference ( $P < 0.0001$ , Kruskal-Wallis; Figure 4). These results indicated that the uremic peritoneum was significantly thicker than normal peritoneum and that the peritoneal thickness progressively increased as the PD treatment prolonged.

### Peritoneal Vasculopathy of Normal, Uremic (Pre-PD) and PD Peritoneum

The incidence of hyalinizing vasculopathy, which was defined by a presence of hyalinosis in the vessel wall, was zero (0%) of nine in normal peritoneums, 29 (20.9%) of 139 in uremic peritoneums, 18 (58.1%) of 31 in peritoneums of PD  $< 4$  yr, 27 (90%) of 30 in peritoneums of PD 4 to 8 yr, and 15 (100%) of 15 in peritoneums of PD  $> 8$  yr. The vascular wall thickness at PCV was  $2.32 \pm 0.90$   $\mu\text{m}$  in normal peritoneums,  $3.71 \pm 1.96$   $\mu\text{m}$  in uremic peritoneums,  $6.59 \pm 5.12$   $\mu\text{m}$  in peritoneums of PD  $< 4$  yr,  $9.88 \pm 5.64$   $\mu\text{m}$  in peritoneums of PD 4 to 8 yr, and  $11.91 \pm 5.22$   $\mu\text{m}$  in peritoneums of PD  $> 8$  yr, showing significant difference ( $P < 0.0001$ , Kruskal-Wallis; Figure 5A). The L/V at PCV also changed significantly:  $0.872 \pm 0.048$  in normal peritoneums,  $0.770 \pm 0.122$  in uremic peritoneums,  $0.630 \pm 0.213$   $\mu\text{m}$  in peritoneums of PD  $< 4$  yr,  $0.441 \pm 0.274$   $\mu\text{m}$  in peritoneums of PD 4 to 8 yr, and  $0.301 \pm 0.274$   $\mu\text{m}$  in peritoneums of PD  $> 8$  yr ( $P < 0.0001$ , Kruskal-Wallis; Figure 5B). These results demonstrated that the hyalinizing vasculopathy was present in uremic peritoneum be-



**Figure 4.** Comparison of APT among normal, uremic, and PD peritoneums. The APT of each category were  $62.4 \pm 52.0 \mu\text{m}$  in normal peritoneums ( $n = 9$ ),  $120.0 \pm 84.4 \mu\text{m}$  in uremic (pre-PD) peritoneums ( $n = 71$ ),  $166.3 \pm 101.7 \mu\text{m}$  in peritoneums of PD <4 yr ( $n = 15$ ),  $261.9 \pm 97.0 \mu\text{m}$  in peritoneums of PD 4 to 8 yr ( $n = 15$ ), and  $466.6 \pm 190.1 \mu\text{m}$  in peritoneums of PD >8 yr ( $n = 10$ ), showing significant difference ( $P < 0.0001$ , Kruskal-Wallis).

fore PD treatment and that its severity became increased as the PD treatment prolonged.

#### Influence of Diabetes on Peritoneal Membrane Alterations

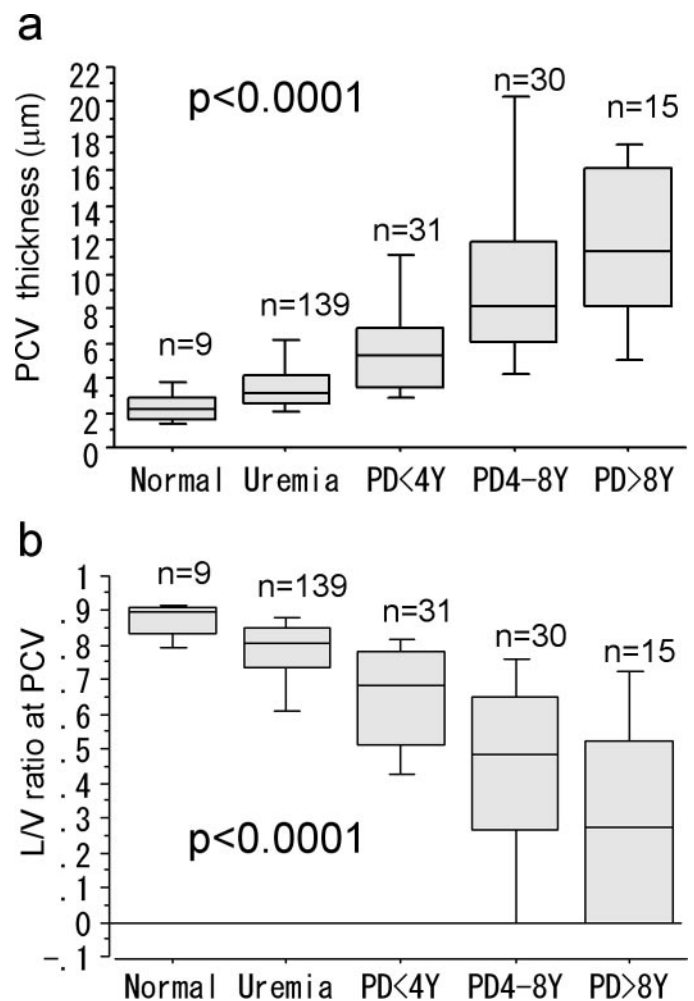
In uremic (pre-PD) patients, the average peritoneal thickness tended to be higher in patients with diabetes ( $148.9.0 \pm 106.8 \mu\text{m}$ ,  $n = 22$ ) than in patients without diabetes ( $107.0 \pm 69.6 \mu\text{m}$ ,  $n = 49$ ) but not significantly different (Figure 6A). The hyalinizing vasculopathy was more frequent in uremic patients with diabetes than in uremic patients without diabetes (frequency 20 [43.5%] of 46 *versus* nine [9.7%] of 93;  $P = 0.001$ ,  $\chi^2$  test). The vascular wall thickness at PCV was increased in uremic patients with diabetes compared with uremic patients without diabetes ( $4.76 \pm 2.67$  *versus*  $3.24 \pm 1.21$ ;  $P = 0.0009$ ; Figure 6B). The L/V was decreased in uremic patients with diabetes compared with uremic patients without diabetes ( $0.708 \pm 0.164$  *versus*  $0.799 \pm 0.079$ ;  $P = 0.0018$ ; Figure 6C).

On the contrary, there was no significant difference in peritoneal thickness and severity of vasculopathy between PD patients (<10 yr of PD treatment) with and without diabetes (Figure 7). We excluded patients with PD duration >10 yr to adjust the PD duration for comparison because the PD durations of all eight patients with diabetes were <10 yr.

Figure 8 shows a representative peritoneal vasculopathy in a uremic (pre-PD) patient with diabetes. Hyalinizing vasculopathy was observed at the PVC in submesothelial interstitium. The thickness of PCV was  $12.1 \mu\text{m}$ , and the L/V was 0.435 (Figure 8).

#### Progression of Peritoneal Sclerosis after PD Induction

In PD patients, the average peritoneal thickness was positively correlated with the PD duration, although its correlation coefficient was low ( $y = 2.231x + 114.74$ ,  $R^2 = 0.433$ ,  $n = 40$ ;

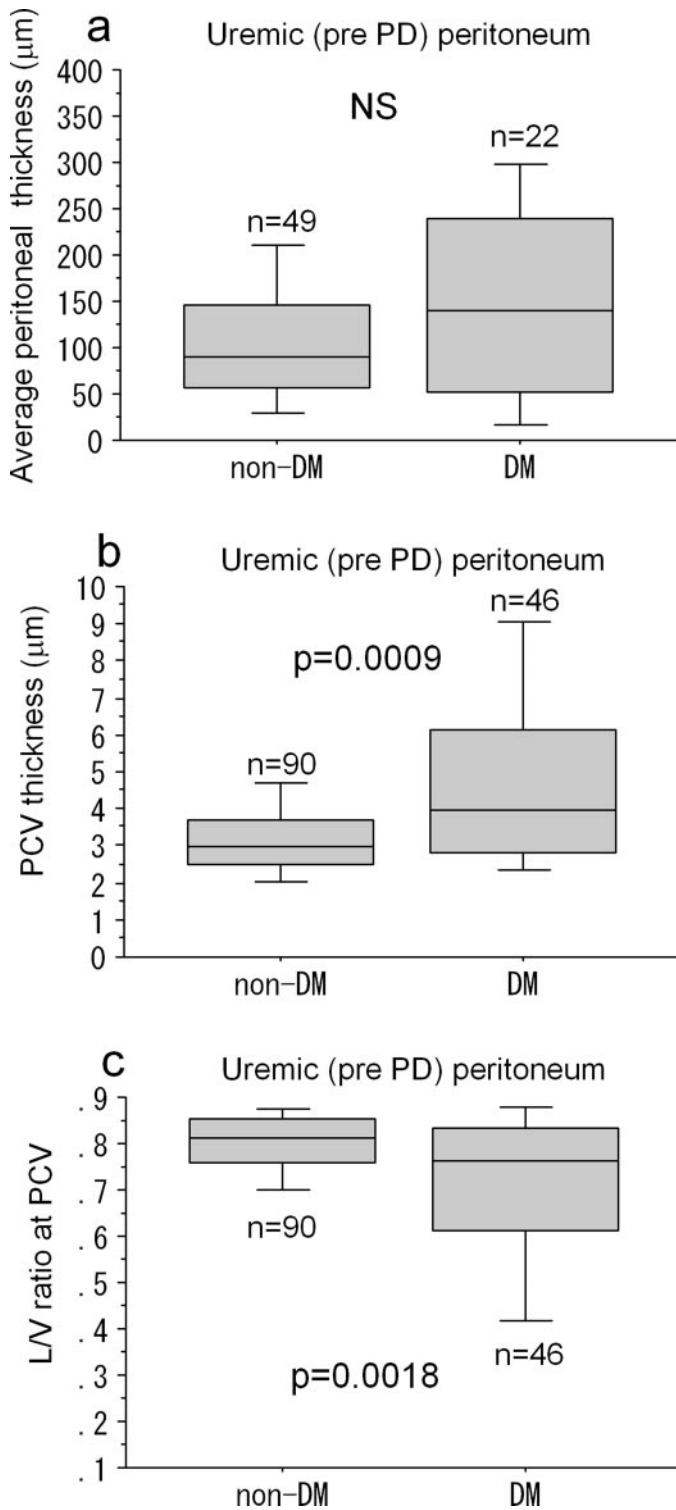


**Figure 5.** Comparison of hyalinizing vasculopathy among normal, uremic, and PD peritoneums. (A) The vascular wall thickness at PCV was  $2.32 \pm 0.90 \mu\text{m}$  in normal peritoneums,  $3.71 \pm 1.96 \mu\text{m}$  in uremic (pre-PD) peritoneums,  $6.59 \pm 5.12 \mu\text{m}$  in peritoneums of PD <4 yr,  $9.88 \pm 5.64 \mu\text{m}$  in peritoneums of PD 4 to 8 yr, and  $11.91 \pm 5.22 \mu\text{m}$  in peritoneums of PD >8 yr, showing significant difference ( $P < 0.0001$ , Kruskal-Wallis). (B) The L/V at PCV also changed significantly:  $0.872 \pm 0.048$  in normal peritoneums,  $0.770 \pm 0.122$  in uremic (pre-PD) peritoneums,  $0.630 \pm 0.213$  in peritoneums of PD <4 yr,  $0.441 \pm 0.274$  in peritoneums of PD 4 to 8 yr and  $0.301 \pm 0.274$  in peritoneums of PD >8 yr, showing significant difference ( $P < 0.0001$ , Kruskal-Wallis).

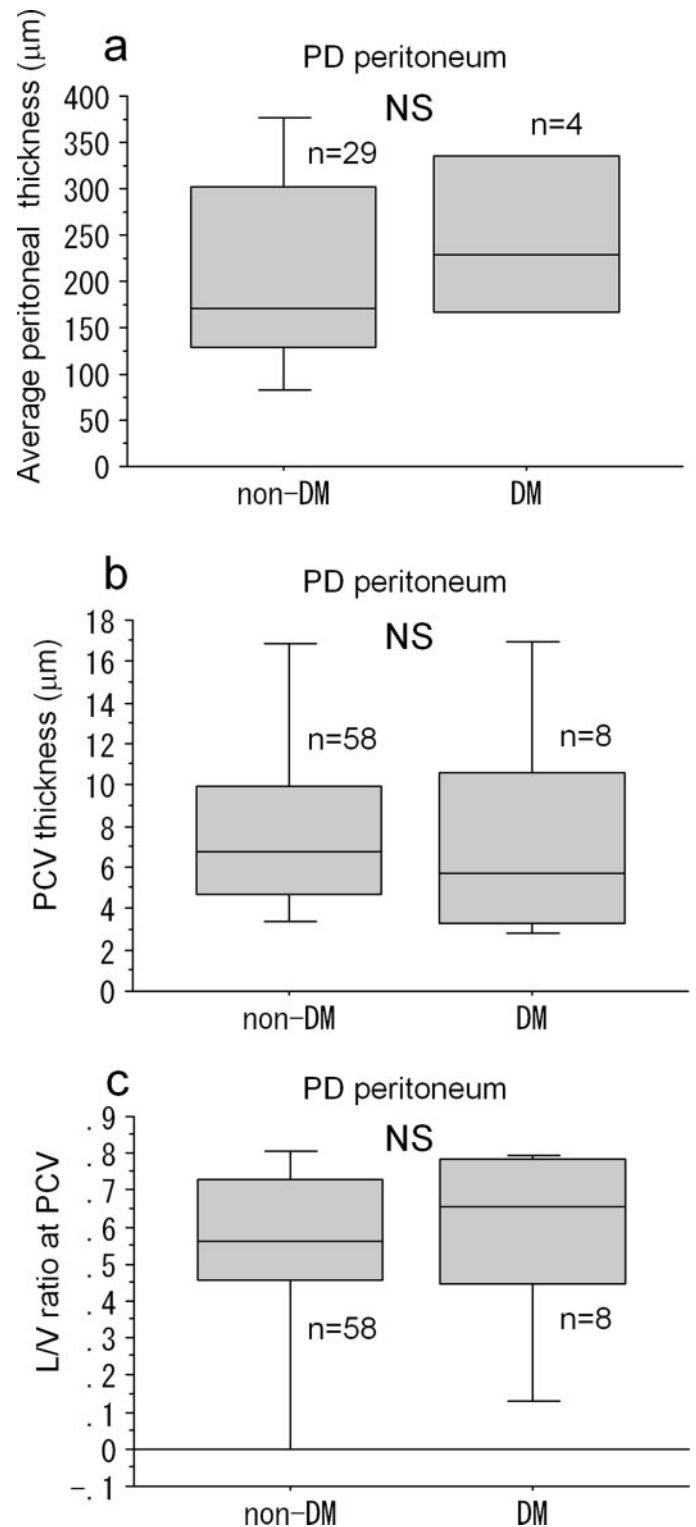
$P < 0.0001$ ; Figure 9A). In addition, there was a positive correlation between the thickness of PCV and PD duration ( $y = 0.064x + 4.959$ ,  $R^2 = 0.243$ ,  $n = 76$ ;  $P < 0.0001$ ; Figure 9B) and an inverse correlation between the L/V and PD duration ( $y = -0.004x + 0.712$ ,  $R^2 = 0.327$ ,  $n = 76$ ;  $P < 0.0001$ ; Figure 9C). These results suggested that the peritoneal fibrosis and vasculopathy progressed gradually as the PD duration prolonged.

#### Relationship between Peritoneal Fibrosis and Hyalinizing Vasculopathy

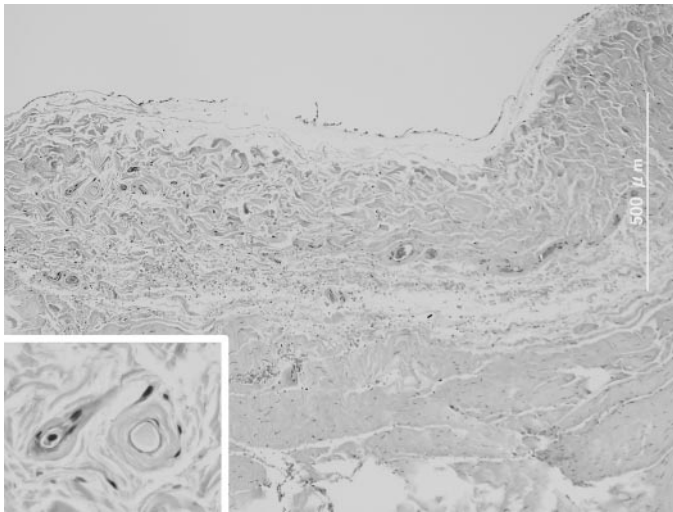
The relationship between peritoneal fibrosis and vasculopathy is shown in Figure 10. In the uremic (pre-PD) patients ( $n =$



**Figure 6.** Comparison of peritoneal sclerosis in uremic (pre-PD) peritoneums between patients with and without diabetes. (A) The APT was higher in uremic (pre-PD) patient with diabetes ( $147.9 \pm 107.0 \mu\text{m}$ ;  $n = 22$ ) than in patients without diabetes ( $107.0 \pm 69.6 \mu\text{m}$ ;  $n = 49$ ) but not significantly. (B) The average PCV thickness was higher in uremic (pre-PD) patients with diabetes ( $4.76 \pm 2.64 \mu\text{m}$ ;  $n = 46$ ) than in patients without diabetes ( $3.24 \pm 1.22 \mu\text{m}$ ;  $n = 90$ ) significantly ( $P = 0.0009$ ). (C) The L/V was decreased in uremic (pre-PD) patients with diabetes ( $0.708 \pm 0.164$ ;  $n = 46$ ) compared with patients without diabetes ( $0.799 \pm 0.079$ ;  $n = 90$ ;  $P = 0.0018$ ).



**Figure 7.** Comparison of peritoneal sclerosis between PD patients (<10 yr) with and without diabetes. (A) The APT was not different between PD patients without ( $212.08 \pm 109.22 \mu\text{m}$ ;  $n = 29$ ) and with diabetes ( $250.62 \pm 104.43 \mu\text{m}$ ;  $n = 4$ ). (B) The average PCV thickness was not different between PD patients without ( $8.23 \pm 5.42 \mu\text{m}$ ;  $n = 58$ ) and with diabetes ( $7.65 \pm 5.70 \mu\text{m}$ ;  $n = 8$ ). (C) The L/V was not different between PD patients without ( $0.528 \pm 0.262$ ;  $n = 58$ ) and with diabetes ( $0.573 \pm 0.273$ ;  $n = 8$ ).



**Figure 8.** Hyalinizing vasculopathy at PCV observed in a pre-PD (uremic) patient with diabetes. Hyalinizing vasculopathy at PCV was observed in the submesothelial interstitium (inset). The thickness of PCV was  $12.1 \mu\text{m}$ , and L/V was 0.435. The peritoneum appeared broadly thickened continuous to subperitoneal dense connective tissue. The thickness of the peritoneum was unable to be measured in this case.

63), there was no significant correlation between peritoneal fibrosis and vascular wall thickness at PCV; however, a significant positive correlation between peritoneal fibrosis and thickness of PCV was observed in the PD patients ( $n = 38$ ,  $y = 0.019x + 4.678$ ,  $R^2 = 0.274$ ;  $P = 0.0005$ ; Figure 10A). An inverse correlation between peritoneal fibrosis and L/V was also shown in the PD patients ( $n = 38$ ,  $y = -0.01x + 0.701$ ,  $R^2 = 0.274$ ;  $P = 0.0005$ ) but not in the uremic (pre-PD) patients (Figure 10B).

#### Relationship between Peritoneal Alterations and UFC

The average peritoneal thickness was significantly increased in the group with impaired UFC ( $372.9 \pm 182.2 \mu\text{m}$ ;  $n = 9$ ) than in the group with maintained UFC ( $211.0 \pm 119.0 \mu\text{m}$ ;  $n = 27$ ;  $P = 0.0041$ ; Figure 11A); however, the PCV thickness was not different between the group with maintained UFC ( $9.24 \pm 5.84 \mu\text{m}$ ;  $n = 50$ ) and the group with impaired UFC ( $9.22 \pm 6.02 \mu\text{m}$ ;  $n = 16$ ; Figure 11B). The L/V also was not different between the group with maintained UFC ( $0.489 \pm 0.283$ ;  $n = 50$ ) and the group with impaired UFC ( $0.463 \pm 0.290$ ;  $n = 8$ ; Figure 11C). The PD duration was not significantly different between the group with impaired UFC ( $78.4 \pm 33.8$  mo;  $n = 16$ ) and the group with maintained UFC ( $57.1 \pm 43.4$  mo;  $n = 53$ ).

## Discussion

Measurement of the thickness of submesothelial compact zone is a method to quantify the severity of peritoneal fibrosis. Williams *et al.* (6) used this method and demonstrated a presence of significant peritoneal fibrosis in uremic and PD patients; however, several difficulties exist to measure the peritoneal thickness properly: Adequacy of the samples and wide variation of the peritoneal thickness at different portions in one

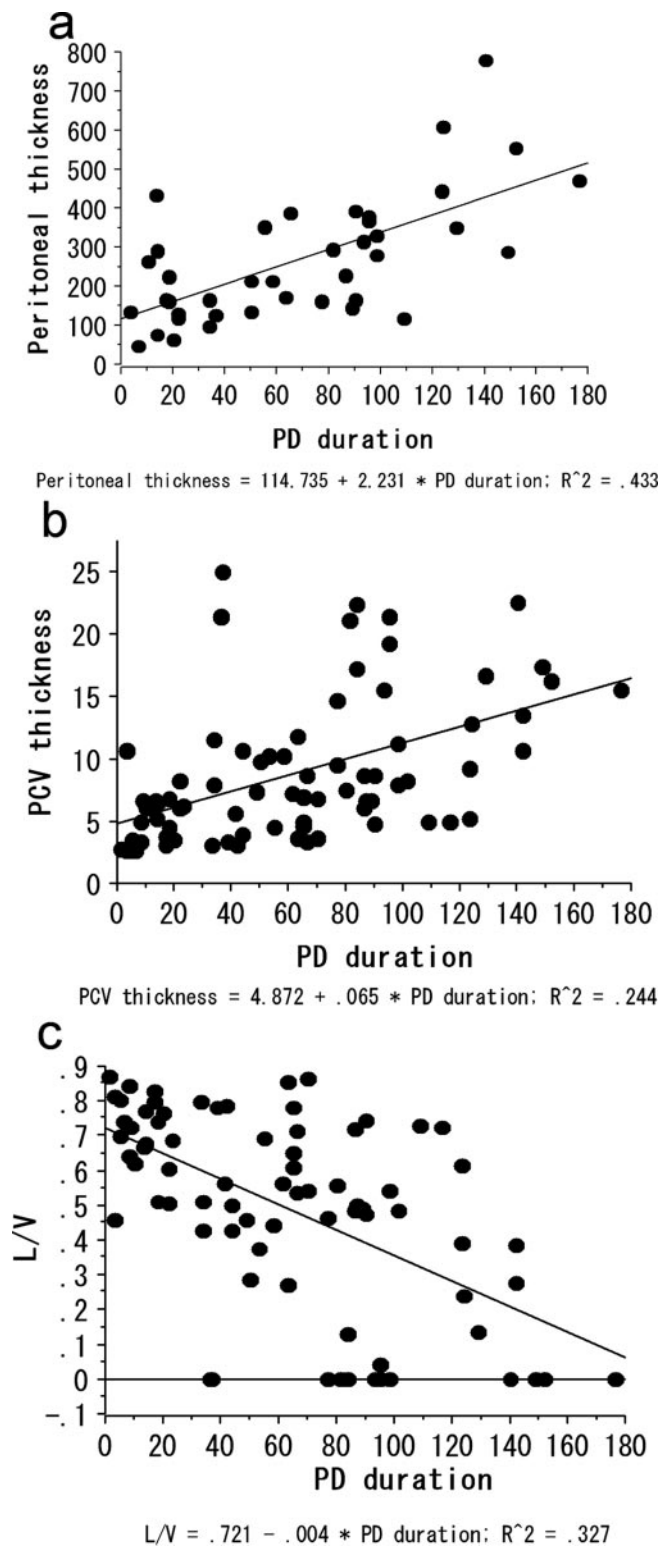
specimen. The average peritoneal thickness as calculated by the five-point measurement method gave us a better estimation of the severity of peritoneal fibrosis by avoiding a selection bias where we chose to measure the thickness. Using this method, we could show the difference of the average peritoneal thickness among normal, uremic, and PD patients with various PD durations. The average peritoneal thickness can be used as a quantitative parameter for the PD-related peritoneal fibrosis.

The method to quantify the severity of vasculopathy has been another concern for the histologic evaluation of the peritoneal alterations. In this study, we measured the wall thickness of the affected PCV and defined the L/V as an index of luminal opening (patency). The L/V was simply correlated with the ratio of luminal area ( $\pi L^2/4$ ) and total vascular area ( $\pi V^2/4$ ). We measured them in short axis of the vessel cut surface to avoid the artificial elongation of luminal and vascular diameter as a result of tangential cutting during the histologic preparation. We selected the PCV within the range of 25 to  $50 \mu\text{m}$  in external vessel diameter for the measurement because the L/V was easily influenced by the size of the vessel. Using this parameter, we could show differences in the severity of vasculopathy among normal, uremic, and PD patients with various PD durations and between uremic (pre-PD) patients with and without diabetes. We could also demonstrate a gradual decrease of the L/V as the PD duration prolonged; therefore, the L/V can be used as a quantitative parameter for PD-related vasculopathy.

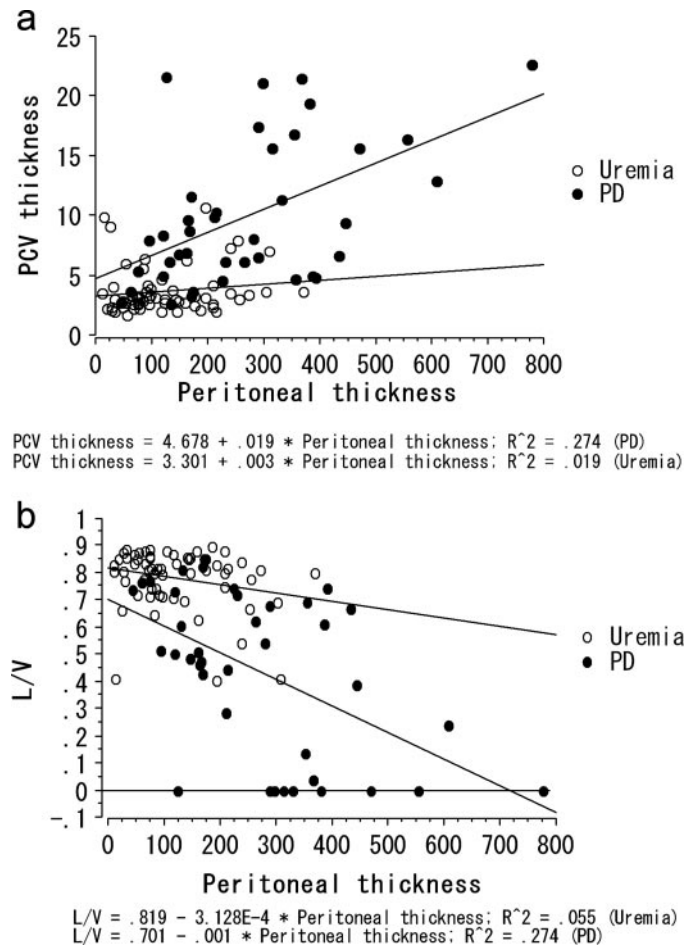
Each peritoneal specimen possessed multiple vessels in one section, and the extents of vasculopathy were heterogeneous in the same sample; therefore, the most severely affected vessel did not necessarily represent the dominant finding of the sample. Because of practical difficulty of evaluating all vessels in the specimen, the previous reports also selected the most severely affected vessel to evaluate vasculopathy (6). Sherif *et al.* (8) demonstrated a heterogeneity of the vasculopathy grade; however, they did not confirm the appropriateness of using the highest grade as a representative of the case. It seems to be better to evaluate all of the vessels and determine the average or dominant value of the quantitative evaluation. Further studies will be required to determine whether such a complicated procedure is necessary or can be simplified and to find the best way to evaluate the vasculopathy.

Using the standardized method, we confirmed that two factors other than PD treatment could affect the development of peritoneal sclerosis in PD patients: Uremia and diabetes. Previous studies demonstrated the presence of peritoneal fibrosis and vasculopathy in uremic pre-PD patients and suggested that the uremia contributed to the pathogenesis of peritoneal sclerosis (6,8). Similarly, we could also demonstrate the presence of peritoneal fibrosis and vasculopathy in the peritoneum before PD induction. This evidence clearly suggested an impact of uremia on the pathogenesis of peritoneal sclerosis.

In comparison of uremic (pre-PD) peritoneum between patients with and without diabetes, uremic patients with diabetes had more severe vasculopathy than uremic patients without diabetes, suggesting an additional adverse effect of diabetes on the development of hyalinizing vasculopathy in uremic perito-



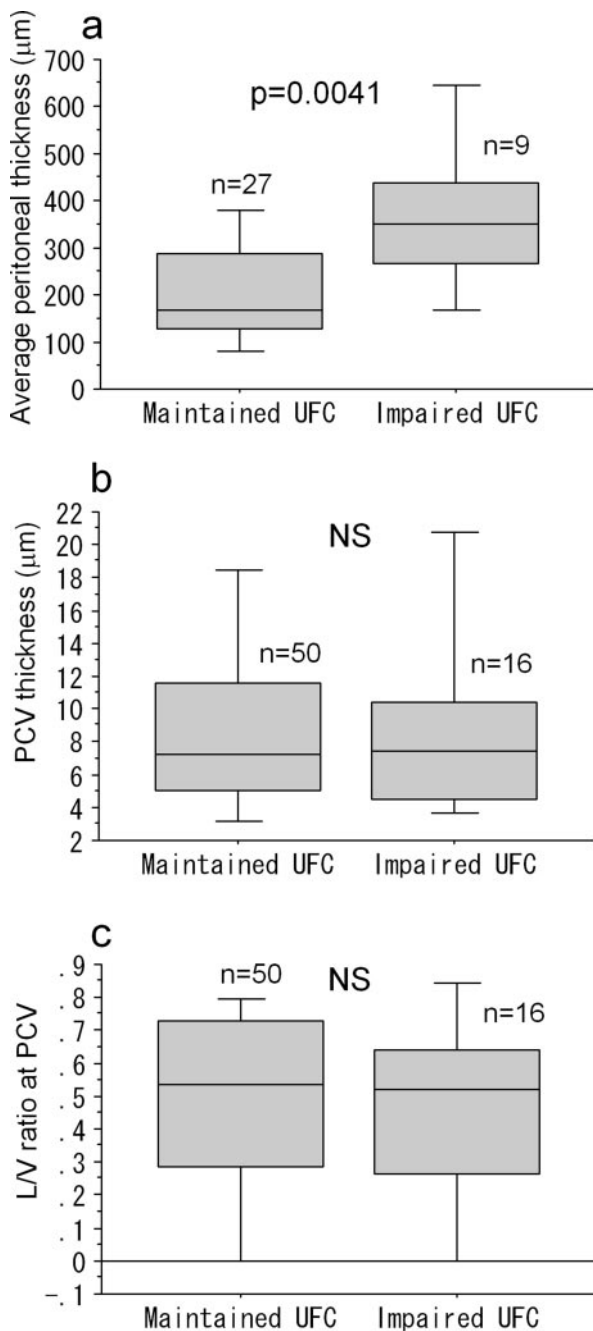
**Figure 9.** Correlation between PD duration and severity of peritoneal sclerosis in PD patients. (A) In PD patients, the APT was positively correlated with the PD duration, although its correlation coefficient was very low ( $y = 2.231x + 114.74$ ,  $R^2 = 0.433$ ,  $n = 40$ ;  $P < 0.0001$ ). (B) There was a positive correlation between PCV thickness and PD duration ( $y = 0.064x + 4.959$ ,  $R^2 = 0.243$ ,  $n = 76$ ;  $P < 0.0001$ ). (C) There was an inverse correlation between L/V at PCV and PD duration ( $y = -0.004x + 0.721$ ,  $R^2 = 0.327$ ,  $n = 76$ ;  $P < 0.0001$ ).



**Figure 10.** Correlation between peritoneal thickness and vasculopathy. (A) There was no significant correlation in uremic (pre-PD) patients ( $\circ$ ;  $n = 63$ ); however, a significant correlation was observed between APT and PCV thickness in PD patients ( $\bullet$ ;  $n = 38$ ;  $y = 4.678x + 0.019$ ,  $R^2 = 0.274$ ;  $P = 0.0005$ ). (B) Similarly, a significant correlation was observed between APT and L/V in PD patients ( $\bullet$ ;  $n = 38$ ;  $y = 0.701 - 0.001x$ ,  $R^2 = 0.274$ ;  $P = 0.0005$ ) but not in uremic (pre-PD) patients ( $\circ$ ;  $n = 63$ ).

neum. AGE and GDP are possible candidates for adverse effectors to the peritoneal membrane, because both of them are very common in uremia and diabetes in various organs, including peritoneum (9–16). In immunohistochemistry, AGE was broadly stained in interstitial fibrous tissue of uremic peritoneum and more intensely stained in PD peritoneum (9–13). The vessel walls of the PD peritoneum were also intensely positive for AGE staining (12,13); therefore, the diabetic condition may promote AGE formation in the peritoneal vessels and facilitate the progression of vasculopathy in pre-PD peritoneum.

The PD treatment itself is undoubtedly considered as a major deteriorating factor of the peritoneal sclerosis. The correlation between PD duration and the severities of peritoneal fibrosis and vasculopathy supported this hypothesis. High concentration of glucose, lactate, and GDP in conventional peritoneal dialysate induced an increased state of carbonyl stress on the peritoneum and promoted the development of peritoneal sclerosis (10,14–16). The reason that we could not demonstrate a



**Figure 11.** Comparison of peritoneal alterations between the patients with maintained and impaired ultrafiltration capacity (UFC). (A) The APT was significantly thicker in the group with impaired UFC ( $372.9 \pm 182.2 \mu\text{m}$ ,  $n = 9$ ) than in the group with maintained UFC ( $211.0 \pm 119.0 \mu\text{m}$ ,  $n = 27$ ;  $P = 0.0041$ ). (B) The average PCV thickness was not different between the group with maintained UFC ( $9.24 \pm 5.84 \mu\text{m}$ ,  $n = 50$ ) and the group with impaired UFC ( $9.22 \pm 6.02 \mu\text{m}$ ,  $n = 16$ ). (C) The L/V at PCV was not different between the group with maintained UFC ( $0.489 \pm 0.283$ ,  $n = 50$ ) and the group with impaired UFC ( $0.463 \pm 0.290$ ,  $n = 8$ ).

deteriorating effect of diabetes on the severity of peritoneal sclerosis after PD induction might be the relatively small number of the PD patients with diabetes in this study. It may also be

speculated that the exposure of peritoneal dialysate that contained a high concentration of glucose and GDP exerted an extremely stronger impact of carbonyl stress on the peritoneum, irrespective of presence or absence of diabetes, once the PD therapy had been initiated.

The peritoneal alterations in patients with impaired UFC were expected to be different from those of patients with maintained UFC. Our comparison revealed that the peritoneal fibrosis was significantly advanced in patients with impaired UFC; however, hyalinizing vasculopathy was not necessarily advanced in patients with impaired UFC. Usually, patients with impaired UFC had a relatively long history of PD treatment; therefore, we often observed advanced peritoneal fibrosis and vasculopathy in patients with impaired UFC. Our results suggested, however, that other factors may be required for the peritoneal membrane to become hyperpermeable and consequently result in ultrafiltration failure. Increased vascular density or vascular surface area and loss of barrier for water and solute transport through the peritoneal membrane are possible factors for the peritoneal hyperpermeability, and further studies are required to elucidate the morphologic and functional relationship in peritoneal disorders in PD patients.

A significant correlation between peritoneal fibrosis and vasculopathy was demonstrated in PD patients but not in pre-PD patients. In pre-PD patients, both peritoneal fibrosis and vasculopathy were in relatively mild degrees; therefore, no significant correlation was seen between two histologic factors. In contrast, when these alterations progressed after the PD treatment was introduced, a significant correlation between peritoneal fibrosis and vasculopathy became apparent, suggesting that these two pathologic events progressed concomitantly. We must wait further investigation to understand how these two factors influence each other in the pathogenesis of peritoneal sclerosis. Impaired blood circulation induced by hyalinizing vasculopathy might promote fibrogenic response to the cellular components of peritoneal interstitium. In addition, hyalinizing vasculopathy can occur not only in PCV but also in small artery. The influence of arterial vasculopathy on the development of peritoneal sclerosis is also important and should be analyzed in future studies.

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## Disclosures

None.

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