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# Predictors of culture-negative peritoneal dialysis-associated peritonitis: a single center, retrospective study

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## Abstract

**Background** Empirical antibiotic treatment against peritoneal dialysis (PD)-related peritonitis should be immediately initiated before PD effluent culture results are obtained. As culture results guide the choice of antibiotics, culture-negative peritonitis (CNP) is a serious issue. In addition, the identification of the causative organism often indicates a possible source of infection. This study aimed to clarify the predictors of CNP.

**Methods** This single-center, retrospective study was conducted from November 2007–December 2018 in patients undergoing PD with peritonitis at our institution, where 204 peritonitis episodes (57 culture-negative, 147 culture-positive) were investigated based on demographics, and clinical parameters. CNP predictors were investigated using logistic regression.

**Results** CNP rate was significantly higher in female and in patients with higher platelet counts, lower dialysate cell counts at peritonitis diagnosis, and higher serum  $\beta_2$ -microglobulin levels. In multivariate logistic regression, female sex (odds ratio [OR] 2.69, 95% confidence interval [CI] 1.31–5.54), dialysate cell count at diagnosis (OR 0.99, 95% CI 0.99–0.99), and serum  $\beta_2$ -microglobulin level (OR 1.04, 95% CI 1.00–1.07) were significantly associated with CNP. The areas under the receiver operating characteristic curve for female patients, dialysate cell counts at diagnosis of peritonitis, serum  $\beta_2$ -microglobulin level, and female patients + dialysate cell counts at diagnosis of peritonitis + serum  $\beta_2$ -microglobulin level were 0.604, 0.694, 0.603, and 0.751, respectively.

**Conclusions** Female sex, dialysate cell counts at peritonitis diagnosis, and serum  $\beta_2$ -microglobulin levels may be predictors of CNP.

**Keywords** Culture-negative peritonitis, Peritoneal dialysis, Gender, Dialysate cell counts,  $\beta_2$ -microglobulin

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## Background

Peritonitis is a major cause of morbidity and mortality in patients undergoing peritoneal dialysis (PD) [1]. Empirical antibiotic treatment against both gram-positive and gram-negative microorganisms should be immediately initiated before PD effluent culture results are obtained. As culture results guide the choice of antibiotics, culture-negative peritonitis (CNP) is a serious issue. In addition, the identification of the causative organism often indicates a possible source of infection. The International Society for Peritoneal Dialysis (ISPD) guidelines recommend that the CNP rate should not exceed 15% [2]. However, few studies have reported the predictors of CNP [3–7]. The objective of this study was to identify the predictors of CNP.

## Methods

### Study population

Based on medical records, there were 243 episodes of PD-associated peritonitis between November 2007 and December 2018 at our institution, of which we excluded 39 episodes: 27 episodes had PD-related peritonitis within 3 months after PD initiation; five episodes, malignancy; four episodes, peritonitis with other infections; one episode, liver cirrhosis; one episode, interferon therapy; and, one episode, immunosuppression therapy. Finally, 204 episodes were registered in our database and have been previously reported [8]. In this study, we used this database to investigate predictors of culture-negative PD-associated peritonitis. CNP was defined by clinical features including peritonitis, dialysate leukocytosis (white blood cell count  $>100/\mu\text{L}$  with neutrophils  $>50\%$ ), and negative dialysate culture [2]. This study was approved by the Ethical Committee of our institution (approval no. 1129) and was conducted in accordance with the principles of the Declaration of Helsinki and Japanese ethical guidelines.

### Data collection

Data including age, sex, body mass index, number of peritonitis episodes per patient-year, recent antibiotic use within 30 days, microorganisms in dialysate cultures, presence of diabetes mellitus and cardiovascular disease, body temperature, blood pressure, and laboratory data at the time of visit diagnosed with peritonitis were collected by reviewing the patients' medical records. Laboratory data included white blood cell count; hemoglobin, platelet, total protein, serum albumin, serum aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, uric acid, sodium, potassium, corrected calcium, phosphate, and C-reactive protein levels; dialysate cell counts; dialysate and plasma creatinine ratio; Kt/V

urea per week; and  $\beta_2$ -microglobulin level. The second dialysate cell count was evaluated 3–5 d after initiating antibiotics.

### PD effluent culture

We inoculated 5 to 10 mL of PD effluent in two (aerobic and anaerobic) blood culture bottles, as recommended by the ISPD guidelines [2]. Then, we transfer the inoculated bottles to the microbiology laboratories outside the hospital.

### Statistical analysis

Data are expressed as mean  $\pm$  standard deviation or median (interquartile range), as appropriate. Categorical variables were evaluated using the chi-squared test or Fisher exact test. Continuous variables were compared using Student's *t* test or Mann–Whitney *U* test, as appropriate. A multivariate logistic regression analysis was performed to determine the independent variables associated with CNP. Odds ratios (ORs) were calculated with their respective 95% confidence intervals (CIs). Receiver operating characteristic (ROC) curves were used to evaluate the strength of the predictive factors. All analyses were performed using JMP, version 16 (SAS Institute Inc., Cary, NC, USA).  $P < 0.05$  was considered to be statistically significant.

## Results

### Patients' clinical and laboratory characteristics

There were 57 culture-negative among a total of 204 peritonitis episodes. The baseline clinical and laboratory characteristics of the patients undergoing PD having culture-negative and culture-positive peritonitis are listed in Tables 1 and 2. Compared with culture-positive peritonitis, CNP episodes were significantly more frequent among female patients (43.9 vs. 23.1%;  $p = 0.0055$ ) and patients with higher platelet counts ( $23.7 \times 10^4/\mu\text{L}$  [ $19.6 \times 10^4/\mu\text{L}$ – $30.2 \times 10^4/\mu\text{L}$ ] vs.  $20.3 \times 10^4/\mu\text{L}$  [ $16.3 \times 10^4/\mu\text{L}$ – $26.4 \times 10^4/\mu\text{L}$ ];  $p = 0.0102$ ), lower dialysate cell counts at peritonitis diagnosis ( $724/\mu\text{L}$  [ $354/\mu\text{L}$ – $1601/\mu\text{L}$ ] vs.  $1959/\mu\text{L}$  [ $709/\mu\text{L}$ – $5230/\mu\text{L}$ ];  $p < 0.0001$ ), and higher serum  $\beta_2$ -microglobulin levels ( $29.8 \mu\text{g/L}$  [ $24.1$ – $34.9 \mu\text{g/L}$ ] vs.  $26.5 \mu\text{g/L}$  [ $19.9$ – $33.2 \mu\text{g/L}$ ];  $p = 0.0235$ ). No significant differences were found among the other characteristics.

### Initial antibiotic regimens

The initial antibiotic regimens for CNP and culture-positive peritonitis are shown in Fig. 1. All CNP episodes were treated with at least two antibiotics. 47 peritonitis episodes were treated with either cefazolin or vancomycin in combination with aminoglycosides.

**Table 1** Clinical characteristics in PD patients with culture-negative or culture-positive peritonitis

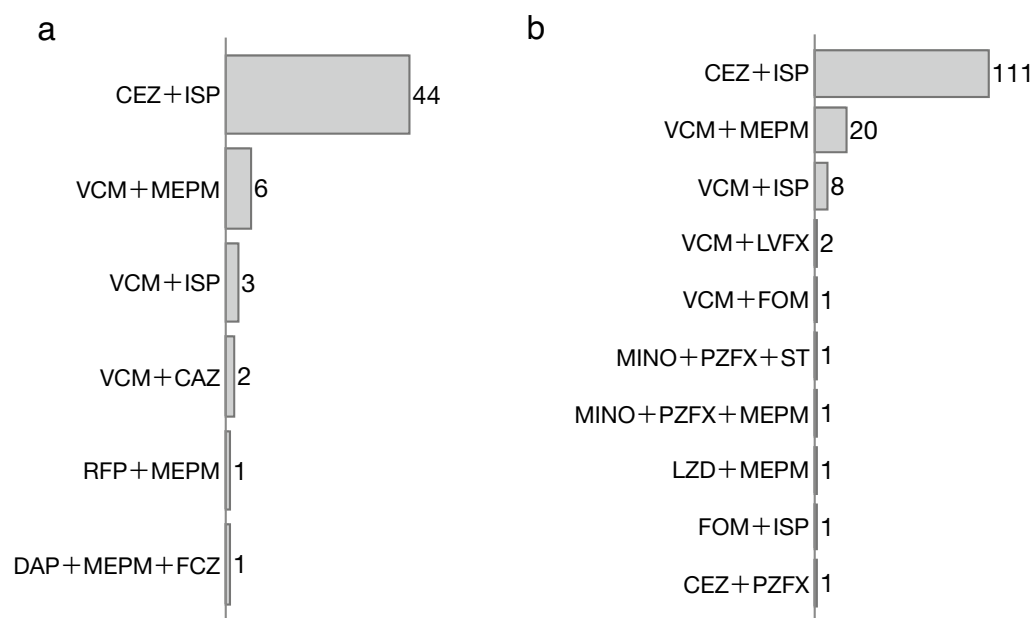
Variables	Culture negative (n = 57)	Culture positive (n = 147)	p
Female, n (%)	25 (43.9)	34 (23.1)	0.0055*
Age at initiation (years)	62.0 ± 13.3	62.6 ± 12.2	0.77
Age at peritonitis (years)	64.7 ± 13.2	65.2 ± 11.9	0.87
Dialysis Duration (days)	747 [287–1554]	644 [320–1329]	0.58
Past history of peritonitis, n (%)	43 (75.4)	92 (62.6)	0.10
Frequency of peritonitis/years from PD initiation	1.1 [0.52–3.5]	1.3 [0.49–2.0]	0.60
Recent antibiotic therapy within 30 days, n (%)	17 (29.8)	29 (19.7)	0.14
Automated PD, n (%)	5 (8.8)	11 (7.5)	0.77
Diabetes mellitus, n (%)	21 (36.8)	74 (50.3)	0.088
Cardiovascular disease, n (%)	22 (38.6)	62 (42.2)	0.75
Body mass index (kg/m <sup>2</sup> )	24.8 ± 3.7	23.7 ± 4.1	0.13
Body temperature (°C)	36.8 [36.4–37.1]	36.8 [36.5–37.4]	0.070
Systolic blood pressure (mmHg)	129 [115–151]	125 [104–148]	0.36
Diastolic blood pressure (mmHg)	78 [64–88]	75 [62–88]	0.53
Primary cause of end-stage renal disease			
Diabetic nephropathy, n (%)	19 (33.3)	66 (44.9)	
Chronic glomerulonephritis, n (%)	10 (17.5)	20 (13.6)	
Other, n (%)	4 (7.0)	21 (14.3)	
Unknown, n (%)	24 (42.1)	40 (27.2)	

\*p &lt; 0.05 when comparing Culture-negative versus Culture-positive group

**Table 2** Laboratory characteristics in PD patients with culture-negative or culture-positive peritonitis

Variables	Culture negative (n = 57)	Culture positive (n = 147)	p
White blood cell (/μL)	8500 [6100–11650]	8100 [5800–10900]	0.65
Hemoglobin (g/dL)	10.7 ± 1.7	10.7 ± 1.6	0.43
Platelet (× 10 <sup>4</sup> /μL)	23.7 [19.6–30.2]	20.3 [16.3–26.4]	0.0102*
Urea nitrogen (mg/dL)	43.0 [35.0–54.4]	43.7 [35.5–52.8]	0.71
Creatinine (mg/dL)	8.6 ± 2.5	8.8 ± 3.1	0.56
Total protein (g/dL)	6.0 ± 0.85	6.0 ± 0.89	0.92
Albumin (g/dL)	2.9 [2.6–3.2]	3.0 [2.5–3.3]	0.76
Aspartate aminotransferase (U/L)	20 [17–28]	19 [15–24]	0.14
Alkaline phosphatase (U/L)	207 [159–301]	258 [189–317]	0.091
Lactate dehydrogenase (U/L)	228 [183–296]	216 [187–252]	0.12
Uric acid (mg/dL)	5.6 ± 1.5	5.3 ± 1.2	0.27
Sodium (mmol/L)	137 [134–140]	138 [135–140]	0.36
Potassium (mmol/L)	3.9 [3.5–4.5]	3.9 [3.4–4.4]	0.31
Corrected calcium (mg/dL)	9.5 ± 0.71	9.3 ± 0.74	0.15
Phosphate (mg/dL)	4.5 [3.7–5.4]	4.1 [3.4–5.1]	0.15
C-reactive protein (mg/dL)	1.4 [0.51–3.4]	1.5 [0.48–4.9]	0.85
Cell counts of dialysate at diagnosis of peritonitis (/μL)	724 [354–1601]	1959 [709–5230]	< 0.0001*
Cell counts of dialysate after antibiotic administration (/μL)	86 [20–216]	91 [27–470]	0.14
β <sub>2</sub> -microglobulin (μg/L)	29.8 [24.1–34.9]	26.5 [19.9–33.2]	0.0235*
D/P Cr	0.69 ± 0.09	0.69 ± 0.10	0.86
Kt/V urea per week	1.8 [1.4–2.2]	2.0 [1.8–2.4]	0.35

\* p &lt; 0.05 when comparing Culture-negative versus Culture-positive group



**Fig. 1** Initial antibiotic regimens of **a** CNP, and **b** culture-positive peritonitis. CEZ, cefazoline; ISP, isepamicin sulfate; VCM, vancomycin; MEPM, meropenem; CAZ, ceftazidime; RFP, rifampicin; DAP, daptomycin; FCZ, fluconazole; LVFX, levofloxacin; FOM, fosfomycin; MINO, minocycline; PZFX, pazufloxacin; ST, sulfamethoxazole trimethoprim; LZD, linezolid

**Table 3** Clinical outcomes in PD patients with culture-negative or culture-positive peritonitis

Variables	Culture negative (n = 57)	Culture positive (n = 147)	p
PD withdrawal, n (%)	9 (15.8)	29 (19.7)	0.69
Death, n (%)	2 (3.5)	2 (1.4)	0.31
Cause of death, n (%)	2 (100)	2 (100)	

### Outcomes of culture-negative peritonitis

The PD withdrawal and mortality rates were similar between culture-negative and culture-positive peritonitis (15.8 vs. 19.7%;  $p=0.69$ , 3.5 vs. 1.4%;  $p=0.31$ , respectively) episodes. All deaths were attributed to peritonitis (Table 3).

### Predictors of culture-negative peritonitis

We performed a multivariate logistic regression analysis to investigate the predictors of CNP. Female sex (OR 2.69, 95% CI 1.31–5.54), dialysate cell counts at peritonitis diagnosis (OR 0.99, 95% CI 0.99–0.99), and serum  $\beta_2$ -microglobulin level (OR 1.04, 95% CI 1.00–1.07) were significantly associated with CNP (Table 4). Furthermore, we evaluated the strength of the predictors of CNP by using ROC curve analysis. The areas under the ROC curve for female patients, dialysate cell counts at diagnosis of peritonitis, serum  $\beta_2$ -microglobulin level, and female patients + dialysate cell counts at

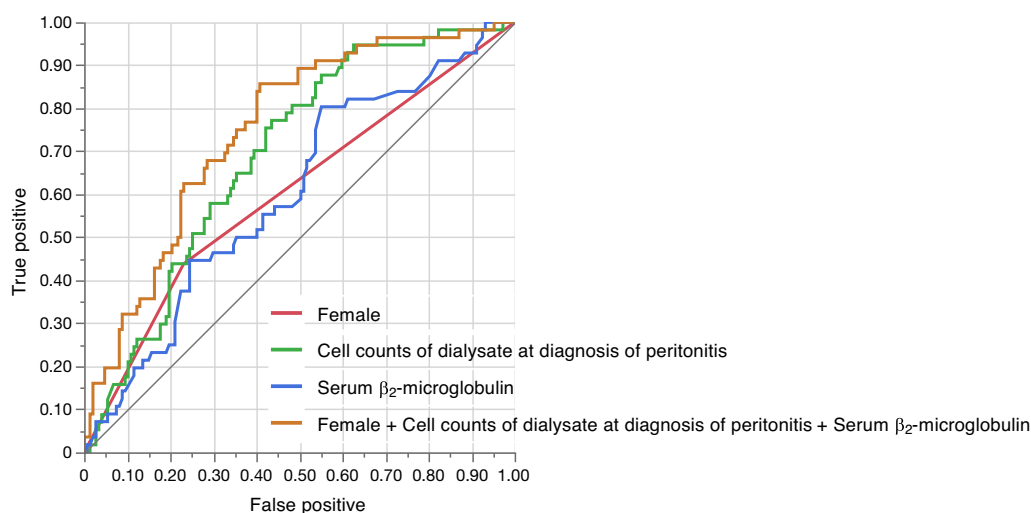
**Table 4** Multivariate logistic regression analyses of predictors for culture-negative peritonitis

Variables	OR (95% CI)	p
Female, n (%)	2.69 (1.31–5.54)	0.0072*
Platelet ( $\times 10^4/\mu\text{L}$ )	1.03 (0.99–1.07)	0.156
Cell counts of dialysate at diagnosis of peritonitis ( $/\mu\text{L}$ )	0.99 (0.99–0.99)	<0.0001*
Serum $\beta_2$ -microglobulin ( $\mu\text{g/L}$ )	1.04 (1.00–1.07)	0.0365*

diagnosis of peritonitis + serum  $\beta_2$ -microglobulin level were 0.604, 0.694, 0.603, and 0.751, respectively (Fig. 2).

### Sub-analysis

We should consider the retrograde menstruation and other gynecologic causes such as ovulation, especially in female patients, which might be related to a lower diagnostic and culture-negative rate [9, 10]. Therefore, we select postmenopausal female cases for sub-analysis. The results were shown in Additional file 1. CNP rate was also significantly higher in female and in patients with higher platelet counts, lower dialysate cell counts at peritonitis diagnosis, and higher serum  $\beta_2$ -microglobulin levels (Additional file 1: Table S1 and S2). In multivariate logistic regression, female sex and dialysate cell count at diagnosis were significantly associated with CNP (Additional file 1: Table S3).



**Fig. 2** The area under the ROC curve for female patients, cell counts of dialysate at diagnosis of peritonitis, serum  $\beta_2$ -microglobulin, and female patients + cell counts of dialysate at diagnosis of peritonitis + serum  $\beta_2$ -microglobulin

## Discussion

To the best of our knowledge, this is the first report revealing a significant relationship between culture-negative PD-related peritonitis, sex, dialysate cell counts at peritonitis diagnosis, and serum  $\beta_2$ -microglobulin level. Female patients with diabetes had a higher CNP rate as well as higher rates of gram-positive and streptococcal peritonitis [11]. In this study, we revealed the effect of sex alone on CNP. The culture-negative rate in female patients without diabetes was similar to that in female patients with diabetes (data not shown). The discrepancy among studies may be related to the smaller sample size and adjustments for comorbidities in our study. The noninfectious causes of CNP were reported due to visceral inflammation, drug reactions, malignancy, and retroperitoneal inflammation [9]. Considering the possible gynecologic causes of CNP [9, 10], we selected postmenopausal female cases and assessed for sub-analysis. The sub-analysis similarly revealed that female sex may be an independent predictor of CNP.

Although the mechanism remains unclear, we revealed that the dialysate cell count at peritonitis diagnosis and the serum  $\beta_2$ -microglobulin level were independently associated with CNP. Automated PD (APD) utilization and extra peritoneal retention for culture sampling often shorten the peritoneal retention time, which may affect lower cell counts and higher CNP rate. However, in this study, APD was not associated with higher rates of CNP. Cytokine release was reported to be significantly lower in CNP than that in culture-positive peritonitis [12]. Therefore, there may be microbiological and immunological differences between culture-negative and culture-positive

peritonitis. Serum  $\beta_2$ -microglobulin level has been used as a predictor of residual renal function and all-cause mortality in patients undergoing hemodialysis [13, 14]. It is also a marker for cellular immune system activation [15]. In patients with PD, a lower serum  $\beta_2$ -microglobulin level, which represents impaired immunity, was independently associated with overall and infection-related mortality [16]. Although the mechanisms responsible for the observations in our study remain unclear, microbiological and immunological alterations in CNP might affect serum  $\beta_2$ -microglobulin levels. Further studies are needed to confirm the relationship between the serum  $\beta_2$ -microglobulin level and CNP.

The major limitation of this study is that CNP accounted for 27.9% of all cases during the study period, which was higher than the proportion reported in previous studies [3, 6, 17, 18]. Most cases of CNP can be explained by recent antibiotic treatment or technical problems during PD effluent culture [4]. Cefazolin sodium and isepamicin sulfate were used in our institution for the empirical treatment of peritonitis, as in the case of CNP. The response to antibiotics and outcomes did not significantly differ between culture-negative and culture-positive peritonitis episodes, supporting bacterial infection as the cause of CNP in this study. A history of antibiotic use within 30 days before peritonitis occurrence was reported as a reason for CNP [3], although this was not the case in our study. We inoculated 5–10 mL of effluent in two (aerobic and anaerobic) blood culture bottles, as recommended by the ISPD guidelines [2]. However, this technique for culture has reasonable sensitivity, and the culture-negative rate is typically about 10–20%

[19, 20], which is lower than that of our institution. The facility-specific reason for the high culture-negative rate may be the delay due to the time taken to transfer the inoculated bottles to the microbiology laboratories because the tests were ordered outside the hospital. The ISPD guidelines recommend the immediate transfer of inoculated bottles to laboratories within 6 h, as this was associated with lower CNP rates [2, 7]. However, at our institution, the inoculated bottles were stored at room temperature for more than 6 h, which may have led to a high culture-negative rate.

## Conclusions

Our study revealed that female sex, dialysate cell counts at diagnosis of peritonitis, and serum  $\beta_2$ -microglobulin levels were independent predictors of culture-negative PD-related peritonitis.

## Abbreviations

PD	Peritoneal dialysis
CNP	Culture-negative peritonitis
ISPD	International Society for Peritoneal Dialysis
ORs	Odds ratios
CI	CI confidence intervals
ROC	Receiver operating characteristic

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s41100-023-00498-9>.

**Additional file 1. Table S1** Clinical characteristics in PD patients with culture-negative or culture-positive peritonitis (male and postmenopausal female). **Table S2** Laboratory characteristics in PD patients with culture-negative or culture-positive peritonitis (male and postmenopausal female). **Table S3** Multivariate logistic regression analyses of predictors for culture-negative peritonitis (male and postmenopausal female).

## Acknowledgements

We thank Taro Banno (Department of Urology, Tokyo Women's Medical University, Tokyo, Japan) for valuable discussion. We thank *Editage* for their technical assistance in editing a draft of this manuscript.

## Author contributions

HS drafted the first manuscript. HS, TO, MT, TI, SW, HB, HA, NI, TO, TD, KO, and JM performed the literature search. TO, MT, TI, MI, SW, TD, KO, and JM coordinated the data analysis and critically commented on the manuscript. HB, HA, NI, TO, TD, KO, and JM helped with writing the manuscript. All authors participated in discussions, and read and approved the final manuscript.

## Funding

None.

## Availability of data and materials

All data generated or analyzed during this study are included in this published article.

## Declarations

### Ethics approval and consent to participate

This study was approved by the Ethical Committee of Kawashima Hospital (approval no. 1129) and was conducted in accordance with the principles of

the Declaration of Helsinki and Japanese ethical guidelines. All patients gave informed consent for their data to be included in this study.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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Received: 2 June 2023 Accepted: 20 August 2023

Published online: 24 October 2023

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