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CASE REPORT

Morphological analysis of biofilm of peritoneal dialysis catheter in refractory peritonitis patient

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Abstract A 66-year-old man undergoing peritoneal dialysis (PD) was admitted to our hospital for treatment of PD-related peritonitis. Culture of the PD fluid revealed the presence of *Citrobacter freundii*, and therapy with ceftazidime was started intraperitoneally. The cell count in PD fluid slowly decreased over time during the first 2 weeks of treatment, but increased again on the 14th hospital day. A second culture of the PD fluid revealed the presence of *Enterococcus* species. A switch in antibiotic therapy to vancomycin did not improve the cell count in the PD fluid. A third culture of the PD fluid revealed the presence of *Stenotrophomonas maltophilia*. The PD was discontinued and the catheter removed on the 28th hospital day. Examination of the catheter revealed that the inner tip was coated with a fibrous sheet of cells, suggesting biofilm formation. Following catheter removal, the patient was administered intravenous ciprofloxacin, and the inflammatory reaction started to disappear immediately and had completely disappeared after 1 week of treatment. Microscopic analysis of the fibrous structure on the catheter revealed multiple layers of various inflammatory cells. Immunostaining revealed the presence of CD44-positive polynuclear cells, indicating neutrophils, facing the catheter lumen. CD68-positive cells, indicating macrophages, were observed in the following layer, and keratin-positive cells, indicating peritoneal mesothelial cells, were present at the bottom of the structure. Based on the immediate improvement of PD-related peritonitis after catheter removal, we presumed that this biofilm contributed to the intractability of the patient's peritonitis. Morphological analysis of catheter revealed that both the mesothelial cells and the various inflammatory cells may have contributed to biofilm development.

Keywords Biofilm · Refractory peritonitis · Peritoneal dialysis

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Introduction

Peritoneal dialysis (PD)-related peritonitis is an important and major complication in patients receiving PD. Although most such patients have a benign course, PD-related peritonitis is sometimes difficult to treat, which is major reason for switching from PD to hemodialysis (HD). When PD-related peritonitis becomes intractable despite appropriate antibacterial therapy, the 2005 guidelines for PD-related infections recommend removal of the catheter [1].

One candidate for the intractability of PD-related peritonitis is biofilm formation at the catheter tip [2, 3]. Biofilm development at the catheter tip may contribute not only to relapse of infection but also to antibiotic resistance [4]. However, the mechanisms related to biofilm development are still not well understood. We report here a case of intractable PD-related peritonitis. Morphological analysis of the catheter tip revealed the presence of biofilm consisting of various inflammatory cell and mesothelial cell layers. Knowledge of the constituent cells of a biofilm and their distribution in the biofilm may contribute to a better understanding of biofilm development and the subsequent intractability of PD-related peritonitis.

Case

A 66-year-old man who had been undergoing PD for end-stage renal failure caused by chronic glomerulonephritis 1 year before was admitted to our hospital for treatment of PD-related peritonitis. The exit-site of the catheter was clear, and neither exudate nor granulomatous tissue was observed. No evidence of intra-abdominal infectious disease, such as diverticulitis, was evident on the computed tomography scan image. The PD fluid was very cloudy, and the cell count was 8,660 cell/μL, with 77.7 % of neurocytes and 14.4 % of monocytes (Fig. 1). Culture of the PD fluid revealed the presence of *Citrobacter freundii*, and the patient was started on intraperitoneal ceftazidime (500 mg once daily). The cell count in the PD fluid slowly decreased over time during the first 2 weeks to 550 cell/μL, but had increased again to 7,670 cell/μL on the 14th hospital day. A second culture of the PD fluid revealed the presence of *Enterococcus* spp, which led us to switch the anti-bacterial agent to intravenous vancomycin administration (500 mg every other day); however, the cell count in the PD fluid did not improve. A third culture of the PD revealed the presence of *Stenotrophomonas maltophilia*. PD was then discontinued and the PD catheter removed on the 28th hospital day. Examination of the removed catheter revealed a fibrous cap at the inner tip (Fig. 2a). Culture of the PD catheter also revealed positive growth of *S. maltophilia*. Following catheter removal, the patient was started on intravenous ciprofloxacin (200 mg twice daily), and the inflammatory reaction completely and immediately disappeared within 1 week.

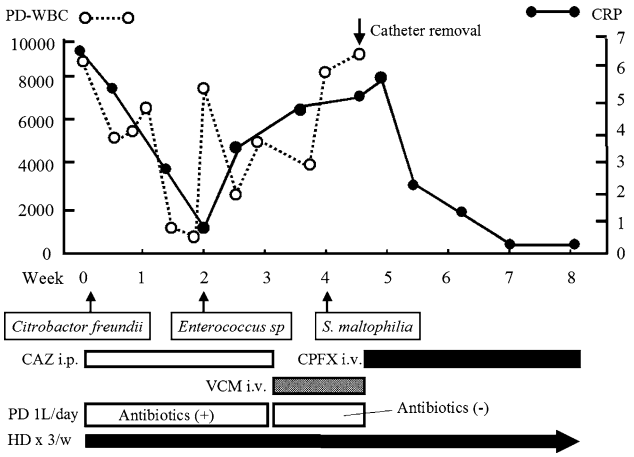

Fig. 1 Clinical course of the patient. 1 L of peritoneal dialysis (*PD*) fluid containing ceftazidime (*CAZ*) was administered once daily. After the switch to the intravenous vancomycin (*VCM*) treatment, 1 L of PD fluid was administered as a rinse of the abdominal cavity once daily. *Arrows* Positive growth of this bacteria in PD fluid culture at the indicated points. *HD* hemodialysis, *PD-WBC* white blood cell count in peritoneal dialysis fluid, *CRP* C-reactive protein, *CPFX* ciprofloxacin, *i.p.* intraperitoneal administration, *i.v.* intravenous administration

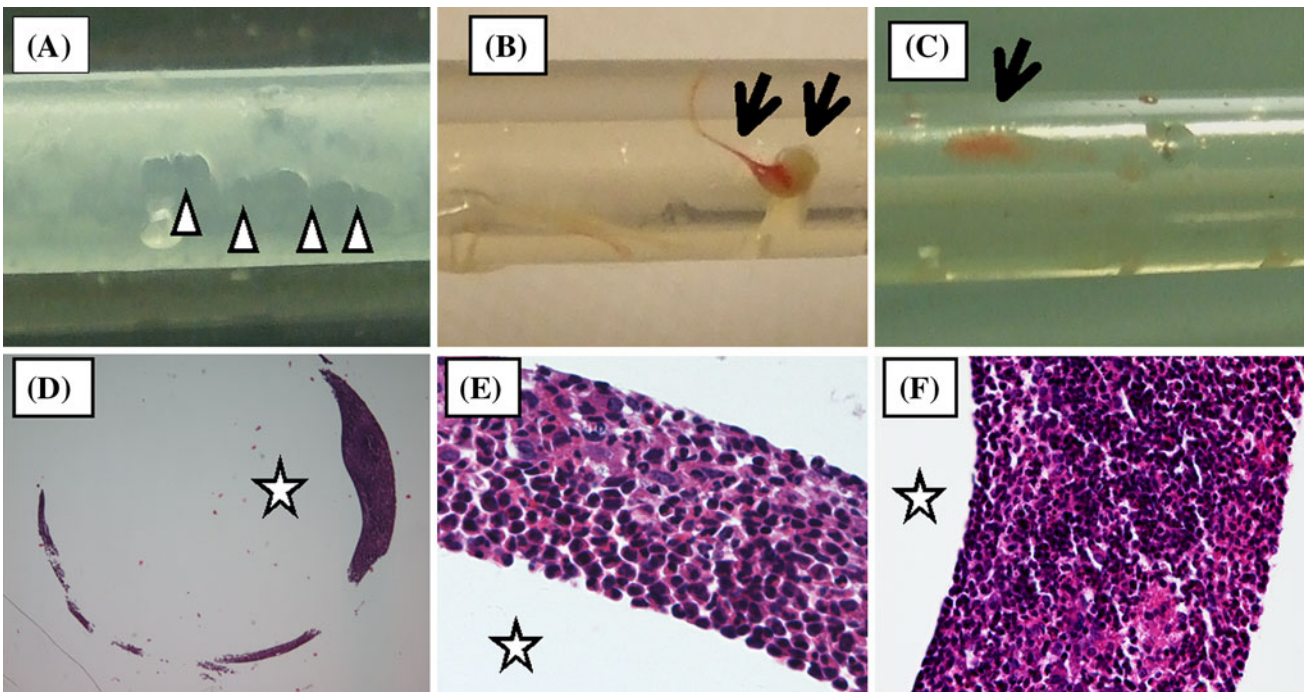

Fig. 2 Macro- and microscopic findings of the catheter tip. **a** Fibrous coat formation was observed on the inner side of the catheter tip, indicating the presence of biofilm (*arrowheads*). **b**, **c** Catheter tips from patients with uncomplicated PD-related peritonitis. Although a small amount of debris was observed on the outer side of catheter (*arrow*), there was no structure on the inner side of the catheter. **d–f** Hematoxylin and eosin staining of the catheter tip. A multilayer structure consisting of poly- and mononucleolar inflammatory cells was observed. *Stars* Catheter lumen side

Microscopic analysis of the fibrous formation on the catheter tip revealed multiple layers of various inflammatory cells (Fig. 2d–f). Immunostaining of the structure revealed the presence of CD44-positive polynuclear cells, indicating neutrophils, facing towards the catheter lumen (Fig. 3a). CD-68-positive cells, indicating macrophages, were observed in the next layer (Fig. 3b), and keratin-positive cells, indicating peritoneal mesothelial cells, were present in the lower layer of the fibrous structure (Fig. 3c). However, none of the microorganisms stained Gram-positive (Fig. 3d).

To investigate whether the development of biofilm is a common occurrence in PD-related peritonitis patients, we analyzed two catheters removed from two other PD-related peritonitis patients. One patient is a 48-year-old woman who had been on PD for 5 years (Fig. 1b); the other patient is 68 years old and had received PD for 1 year (Fig. 1c). As both patients suffered from PD-related peritonitis with exit site infection, a catheter exchange was performed. Small amount of debris were observed on the outer side of both catheter tips. However, no fibrous structure was observed on the inner side of the catheter lumen. Microscopic examination of paraffin sections of these catheter tips revealed nothing out of the ordinary.

Discussion

PD-related peritonitis is one of the major complications of PD and remains the primary causal factor for patients switching from PD to HD. Many theories have been put forth to explain the source of bacteria, including contamination of the external aspect of the catheter at the time of PD exchange, migration of bacteria from the skin through the exit-site and tunnel, and the transbowel migration of enteric microorganisms [5, 6]. One possible causal factor for PD-related peritonitis, especially in patients with multiple episodes of infection, is the release of planktonic bacteria from biofilm.

Biofilm is considered to be a microbial derived sessile community characterized by cells that are irreversibly attached to a substratum or interface and to each other and embedded in a matrix of extracellular polymeric substances

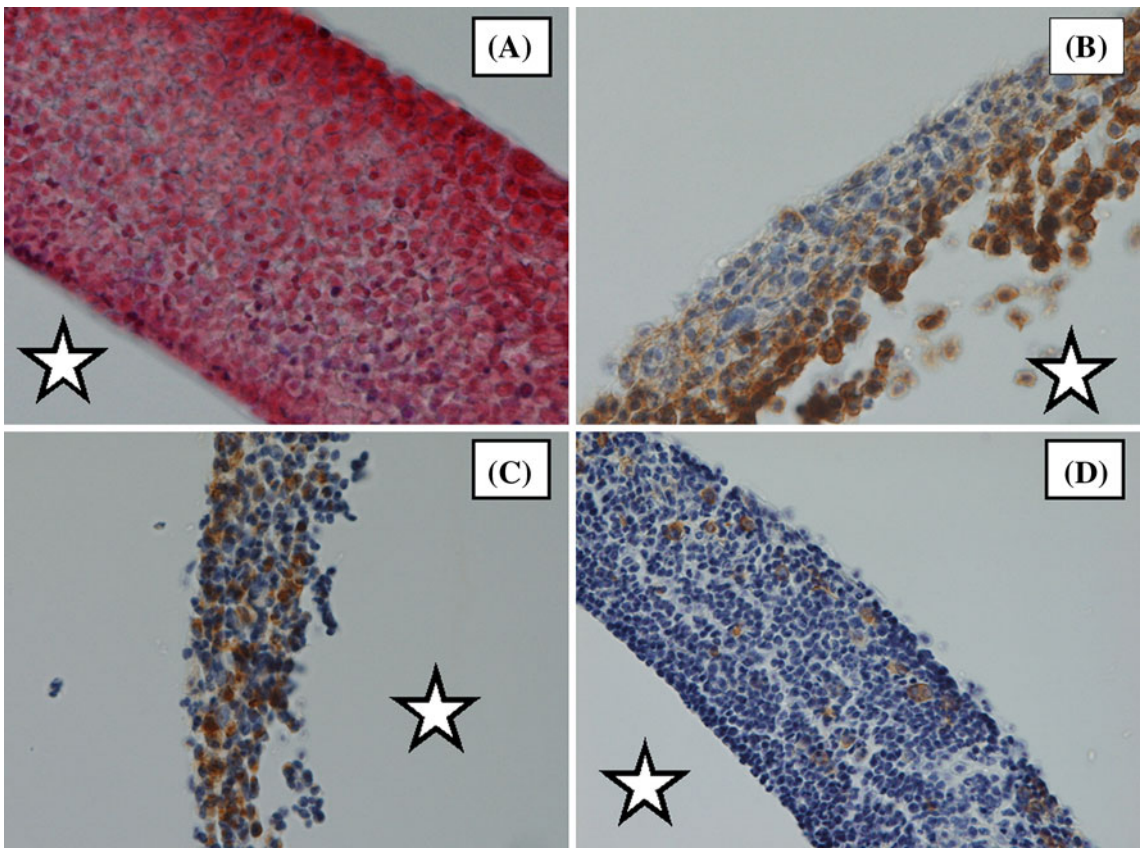

Fig. 3 Gram stain and immunohistochemical analysis of catheter tip. *Star* marks the lumen side of the catheter. **a** Immunostaining with anti-CD44 antibody; CD44-positive polynucleolar cells, indicating neutrophils, are present in the layer facing the catheter lumen. **b** Immunostaining with anti-CD68 antibody. CD68-positive cells, indicating macrophages, were observed in the next layer. **c** Immunostaining with anti-keratin antibody; keratin-positive cells, indicating peritoneal mesothelium cells, were observed on the catheter side of the biofilm. **d** Gram staining of the catheter tip; no Gram-positive microorganisms were observed in the biofilm

that the cells have produced. Although biofilm is generally thought to contribute to the pathogenesis and/or persistence of PD-related peritonitis, the relationship between the biofilm and the occurrence of overt infection is still controversial. Swartz et al. [7] and Dasgupta et al. [8] verified that biofilm was present in the PD catheter even when overt infection was absent. These findings indicate that the matrix accumulating on chronic PD catheters may not always be formed from colonizing microorganisms but may also partially be the result of host responses to the catheter [7]. Alternatively, Nodaira et al. [9] reported that biofilm formation was never found in PD catheters from patients without infection, whereas biofilm was observed in all catheters from patients with infection. In our patients, although all patients presented overt infection, the biofilm was observed in only one catheter. On this basis, the current understanding is that biofilm is just one of the candidate risks for developing overt infection or relapsing PD-related peritonitis.

The mechanisms for developing biofilm in PD catheters are not well understood. Swartz et al. [7] demonstrated that mesothelialization of the PD catheter surface, similar to that observed in the catheter of our patient, occurred in 66 % of their patients. These findings are in accordance with the theory of biofilm progression. First, following implantation of a device, a conditioning film, consisting of ionic constituents, albumin, and fibrin, immediately covers the surface [3]. This initial coating may enable not only bacteria but also peritoneal mesothelial cells to adhere to the device. The next step in infection involves the production of extracellular slime substances, resulting in the encasement of multiple layer of bacteria [10]. Inflammatory cells, such as macrophages and neutrophils, collect around this structure, as observed in our analysis, which functions as a barrier against the penetration of antibodies or antibiotics [10, 11].

Methods for preventing the development of biofilm, as well as their clinical benefit, are still controversial. One possible intervention is the intraperitoneal administration of urokinase. Urokinase is a plasminogen activator that has been used to dissolve biofilm, thereby allowing better penetration of the antibiotics. There have been some reports of the use of urokinase for resistant and relapsing PD-related peritonitis resulting in a higher rate of the catheter salvage [12–14]. However, opposite results have also been reported; in some studies, the use of urokinase did not provide any clinical benefit, such as a lower relapse rate, catheter removal rate, and mortality rate [15, 16]. Based on these previous reports, the use of urokinase for relapsing PD-related peritonitis can be considered to be beneficial and suitable for a limited number of specific cases, but not just for preventing biofilm development.

We were unable to confirm the presence of bacteria by Gram staining of the catheter tip; however, culture examination revealed *S. maltophilia* colonization. A higher number of positive results were obtained for the biofilm culture tests than for the PD fluid [2]. PD fluid culture identifies free-floating planktonic bacteria, and their concentration is thought to be low under conditions of multiple antibiotic therapy. On the contrary, in the PD catheter bacteria are protected against antibiotics due to the presence of the biofilm structure, resulting in a high concentration of bacteria and an increased sensitivity of the culture test. Based on these considerations, and even though peritonitis due to *S. maltophilia* is quite rare, we confirmed this organism to be the causal bacteria and not an error due to contamination. Furthermore, the rapid recovery of the patient following catheter removal indicates that the persistent intraperitoneal infection was caused by the biofilm of the various microorganisms.

Gram-positive microorganisms, particularly *Staphylococcus aureus* or *S. epidermidis*, are the most frequent causative pathogens in PD-related peritonitis and *Enterococcus* species are also frequently isolated [3, 17]. However, *Citrobacter freundii* and *Stenotrophomonas maltophilia* are quite uncommon pathogens in PD-related peritonitis. *Citrobacter* species are Gram-negative bacilli belonging to the family Enterobacteriaceae and are frequently found in the gastrointestinal flora of humans [18]. Gram-negative bacilli causing PD-related peritonitis frequently move from the gastrointestinal tract to the dialysate in the peritoneum [19]. Despite the absence of any evidence of intra-abdominal infectious focus, we consider *Citrobacter freundii* to have originated from the gastrointestinal tract in our patient.

Stenotrophomonas maltophilia has risen to prominence over the last decade as an important nosocomial pathogen in certain patient populations, particularly in individuals who are severely debilitated or immunosuppressed, or who use an indwelling catheter [20, 21]. In previous studies on PD-related peritonitis due to *S. maltophilia*, the use of a broad spectrum of antibiotics was considered to be a major risk factor [22, 23]. In terms of treatment, in one study the outcome with medical treatment alone was poor, and early removal of the peritoneal catheter was required because *S. maltophilia* is usually resistant to many of the currently available broad-spectrum antimicrobial agents [23]. The bacteria detected in our patient were susceptible only to ciprofloxacin; consequently, ciprofloxacin therapy was combined with catheter removal, resulting in immediate improvement of the infection. Nevertheless, we recommend early removal of catheter earlier so as not to develop the PD-related peritonitis caused by nosocomial pathogens such as *S. maltophilia.*

Conclusion

We have demonstrated the likely clinical impact of biofilm in this patient with intractable PD-related peritonitis. Morphological analysis of the catheter revealed that both mesothelial cells and various inflammatory cells may contribute to biofilm development.

Conflict of interest None.

References

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