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Original Article

Peritoneal macrophage infiltration is correlated with baseline peritoneal solute transport rate in peritoneal dialysis patients

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Abstract

Background. High baseline peritoneal solute transport rate is reportedly associated with reduced patient and technique survival in continuous peritoneal dialysis (PD) patients. However, the determinants of baseline peritoneal solute transport rate remain uncertain. The aim of this study was to investigate the relationship between peritoneal local inflammation, angiogenesis and systemic inflammation and baseline peritoneal permeability.

Methods. Peritoneal biopsy specimens from 42 pre-dialysis uraemic patients and 11 control individuals were investigated. Immunohistochemistry for CD68-positive macrophages, chymase- and tryptase-positive mast cells, interleukin-6 (IL-6)-positive cells, CD3-positive T cells, CD20-positive B cells, neutrophils and CD31- and pathologische anatomie Leiden-endothelium (PAL-E)-positive blood vessels in the peritoneum was performed. Baseline dialysate-to-plasma ratio for creatinine (D/P Cr) was determined within 6 months of PD induction. Clinical and laboratory parameters were measured at the time of peritoneal biopsy. Factors associated with peritoneal permeability were assessed by multiple linear regression analysis.

Results. Pre-dialysis uraemic peritoneum showed infiltration by CD68-positive macrophages, and mast cells, as compared with controls. Baseline D/P Cr was correlated with density of CD68-positive macrophages ($P < 0.001$), IL-6-positive cells ($P < 0.001$), CD31-positive ($P < 0.05$) and PAL-E-positive blood vessels ($P < 0.05$) and serum albumin ($P < 0.05$). However, baseline peritoneal permeability was not correlated with infiltration by mast cells, B cells, T cells, neutrophils, serum C-reactive protein or other clinical factors. On multiple linear regression analysis, the number of CD68-positive macrophages in peritoneum was an independent predictor for baseline peritoneal permeability ($P = 0.009$).

Conclusions. Peritoneal macrophage infiltration is predominant in uraemic patients and is an important factor in predicting baseline peritoneal permeability.

Keywords: blood vessel; D/P Cr; inflammation; macrophage; mast cell

Introduction

Peritoneal dialysis (PD) is an important method for renal replacement therapy. However, the decrease in ultrafiltration capacity associated with high peritoneal transport seen after prolonged PD treatment is a major reason for discontinuation [1–3]. Several studies have shown that a higher peritoneal solute transport rate is also associated with reduced patient survival in PD patients [1,2,4]. Peritoneal solute transport rate in PD patients is determined by baseline peritoneal permeability and acquired factors after initiation of PD. In the latter, bioincompatibility of dialysis solutions with high glucose, high osmolality, advanced glycation end products, glucose degradation products and bacterial peritonitis including complement activation contributes to functional and histological alterations in the peritoneal membrane [5,6].

Approximately half of the patients (47.4 to 66.5%) are high or high-average peritoneal transporters, which may be a poor prognostic factor, from the beginning of PD [2,7]. However, factors that determine peritoneal transport status at the initiation of PD remain unclear. It is thus important to explore the mechanisms that determine baseline peritoneal permeability in order to establish new strategies for improving patient and technique survival. Previous studies have suggested that baseline peritoneal permeability is related to age, gender, diabetes, comorbid disease, ethnic origin and lower body mass index (BMI) [1,7,8]. Systemic inflammation and nutritional indices have consistently been shown to predict outcome in PD, just as they have in chronic kidney disease in general [9,10]. Some studies have suggested that an increased peritoneal solute transport rate is linked to malnutrition and inflammation [11,12], while other groups have reported that systemic inflammation is not associated with high baseline peritoneal transport rate [13, 14]. Thus, the relationship between sys-

temic inflammation and baseline peritoneal permeability has remained controversial, and although local intraperitoneal inflammation has been shown to affect baseline peritoneal permeability in studies measuring IL-6 concentration in PD effluent and peritoneal tissue [12,15,16], no detailed information on pathological peritoneal local inflammation and the extent of angiogenesis in these issues has been reported to date.

The aim of this study was therefore to investigate the relationship between pathological peritoneal local inflammation and vessel density and baseline peritoneal permeability. We performed immunohistochemistry to detect macrophages, mast cells, T cells, B cells, neutrophils, IL-6-positive cells and vessels positive for CD31 and pathologische anatomie Leiden-endothelium (PAL-E), which is a more specific marker of vascular endothelial cells than CD31 [17], in human frozen peritoneal tissues. In addition, we analysed the correlations between these markers and systemic inflammatory parameters. The peritoneal samples used in this study were obtained at initial catheter insertion in order to exclude any acquired effects by PD-related factors.

Materials and methods

Patient profiles and demographic data (Table 1)

A total of 53 peritoneal specimens biopsied between December 2005 and May 2010 at the Department of Nephrology and Renal Replacement Therapy of Nagoya University Hospital (Nagoya, Japan) or at affiliated hospitals, including Handa Municipal Hospital (Handa, Japan) and Yokkaichi Municipal Hospital (Yokkaichi, Japan), were studied. All patients were Japanese aged over 18 years. Peritoneal tissue samples were obtained from 42 pre-dialysis chronic renal failure patients at the time of PD catheter insertion ('pre-dialysis uraemic group'). Peritoneal biopsy samples with normal renal function ($n = 11$) were taken from living kidney donors with no history of abdominal surgery ('control group'). Collected data consisted of information on underlying causes of end-stage renal disease, demographic details (age, gender), comorbidity (presence of diabetes, hypertension, coronary artery disease, peripheral vascular disease and cerebrovascular disease) and measurements of height, weight, laboratory variables [serum albumin, C-reactive protein (CRP) and serum creatinine] and creatinine concentration ratios in dialysate and plasma (D/P Cr) at 4 h. Causes of renal failure were diabetic nephropathy ($n = 24$), chronic glomerulonephritis ($n = 10$) and nephrosclerosis ($n = 8$). Patients who had systemic inflammatory disease (e.g. vasculitis or pulmonary inflammatory disease) were excluded. BMI was calculated from height and weight data. Blood was taken before surgery in order to assess renal function [estimated glomerular filtration rate (eGFR)], systemic inflammation (CRP and serum albumin) and nutritional status (serum albumin). Markers were measured using the Creatinin-kit (Shino-test, Sagamihara, Japan), Albumin-kit (Shino-test) and CRP-kit (Denka Seiken, Niigata, Japan). eGFR was calculated as described previously [18]. Fast PET was performed within 6 months of induction of PD (range, 28–124 days; mean, 35.2 ± 16.3 days) using 2.27% glucose-based dialysis solution (Dianeal-N PD-4, Baxter), as described by Twardowski [19], and D/P Cr values at 4 h were defined as 'baseline D/P Cr', as described previously [8,13]. All patients were free from infections and catheter-related complications until PET was performed. All studies were approved by the ethics committee for Human Research of the Faculty of Medicine, Nagoya University (approval number 299, Nagoya, Japan), and all patients provided informed consent prior to participation in the study.

Processing of biopsy samples and immunohistochemistry

Parietal peritoneal samples were biopsied according to standard methods and were processed as reported previously [20,21]. Parietal peritoneum (5–10 × 10–20 mm) was obtained from the anterior abdominal wall. To avoid detachment of mesothelial cells, all procedures were carried out with due care. Immunostaining was performed on frozen tissues, and some of the tissues were embedded in OCT compound (Sakura Fine Technical, Tokyo, Japan), frozen in liquid nitrogen and stored at −80°C for immunohistochemistry. Immunohistochemistry was performed as described previously [22,23]. Briefly, 4-μm sections were cut with a cryostat, air-dried and fixed in acetone at room temperature for 10 min. Endogenous peroxidase activity was inhibited using 0.1% NaN_3 and 0.3% hydrogen peroxide in phosphate-buffered saline (PBS), and nonspecific protein-binding sites were blocked with normal goat serum (Dako, Glostrup, Denmark). Sections were then incubated with primary murine monoclonal antibodies against CD31 (JC/70A; Dako), PAL-E (Abcam, Cambridge, UK), CD68 (EBM11; Dako), mast cell chymase (Millipore, Temecula, CA), MC tryptase (AA1; Dako), mesothelial cells (HBME-1; Dako), interleukin-6 (IL-6; Lifespan Biosciences, Seattle, WA), CD3 (F7. 2. 38; Dako), CD20 (L26; Dako) and rabbit polyclonal antibodies against myeloperoxidase (Dako) overnight at 4°C. After washing with PBS, sections were treated with a conjugate of polyclonal goat anti-mouse IgG antibodies or goat anti-rabbit IgG antibodies and horseradish peroxidase-labelled polymer (Histofine Simple Stain; Nichirei, Tokyo, Japan) as secondary reagent. Enzyme activity was detected by 3-amino-9-ethyl-carbazole (Dako).

Morphological analysis

CD31-positive vessels, PAL-E-positive vessels, CD68-positive macrophages, chymase-positive mast cells, tryptase-positive mast cells, CD3-positive T cells, CD20-positive B cells and myeloperoxidase-positive neutrophils were identified and counted using Zeiss Z1 image microscopy and Axiovision Windows software version 4.4 (Carl Zeiss, Oberkochen, Germany). All tissue samples were evaluated under a microscope at ×200

Table 1. General characteristics and biochemical data

	Control group	Pre-dialysis uraemic group	P
Number	11	42	
Age (year)	56.1 ± 10.3	62.8 ± 13.9	0.092
Men (*n*, %)	5 (45.5%)	33 (78.6%)	0.056
BMI (kg/m^2)	24.9 ± 3.7	23.0 ± 2.8	0.144
Diabetes mellitus (*n*, %)	1 (9.1%)	24 (57.1%)	0.006
Hypertension (*n*, %)	5 (45.5%)	27 (64.3%)	0.310
Coronary artery disease (*n*, %)	0 (0%)	9 (21.4%)	0.177
Peripheral vascular disease (*n*, %)	0 (0%)	3 (7.1%)	0.490
Cerebrovascular disease (*n*, %)	0 (0%)	10 (23.8%)	0.098
eGFR (mL/min/1.73 m^2)	91.8 (64.2–102.1)	6.7 (5.2–7.5)	<0.001
Serum albumin level (g/dL)	4.04 ± 0.19	3.29 ± 0.73	<0.001
Serum CRP level (mg/dL)	0.07 (0.02–0.09)	0.20 (0.08–0.71)	0.006
D/P Cr	–	0.72 ± 0.13	–

Results are expressed as means ± SD or numbers (percent). Variables without a normal distribution are given as medians (interquartile range).

magnification in the submesothelial compact zone [26]. The submesothelial compact zone was identified as the membrane area extending from surface mesothelium down to the upper limit of the looser connective tissue containing widely spaced collagen fibres, vessels, fibroblast-like cells, mononuclear cells and adipose tissue [26]. At least 10 fields were assessed, even if the tissues were small fragments of the peritoneum. Densities of inflammatory cells and blood vessels were calculated and expressed as number per peritoneal surface length (/mm). IL-6 expression was analysed in the submesothelial compact zone, except in blood vessels. IL-6 expression in mesothelial cells was analysed and semiquantitatively classified into four groups based on positive staining intensity: (0) no staining, (1) mild staining, (2) moderate staining and (3) pronounced staining. Vasculopathy was classified as grades 0–4 according to the grading scale of Williams *et al.* [26], and the thickness of vascular wall and severity of luminal narrowing at the level of post-capillary venules were assessed using the method of Honda *et al.* [20]. Morphometric analyses were performed by two investigators blinded to the identity of the samples.

Statistical analysis

Shapiro–Wilk test was applied to test normal distributions. Variables without a normal distribution were either analysed by nonparametric test or transformed into the logarithmic scale and subjected to parametric test. Variables with a normal distribution are expressed as mean values ± SD, and asymmetrically distributed data are given as median and interquartile range. Comparisons between two groups were performed using Student's *t*-test or Mann–Whitney *U*-test. The chi-squared test was used for categorical variables. Correlation coefficients were calculated by Pearson correlation analysis. We performed additional analyses using the Bonferroni method to minimize the risk of type 1 statistical errors. This methodology compares the P-values of all (k) comparisons with an α-value of $0.05/k$; if P-values are $<0.05/k$, the association is considered to be statistically significant. Multiple linear regression analysis was also performed in order to examine the factors related to baseline peritoneal solute transport rate. Differences were considered to be statistically significant at $P < 0.05$. All analyses were performed using SPSS (Chicago, IL).

Results

Clinical characteristics (Table 1)

Pre-dialysis uraemic patients had significantly higher CRP concentrations and lower serum albumin concentrations than control individuals. End-stage renal failure was caused by diabetic nephropathy in 57.1% of cases. eGFR levels in the control group and the pre-dialysis renal failure group were 91.8 and 6.7 mL/min/1.73 m^2, respectively. There were no statistically significant differences between these two groups in other parameters.

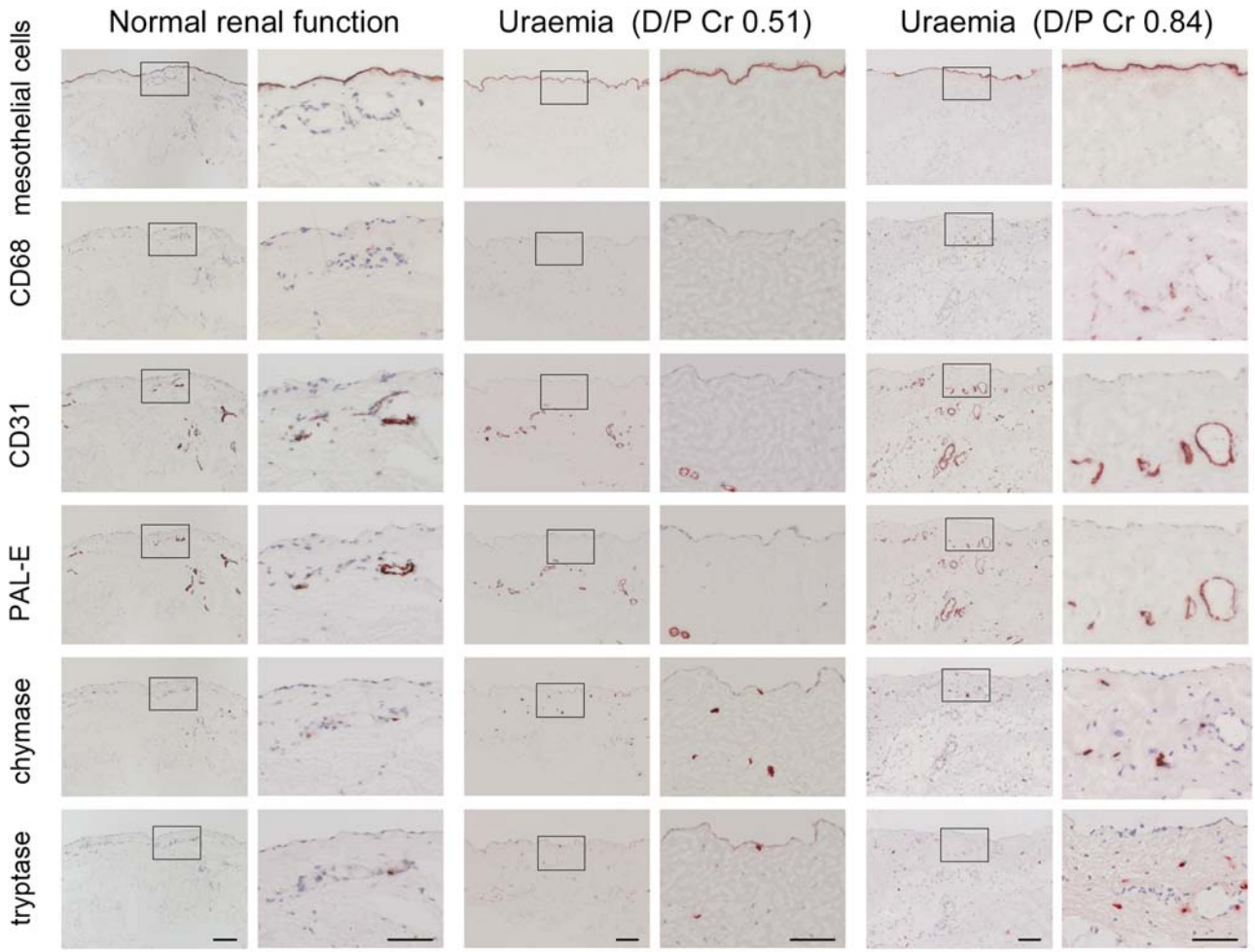

Fig. 1. Pathological findings in human peritoneum of controls and patients in the low-average and high categories. Immunohistochemistry for mesothelial cells, CD68, CD31, PAL-E, chymase and tryptase was performed in serial sections. CD68-positive cells and mast cells were scarcely detected in control peritoneum. CD68-positive cell infiltration and blood vessel density were predominant with higher peritoneal transport rates. Control peritoneum (normal renal function), PET low-average category (D/P Cr 0.51) uraemic peritoneum and PET high category (D/P Cr 0.84) uraemic peritoneum. Scale bars = 100 μm.

Immunohistochemistry for inflammatory cells and blood vessels

Mesothelial cells were observed as a monolayer covering the surface of the peritoneum in all peritoneal tissues (Figure 1). We evaluated the inflammatory cells (macrophages, mast cells, IL-6-positive cells, T cells, B cells and neutrophils) and blood vessel density (CD31 and PAL-E) in both control and pre-dialysis peritoneum (Figures 1 and 2). CD68-positive macrophages, CD31- and PAL-E-positive blood vessels and chymase- and tryptase-positive mast cells were predominant in the peritoneal membrane with high peritoneal solute transport rates; in contrast, these were scarcely detectable in control peritoneum (Figure 1). IL-6 was expressed in mesothelial cells, vessel walls and submesothelial cells, including CD68-positive macrophages (Figure 2A). The number of IL-6-positive cells outside of blood vessels in the submesothelial compact zone was greater in patients with high peritoneal permeability (Figure 2B–D). On the other hand, the extent of IL-6 expression in mesothelial cells tended to be elevated in the high permeability group, but the difference was not statistically significant (data not shown).

Infiltration of macrophages and mast cells was predominant in the uraemic peritoneum

The number of CD68-positive macrophages and chymase- and tryptase-positive mast cells in the peritoneum was significantly higher in pre-dialysis uraemic peritoneum than in control peritoneum. On vessel density analysis by CD31 and PAL-E staining, IL-6-positive cells, CD3-positive T cells and neutrophils were not higher in uraemic peritoneum than in control peritoneum. CD20-positive B cells were rarely detected in either control or uraemic peritoneum (Figure 3). No vasculopathy was observed in control peritoneum, and 10% of uraemic peritoneum showed grade 1 vasculopathy. There were no significant differences between control and uraemic peritoneum in the thickness of the vascular wall [3.43 (3.03–4.49) μm in control, 4.24 (3.46–4.80) μm in uraemic peritoneum; P = 0.079] and severity of luminal narrowing of postcapillary venules [0.78 (0.77–0.83) in control, 0.75 (0.69–0.80) in uraemic peritoneum; P = 0.069]. Immunostaining for CD31 and PAL-E was positive in grade 1 hyalinized vessels.

Predictors of baseline peritoneal solute transport rate

On pathological analysis, the number of CD68-positive macrophages, IL-6-positive cells and CD31- and PAL-E-positive blood vessels was significantly correlated with baseline solute transport rate (Figure 4A–D). We did not observe any correlations between tissue density of chymase-positive mast cells (P = 0.697), tryptase-positive mast cells (P = 0.253), CD3-positive T cells (P = 0.053), neutrophils (P = 0.573) and baseline D/P Cr. Serum albumin concentrations were correlated with baseline peritoneal solute transport rate. In contrast, serum CRP levels had no relationship with baseline peritoneal permeability (Figure 4E, F). The number of CD68-positive macrophages was also

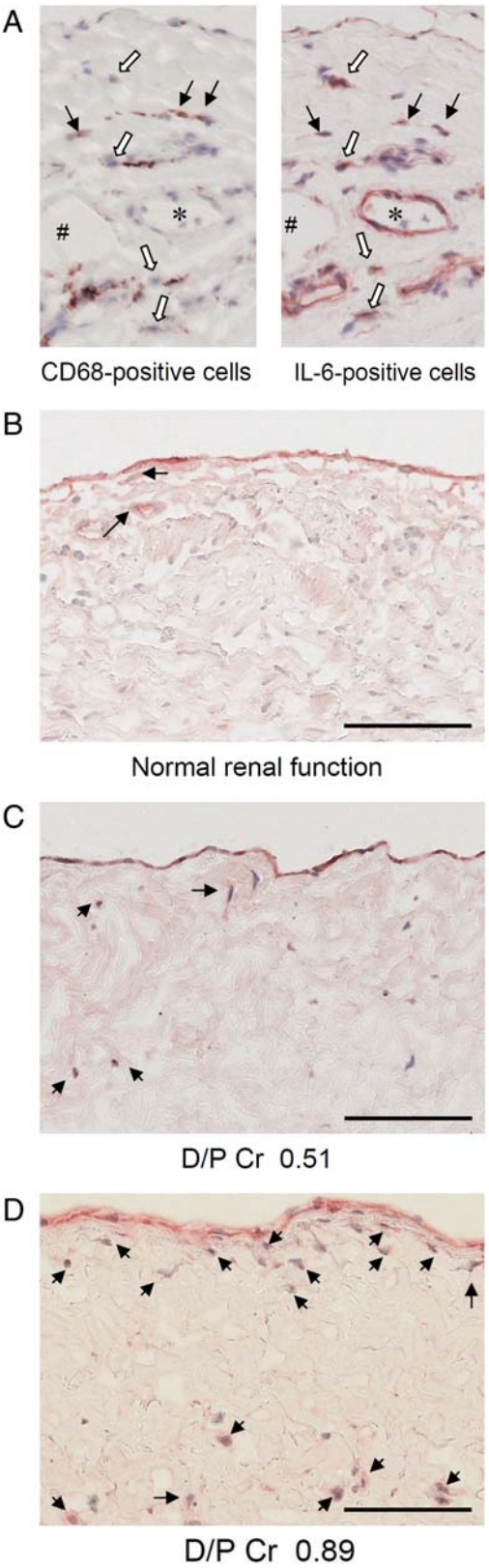

Fig. 2. Interleukin-6 (IL-6) expression in peritoneum. (**A**) IL-6 is expressed in vessels (*), CD68-positive cells (black arrows) and non-CD68-positive cells (open arrows). IL-6 staining is negative in some parts of the vessels (#). IL-6 expression in representative samples; (**B**) normal renal function, (**C**) low-average PET category (D/P Cr 0.51) and (**D**) high PET category (D/P Cr 0.89). Scale bars = 100 μm.

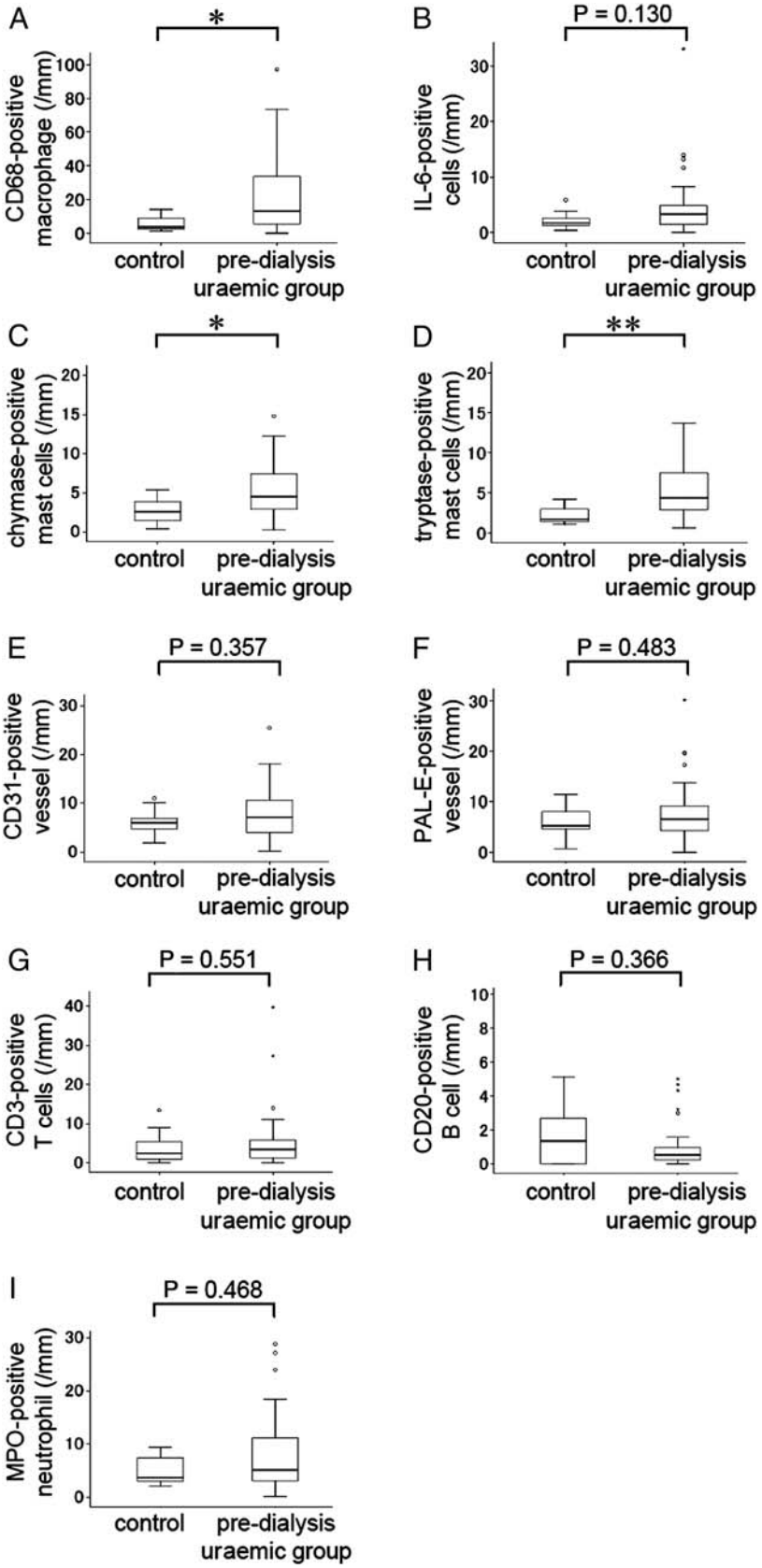

Fig. 3. Immunohistochemical analysis between control group vs pre-dialysis uraemic group. Number of CD68-positive macrophages (**A**) and chymase- (**C**) and tryptase- (**D**) positive mast cells in the peritoneum was significantly higher in pre-dialysis uraemic peritoneum than in control peritoneum. Vessel density based on both markers was not higher in uraemic peritoneum than in control peritoneum (**E**, **F**). IL-6-positive cells (**B**), CD3-positive T cells (**G**), CD-20 positive B cells (**H**), and neutrophils (**I**) were not higher in uraemic peritoneum than in control peritoneum. *$P < 0.05$, **$P < 0.005$.

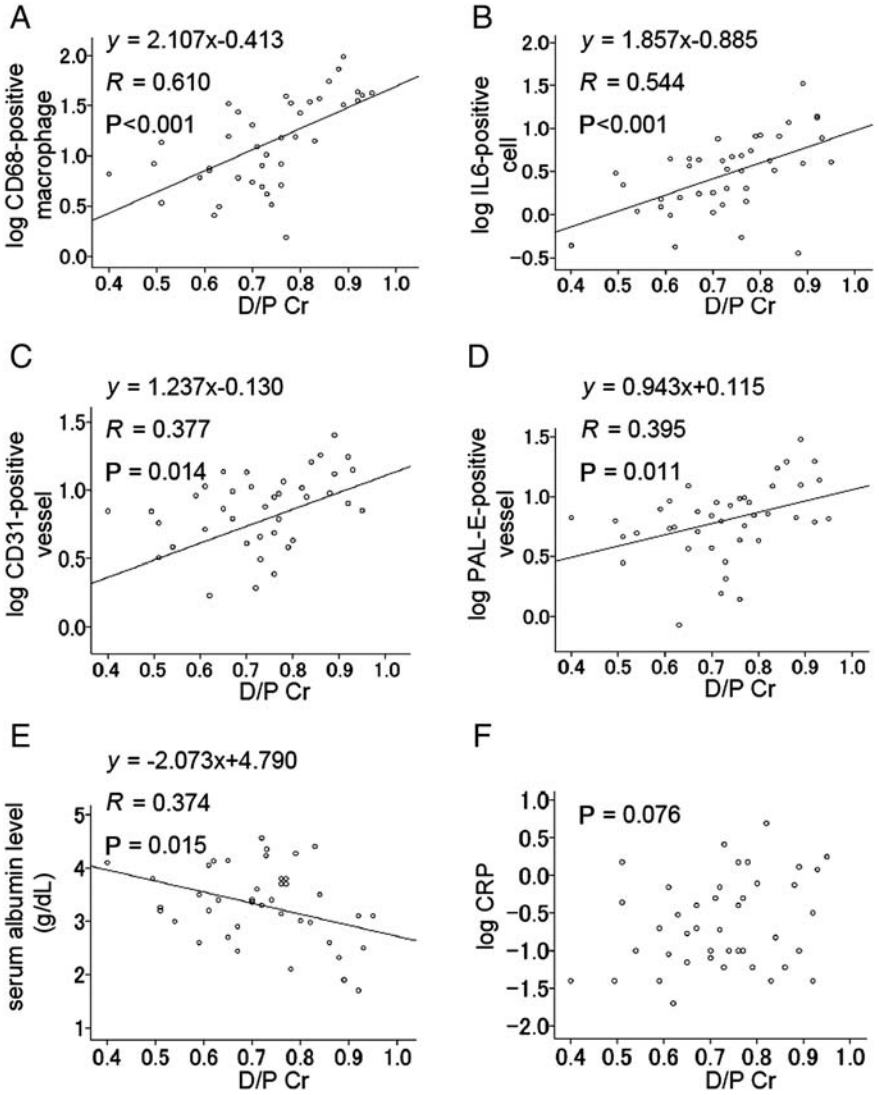

Fig. 4. Correlation between baseline D/P Cr and clinical and pathological parameters. (**A**) Number of CD68-positive cells was correlated with peritoneal solute transport rate. (**B**) Number of IL-6-positive cells in submesothelial compact zone other than vessels was correlated with baseline D/P Cr. (**C**, **D**) Positive correlation was observed between CD31- and PAL-E-positive blood vessels and baseline peritoneal transport D/P Cr. (**E**) Serum albumin concentrations were correlated with baseline peritoneal solute transport rate. (**F**) Serum CRP levels had no relationship with baseline peritoneal permeability.

correlated with CD31- and PAL-E-positive vessels, mast cells, IL-6-positive cells and serum albumin concentrations (Figure 5). Correlations between D/P Cr and all biochemical parameters or pathological parameters are summarized in Table 2. After applying Bonferroni correction, CD68-positive macrophages and IL-6-positive cells were correlated with baseline permeability (Table 2). On Student's *t*-test, there was no relationship between baseline D/P Cr and gender (P = 0.667), diabetes (P = 0.639), hypertension (P = 0.167), coronary artery disease (P = 0.394), peripheral vascular disease (P = 0.226) or cerebrovascular disease (P = 0.342) (Table 3). We then performed multiple regression analysis in order to identify factors that independently predict baseline peritoneal solute transport rate. CD31-positive vessels were excluded from the covariates because of co-linearity with PAL-E-positive vessels ($R = 0.864$, $P < 0.001$). Even after adjusting for other factors identified as having significant correlations with baseline D/P Cr, CD68-positive macrophages remained a significant factor in predicting baseline solute peritoneal transport rate (Table 4).

Comparison of baseline D/P Cr, inflammatory cell density, blood vessel density and clinical parameters between diabetic and non-diabetic uraemic patients (Table 5)

Finally, we analysed the differences in these markers between diabetic and non-diabetic uraemic patients. We did not find any significant differences in age between the diabetic and non-diabetic groups. There were no differences in baseline peritoneal solute transport rate (D/P Cr), serum CRP concentrations or density of macrophages, IL-6-positive cells, blood vessels, mast cells, lymphocytes and neutrophils between DM and non-DM patients. Only serum

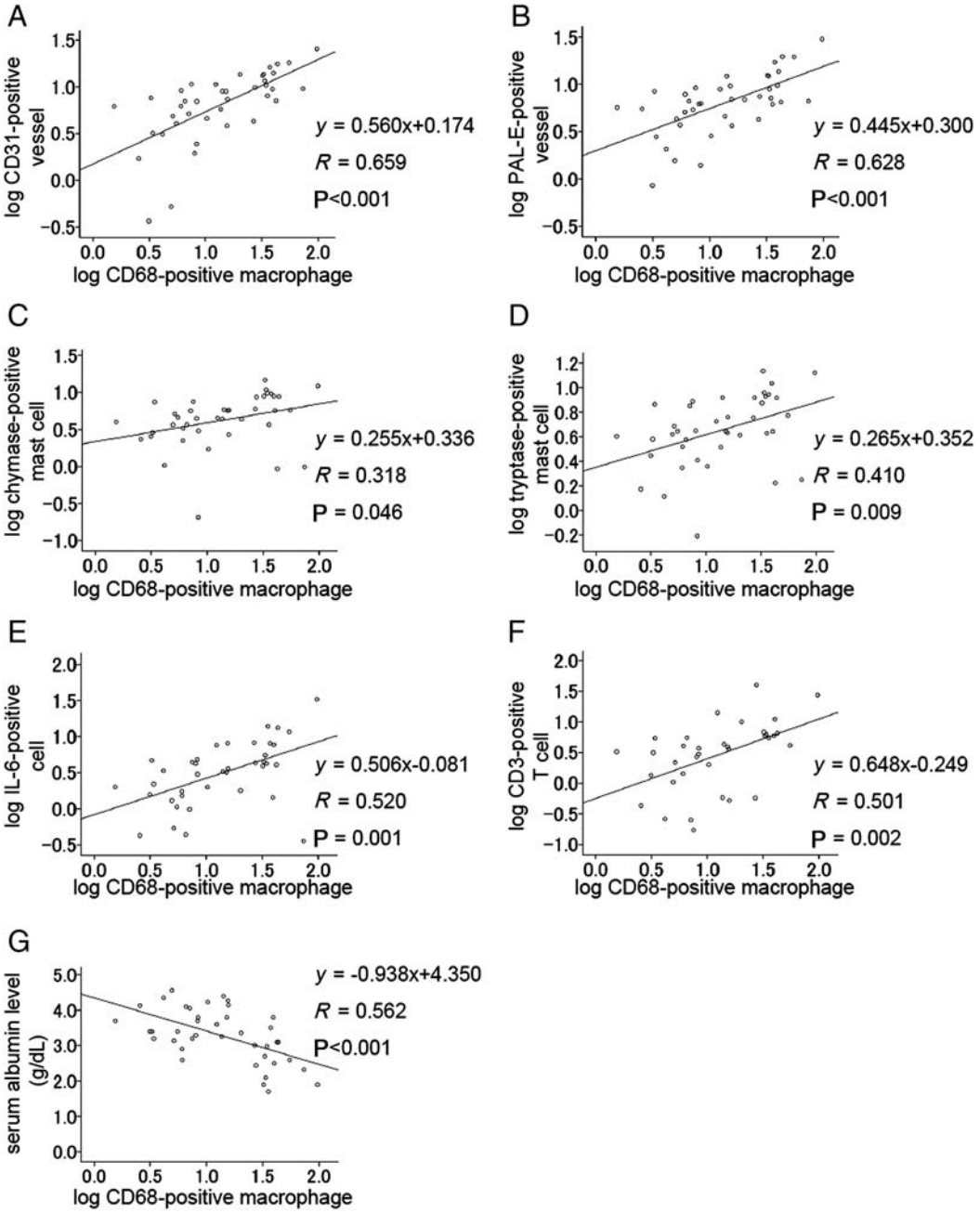

Fig. 5. Correlation between CD68-positive macrophages and clinical and pathological parameters. (**A**, **B**) A significant correlation was observed between CD68-positive macrophages and CD31-positive (A) and PAL-E-positive (B) vessels. (**C**, **D**) Correlation between chymase- and tryptase-positive mast cells and CD68-positive macrophages. (**E**, **F**) Number of IL-6-positive cells and CD3-positive T cells was correlated with CD68-positive macrophages. (**G**) Number of CD68-positive macrophage was correlated with serum albumin levels.

albumin levels were significantly higher in the non-diabetic group (P = 0.003).

Discussion

We investigated peritoneal local inflammation and blood vessel density in pre-dialysis uraemic patients and living kidney donors using immunohistochemical techniques and analysed the relationship between peritoneal local inflammation, systemic inflammation and vessel density and baseline peritoneal permeability. We used the D/P Cr data measured within 6 months after initiation of PD as 'baseline D/P Cr' [8,13], and this may have been slightly affected by short-term PD treatment. Indeed, eGFR was significantly higher in the control group, and prevalence of diabetes was higher in the uraemic group in a comparison between the normal renal function control and pre-dialysis uraemic groups. We found that the uraemic state is associated with significantly lower serum albumin levels, increased CRP and infiltration of CD68-positive macrophages and mast cells in the peritoneum (Table 1, Figure 3). However,

Table 2. Correlation between D/P Cr and biochemical or pathological parameters

	Age	BMI	eGFR	Alb	CRP	CD68	IL-6	CD31	PAL-E	Chymase	Tryptase	CD3	MPO
D/P Cr				−0.374*		0.610##	0.544##	0.377*	0.395*				
Age	–		0.375*										
BMI		–											
eGFR			–		0.340*								
Alb				–		−0.562##	−0.364*	−0.437**	−0.432**			−0.479**	
CRP					–								
CD68						–	0.520##	0.659##	0.628##	0.318*	0.410#	0.501**	
IL-6							–	0.441**	0.457**	0.313*	0.395*	0.363*	0.328*
CD31								–	0.864##	0.509##	0.568##	−0.446#	0.503**
PAL-E									–	0.586##	0.602##	0.483 **	0.347*
Chymase										–	0.953##		0.347 *
Tryptase											–		0.331 *
CD3												–	

Only statistically significant Pearson's correlation coefficients are shown (* <0.05, ** <0.005, # <0.01, ## <0.001). Statistically significant correlations indicated by underlining were determined using the Bonferroni correction method. Variables without normal distribution (eGFR, CRP, CD68, IL-6, CD31, PAL-E, chymase, tryptase, CD3 and MPO) were logarithmically transformed.

Table 3. Comparison of baseline D/P Cr and demographic data and co-morbidities

Variable	Baseline D/P Cr		P
	+	−	
Male gender	0.72 ± 0.12	0.74 ± 0.18	0.667
Diabetes	0.73 ± 0.14	0.71 ± 0.13	0.639
Hypertension	0.75 ± 0.13	0.69 ± 0.13	0.167
Coronary artery disease	0.76 ± 0.10	0.72 ± 0.14	0.394
Peripheral vascular disease	0.81 ± 0.16	0.72 ± 0.13	0.226
Cerebrovascular disease	0.76 ± 0.12	0.71 ± 0.13	0.342

Note: Results are expressed as means ± SD.

no association with blood vessel density was noted (Figure 3). These findings suggest that uraemia is linked with systemic inflammation and local peritoneal inflammation but does not directly contribute to angiogenesis in the peritoneum. Analysis of the pre-dialysis uraemic peritoneum clearly showed that the number of CD68-positive macrophages and IL-6-positive cells, as well as CD31-positive cells and PAL-E-positive blood vessel density, was correlated with baseline peritoneal permeability (Figure 4). However, there was no relationship between the number of mast cells, CD3-positive T cells and neutrophils and baseline D/P Cr. On multiple linear regression analysis, macrophage infiltration remained an independent factor for predicting baseline D/P Cr (Table 4). In addition, there was a significant correlation between peritoneal macrophage infiltration and blood vessel density (Figure 5). High peritoneal solute transport rates in association with angiogenesis may be linked with local macrophage infiltration. Vascular endothelial growth factor and IL-6, which are detected in PD effluent and may be produced by macrophages and mesothelial cells, are thought to play a role in the regulation of vascular density and permeability in the uraemic peritoneum [12,15,27–34].

The relationship between systemic inflammation and peritoneal permeability remains controversial [12–15, 35,36], and the interaction between systemic inflammation and peritoneal pathological findings has not been elucidated to date. Albumin is a widely accepted marker of inflammation and nutritional status and is a predictor of PD or CKD patient outcome [37]. In this analysis, serum albumin levels at catheter insertion were weakly correlated with baseline peritoneal transport rate (Figure 4). In addition, low serum albumin concentrations were associated with infiltration by peritoneal macrophages and IL-6-positive cells (Table 2, Figure 5, Supplementary Figure 1). Our results suggest that systemic inflammation is related to peritoneal local macrophage infiltration and peritoneal permeability. In contrast, serum CRP levels were not related to either baseline solute peritoneal transport rate or other pathological parameters (Table 2, Supplementary Figure 1). However, we did not use high-sensitivity CRP assay and analysed only CRP and albumin as systemic inflammatory parameters, which may have affected our results. The fact that dialysis patients in Asian countries reportedly have lower levels of CRP may also affect the present observations [10,38–40]. Taken together, the present data suggest that systemic inflammation has a

Table 4. Multiple linear regression analysis of factors associated with D/P Cr (R = 0.676, P < 0.001)

	Regression coefficient	95% CI	β	P
Serum albumin level (g/dL)	−0.010	−0.063–0.042	−0.062	0.691
CD68-positive macrophages (log scale)	0.144	0.039–0.249	0.517	0.009
IL-6-positive cells (log scale)	0.074	−0.014–0.162	0.260	0.095
PAL-E-positive vessels (log scale)	−0.032	−0.165–0.101	−0.081	0.631

CI, confidence interval.

Table 5. Comparison between diabetic and non-diabetic patients in age, baseline peritoneal transport D/P Cr, systemic factors and peritoneal pathological factors

	Diabetics	Non-diabetics	P
Number	24	18	
Age (year)	62.0 ± 13.7	64.6 ± 15.1	0.468
Men (*n*, %)	20 (83.3%)	12 (66.7%)	0.168
BMI (kg/m^2)	23.3 ± 2.7	22.4 ± 3.0	0.174
D/P Cr	0.73 ± 0.14	0.71 ± 0.13	0.639
eGFR (mL/min/1.73 m^2)	6.5 (5.1–7.7)	7.0 (5.2–8.0)	0.703
Serum albumin level (g/dL)	3.03 ± 0.74	3.66 ± 0.55	0.003
Serum CRP level (mg/dL)	0.20 (0.06–1.08)	0.18 (0.09–0.71)	0.889
CD68-positive macrophages (/mm)	13.9 (5.2–34.3)	11.3 (6.2–29.5)	0.959
IL-6-positive cells (/mm)	4.1 (1.3–7.2)	3.1 (1.4–4.4)	0.374
CD31-positive vessels (/mm)	8.5 (3.9–12.8)	7.1 (4.0–9.5)	0.360
PAL-E-positive vessels (/mm)	7.1 (4.7–12.28)	6.4 (3.5–8.6)	0.286
Chymase-positive mast cells (/mm)	5.4 (3.3–8.8)	4.0 (2.4–5.7)	0.140
Tryptase-positive mast cells (/mm)	4.7 (3.3–8.3)	4.1 (2.5–5.7)	0.227
CD3-positive cells (/mm)	3.8 (1.4–6.0)	2.9 (0.7–5.8)	0.460
MPO-positive cells (/mm)	4.9 (3.7–11.2)	5.2 (1.1–13.7)	0.723
PCV thickness (μm)	4.2 (3.5–4.6)	4.5 (3.4–5.0)	0.622

Results are expressed as means ± SD or number (percent). Variables without normal distribution are given as median (interquartile range). MPO, myeloperoxidase; PCV, post-capillary venule.

lower impact on peritoneal permeability than peritoneal local macrophage infiltration.

Mast cells are thought to participate in the inflammatory response and fibrosis through their ability to secrete various inflammatory mediators [41,42], and some mast cell mediators are known to promote angiogenesis [42]. Tryptase may contribute to vascular permeability by direct or indirect generation of bradykinin from kininogens [43]. Chymase may also play a role in tissue fibrosis through angiotensin II generation and TGF-β activation [41]. Interestingly, vasculature quantity and thickness in omentum after exposure to high-glucose PD fluid were significantly reduced in mast cell-deficient rats when compared with wild-type rats [44]. Mast cells maturing in different tissue microenvironments can vary widely in the types and amounts of tryptases and chymases expressed [45]. Therefore, we analysed both tryptase-positive mast cells and chymase-positive mast cells in normal and uraemic peritoneum. However, in the peritoneal membrane, mast cell infiltration is not likely to be an important factor in baseline peritoneal permeability, nor is the presence of T cells, B cells and neutrophils, as compared with macrophages (Figure 4).

Numerous other factors may be involved in determining the baseline peritoneal permeability of PD patients. There are many reports supporting the notion that diabetes affects peritoneal transport rate [46,47]. On the other hand, it has also been reported that high peritoneal transport status is not linked with the presence of diabetes [8,48,49]. In our analysis, we found that diabetic patients had significantly lower serum albumin levels than non-diabetic patients; however, there were no differences in peritoneal permeability, density of inflammatory cells and blood vessels and CRP levels between the two groups (Table 5). Taken together, these data suggest that diabetes is not the major determinant of baseline peritoneal transport.

In conclusion, we showed that local macrophage infiltration is associated with uraemia and baseline high peritoneal solute transport. Further *in vivo* and *in vitro* studies are required in order to clarify the role of local macrophage infiltration in baseline peritoneal membrane conditions for PD.

Supplementary data

Supplementary data is available online at http://ndt.oxfordjournals.org.

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Conflict of interest statement. None declared.

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