

Fungal peritonitis in patients on peritoneal dialysis: Twenty five years of experience in a teaching hospital in Argentina

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ABSTRACT

Fungal peritonitis is a rare but serious complication of peritoneal dialysis. The aim of this study was to analyze peritonitis rates, associated factors, clinical course, microbiological aspects, therapeutic regimens, and outcome of patients with fungal peritonitis in the dialysis center of a teaching hospital over the last 25 years. A hundred and eighty three episodes of peritonitis were detected and microbiologically documented in 57 patients. Fungi were identified in eight episodes (4.37%) occurring in seven female patients. The fungal peritonitis rate was 0.06 episodes/patient-year. Gram and Giemsa stains were positive in five out of eight dialysate fluids. The causative microorganisms were: *Candida albicans* in five episodes, and *Candida parapsilosis*, *Candida glabrata*, and *Neosartorya hiratsukae* in the remaining three. Antibiotics were administered to all but one patient, within 3 months before fungal peritonitis was detected. All patients required hospitalization, and antifungal therapy was administered in all episodes. The Tenckhoff catheter was removed in seven out of eight fungal peritonitis. All patients recovered from the fungal episodes. In the group of patients studied, it is concluded that recent exposure to antibiotics and female sex, were strongly associated with the development of fungal peritonitis by yeasts. The peritonitis caused by the environmental filamentous fungus did not require antibiotic pressure. Direct microscopy of the dialysate pellet was extremely useful for the prompt management of the fungal episode. Fungal peritonitis preceded by multiple episodes of bacterial peritonitis always determined the definitive dropout of the patient from the peritoneal dialysis program. Patients with *de novo* yeast-related peritonitis could continue on the program.

Key words: peritoneal dialysis, fungal peritonitis, peritonitis rates, causative microorganisms, *Candida albicans*, *Candida* spp.

RESUMEN

Peritonitis fúngica en pacientes en diálisis peritoneal: la experiencia de 25 años en un hospital universitario de la Argentina. La peritonitis fúngica es una complicación infrecuente pero grave de la diálisis peritoneal. Los objetivos de este trabajo fueron el análisis de las tasas de peritonitis, factores asociados, aspectos clínicos y microbiológicos, esquemas terapéuticos y evolución de los pacientes afectados. Se detectaron y documentaron microbiológicamente 183 episodios de peritonitis en 57 pacientes. Se identificaron hongos en ocho episodios (4,37%) en siete pacientes, todos ellos de sexo femenino. La tasa de peritonitis fúngica fue 0,06 episodios/paciente-año. Las coloraciones de Gram y Giemsa revelaron la presencia de microorganismos en cinco de los ocho líquidos de diálisis evaluados. Los microorganismos causales fueron *Candida albicans* en cinco episodios y *Candida parapsilosis*, *Candida glabrata* y *Neosartorya hiratsukae* en los otros tres. Todos estos pacientes, excepto uno, habían recibido antibióticos en los tres meses previos al episodio de peritonitis fúngica. El catéter de Tenckhoff fue extraído en siete de los ocho episodios. Todos los pacientes evolucionaron favorablemente. Concluimos que en la población estudiada el sexo femenino y la administración reciente de antibióticos estuvieron estrechamente relacionados con el desarrollo de peritonitis fúngicas por levaduras. Sin embargo, la peritonitis causada por el hongo filamentoso ambiental no requirió de la presión antibiótica. La microscopía del sedimento del líquido de diálisis fue útil en el manejo precoz del episodio. La peritonitis fúngica precedida por múltiples episodios de peritonitis bacteriana determinó siempre la exclusión definitiva del paciente del programa de diálisis peritoneal. Los pacientes con peritonitis *de novo* por levaduras, en cambio, pudieron continuar en él.

Palabras clave: diálisis peritoneal, peritonitis fúngica, tasas de peritonitis, microorganismos causales, *Candida albicans*, *Candida* spp.

INTRODUCTION

Infectious peritonitis continues to be one of the most frequent complications in patients with end-stage renal disease treated with long-term peritoneal dialysis. It is a

significant cause of morbidity, hospitalization, catheter removal with transient or permanent transfer to hemodialysis, and even death (3, 6, 14, 15). In recent years, since the 1990s, the rate of peritonitis has dropped due to improvements in dialysis equipment and a better ap-

proach to treatment (7, 20). Fungal peritonitis, although uncommon (the reported incidence is up to 15%), is often severe and still nowadays presents several management problems (1, 5, 19).

Current treatment recommendations from the International Society of Peritoneal Dialysis include immediate catheter removal after fungi identification by microscopy or culture, and the administration, as initial therapy, of a combination of amphotericin B and 5-flucytosine until culture and susceptibility results become available (12).

In this study, we analyzed the prevalence, associated factors, clinical course, microbiological aspects, therapeutic regimens, and outcome of patients with fungal peritonitis in a single dialysis center over the last 25 years.

MATERIALS AND METHODS

Patients and methods

Medical records of all patients with fungal peritonitis between January 1981 and December 2005 were retrospectively reviewed and studied in 57 patients (38 women and 19 men) with end-stage renal disease. Patients underwent peritoneal dialysis treatment through continuous ambulatory peritoneal dialysis (CAPD) and continuous cycler-assisted peritoneal dialysis (CCPD), for more than 30 days at the Nephrology Department in the Instituto de Investigaciones Médicas Alfredo Lanari of the University of Buenos Aires, Argentina. Demographic features and clinical manifestations were reviewed, and the following specific data were recorded: age, gender, cause of end-stage renal disease, type of peritoneal dialysis, presence of comorbid diseases, number of months on the peritoneal dialysis program, peritonitis rates, prior history of fungal or bacterial peritonitis, antibiotic use within 3 months before fungal peritonitis, presence of another fungal infection site, signs and symptoms of peritonitis, peritoneal fluid white blood cells (WBC) count, Gram stain and culture, fungus species causing peritonitis, type of antifungal agents administered, complications and outcome.

In all patients, double-cuff silastic Tenckhoff catheters were implanted by using standard surgical techniques. Moreover, patients were exhaustively trained in chronic peritoneal dialysis methods. The Manual-Spike Method (STS) (Baxter[®], USA) was used up to 1995 whereas the Twin-bag Disconnect System (YTS) (Ultrabag[®], Baxter[®], USA) and the Home Choice Automatic Peritoneal Dialysis System (APD) (Baxter[®], USA) were used until 2005. Patient evaluation to be included in the peritoneal dialysis program involved a maximum score of 10 points and took the following data into account: intellectual level, vision capacity, manual dexterity, communicational capability, motivation, personal hygiene, non-diabetic condition, non-systemic diseases, non-immunosuppressive drugs, and environment.

The fungal peritonitis diagnosis was based on the presence of: a) cloudy peritoneal effluent with 100 WBC/ μ l or greater, with at least 50% polymorphonuclear (PMN) cells, b) abdominal pain or tenderness, and c) isolation (or Gram/Giemsa stains) of fungi from the peritoneal dialysis fluid.

Microbiological procedures

The processing of the peritoneal dialysis effluent included a leukocyte count, and microbiological cultures. Briefly, between 1981 and 1989, 10 ml and the rest of the effluent (approximately 2000 ml) were simultaneously cultured as was previously described (14). Since 1990, 10 ml were inoculated in an anaerobic blood culture bottle (until 2000, Hemo-100 Laboratorios Britania, BA, Argentina and as from 2001, BD Lytic/10 Anaerobic/F, Becton Dickinson and Co, Sparks, Md, USA), and 100 ml aliquots of the

dialysate fluid were centrifuged at 3000 rpm for 15 minutes. After discarding the supernatant, the pellet was washed twice with phosphate-buffered saline, resuspended in about 5 ml of it and Gram, Giemsa, and Ziehl-Neelsen stains were done. When necessary, a wet mount was examined. The pellet was cultured onto brain heart infusion agar (Oxoid, Ltd, Basingstoke Hampshire, England) with 5% sheep blood (Laboratorio Gutiérrez, BA, Argentina) and Sabouraud dextrose agar (home made), both media with antibiotics, gentamicin (Sigma, St. Louis, USA) and chloramphenicol (Sigma, St. Louis, USA) at 100 mg/l each one. A pair of tubes were incubated at 28 °C and 35 °C and reviewed every 24 h for the first 3 days and then, biweekly for 8 weeks for evidence of growth. When yeasts were seen through direct examination, a CHROMagar Candida[®] plate (Paris, France) was added. To complete the study, a blood agar plate (Biokar Diagnostics, Beauvais, France) with 5% sheep blood, and an eosin-methylene blue agar (Laboratorios Britania, BA, Argentina) were used as plating media, and thioglycolate broth (Difco/Becton Dickinson and Co, Sparks, Md, USA) and an aerobic blood culture bottle (BD Standard/10 Aerobic/F, Becton Dickinson and Co, Sparks, Md, USA) were inoculated as enriched liquid media. They were incubated at 35 °C up to 14 days.

For mycobacteria, solid media as Lowenstein-Jensen and Stonebrink (both from Laboratorios Britania, BA, Argentina), and liquid medium as BD Myco/F lytic (Becton Dickinson and Co, Sparks, Md, USA) were inoculated, when clinical and epidemiological data required it. All microorganisms were identified according to standard methods; for yeasts: germ tube test and growth at 45 °C for presumptive identification of *Candida albicans*, malt extract agar, urease (Christensen agar), and the use of the API ID 32C (bioMérieux, Marcy l'Étoile - France) were included whereas for certain molds, the scanning electron microscopy and nucleic acid sequencing were necessary (performed by Dr. Josep Guarro-Artigas *et al.*). Antifungal susceptibility testing was not routinely performed.

Statistical analysis

Comparisons of peritonitis rates were analyzed by using the Chi-square test. A *p* value less than or equal to 0.05 was considered significant.

RESULTS

During the 25-year study period, 183 episodes of peritonitis were observed. In eight of these episodes (4.37%) occurring in seven patients, all of them women, fungi were identified as etiologic agents. One patient had two fungal peritonitis episodes, both of them by *C. albicans*, in a 26-month period. The median duration of peritoneal dialysis before contracting the fungal peritonitis was 19.78 months (range, 4.77 to 60.67 months). The peritonitis rate in patients who developed fungal infection was one episode every 5.7 patient-months, while it was one episode every 7.2 patient-months in those patients with bacterial peritonitis who did not develop fungal peritonitis (*p* > 0.05), at any way statistically non-significant. The fungal peritonitis rate was 0.06 episodes/patient-year. Two episodes developed in 1986, one in 1993, two in 1995, one in 2002, one in 2003 and the last one in 2004.

Microbiology

All fungal peritonitis episodes were microbiologically documented, and the preliminary microscopy identification was made at a median of 4 h (range, 2 to 6 h) after

the diagnosis of peritonitis. Gram and Giemsa stains were positive in five of eight dialysate fluids (62.50%). All cultures were positive (100%) between 18 and 48 h, being the causative agents: *Candida albicans*, five; *Candida parapsilosis*, one; *Candida glabrata*, one; and *Neosartorya hiratsukae*, one. Three episodes were mixed infections with *Pseudomonas aeruginosa*, *Pseudomonas* Group 1, and *Acinetobacter lwoffii*.

Seven out of eight catheters were removed and microbiologically studied. In all of them, the same fungi as in the dialysis fluids were isolated including the filamentous *N. hiratsukae*.

Demographic and Clinical Features

The clinical characteristics of patients with fungal peritonitis are listed in Table 1.

Antibiotic use within 3 months before fungal peritonitis was detected in all but one patient, due to bacterial peritonitis (4 episodes), bronchitis and pneumonia (2 episodes) and urinary tract infection and vaginal discharge (1 episode). Five of the eight episodes were in patients who had had at least one prior episode of bacterial peritonitis, at a mean of 4.8 episodes (range, 1 to 9 episodes), and de novo fungal peritonitis occurred in the three remaining patients. One of these patients developed two fungal episodes, the first of which was de novo, and the second one resulting after four bacterial peritonitis episodes by gram-positive cocci (three by *Staphylococcus epidermidis* and one by *Staphylococcus haemolyticus*). The signs and symptoms of fungal peritonitis were not different from those of bacterial peritonitis. At presenta-

tion, all patients complained of abdominal pain and presented cloudy dialysates. Peritoneal cell count at the time of the fungal episode varied from 120 to 23,600 WBC/ μ l, with a median value of 620 WBC/ μ l and more than 90% PMN.

Treatment and Outcome

All patients required hospitalization, and no associated fungemia was detected. In all cases, antifungal therapy was administered as soon as fungal peritonitis was diagnosed. The agents and routes used have changed over these 25 years, but patients received antifungal therapy for at least 2 weeks or continued to complete 4 to 6 weeks. Four episodes were treated with IV amphotericin B to complete a cumulative dose of 500 mg for *Candida* species, and in one episode, a cumulative dose of 800 mg of IV amphotericin B was administered to treat *N. hiratsukae*. In two *C. albicans* episodes, oral ketoconazole 200 mg/d was used for 2 weeks, and oral fluconazole 200 mg/d was administered to complete 6 weeks, respectively. *C. parapsilosis* was treated with a combination of IV amphotericin B to complete 500 mg plus oral fluconazole 100 mg/d for 2 weeks. In our country, 5-flucytosine was not available.

The Tenckhoff catheter was removed in seven of the eight fungal peritonitis episodes, after a median duration of 7 days (range, 2 to 30 days). Only one patient with a de novo episode of *C. albicans* peritonitis, whose dialysate cleared at 48 h, resolved the infection just with the antifungal treatment and continued on CAPD.

Even though all patients recovered from the fungal episodes, four patients were definitively transferred to

Table 1. Demographic data of the seven patients with fungal peritonitis

Demographics	Fungal peritonitis patients (n = 7)
Mean age, yr (range)	59.43 \pm 18.14 (33 – 77)
Females	7 (100%)
Evaluation score	7.29 \pm 1.49 (5 – 9)
Etiology of ESRD	
Diabetes mellitus	2
Hypertensive nephropathy	1
Unknown	4
Comorbid disease	
Hypertension	3
Diabetes mellitus	2
Cardiac failure FC III (NYHA) ⁽¹⁾	1
Advanced breast cancer with bone metastasis	1
History of use of immunosuppressive agents	1
Dialysis exchange system (during FP episode)	
Manual-Spike method (STS)	4
Twin-bag (YTS)	4

ESRD: end-stage renal disease; FP: fungal peritonitis.

⁽¹⁾ Cardiac failure functional class III, according to the New York Heart Association.

hemodialysis and three continued on the peritoneal dialysis program. The only two patients with de novo yeast-related peritonitis (*C. albicans*) that had resolved the episodes with antifungal therapy and the removal of the Tenckhoff catheter, continued on CAPD. All four patients with peritonitis by yeasts preceded by multiple episodes of bacterial peritonitis (3 to 9), by gram-negative, gram-positive, or mixed episodes, including *Mycobacterium tuberculosis*, had to definitively abandon the peritoneal dialysis treatment. The peritonitis caused by *N. hiratsukae*, which was preceded by only one bacterial peritonitis episode (by *Enterobacter agglomerans*) one year before, had a better clinical outcome with antifungal therapy and removal of the Tenckhoff catheter, being this woman the third patient that could continue on CAPD.

DISCUSSION

Fungal peritonitis continues to be a rare but serious complication of peritoneal dialysis, associated with high morbidity, inability to continue on the program, and contributing to mortality (3, 4, 11).

In contrast to bacterial peritonitis, Gram and Giemsa stains which were available a few hours after the diagnosis of the infectious episodes, were extremely useful to promptly manage the fungal episode; to establish the early antifungal therapy and to remove the Tenckhoff catheter as soon as possible, as was recommended (12, 14, 16). In the last 25 years, 4.37% of all peritoneal dialysis-related peritonitis episodes in our center were caused by fungi, compared with a percentage of 1 to 15% reported in the literature (1, 3-5, 11, 13, 19). In our series and similarly to other ones, *Candida* species were responsible for the majority of fungal isolates, with *C. albicans* being by far the most common, being one episode caused by *N. hiratsukae*, an extremely rare mold. Of all male and female patients on the peritoneal dialysis program, only females developed fungal peritonitis episodes, primarily by yeasts. All these patients but one, who had suffered the *N. hiratsukae* peritonitis, had recently received antibiotics due to bacterial peritonitis episodes or other infections. It is well known that fungi enter the peritoneal cavity intraluminally or periluminally, but rarely through the vaginal route (9). One of our patients with de novo *C. albicans* peritonitis, who had used ampicillin due to a urinary tract infection, also experienced persistent vaginal discharge, which was never microbiologically studied. Selective pressure of antibiotics may help to produce fungal overgrowth on the skin, the mucous membranes and the bowel, thus producing in these patients a better environment for yeast colonization, infection or both (10). It could be hypothesized that in these patients, Douglas sack funds might be colonized with *C. albicans* or *C. glabrata*, and the vaginal route would be considered. In the case of *C. parapsilosis*, the periluminal via through the skin is the

most frequent. Although *Candida* peritonitis is uncommon but with remarkable consequences at personal level, it would probably be necessary to study the genitalia of those female patients on peritoneal dialysis program who had been receiving antimicrobials for long periods of time. It would be important in order to establish local fungal prophylaxis, at least to prevent this route of infection, or oral fungal prophylaxis during antibiotic therapy in programs having high rates of fungal peritonitis (12). Peritonitis due to fungi is very aggressive, particularly that by *C. albicans* where peritoneal immunosuppression is observed because of the inhibition of the expression of the specific receptor DC-SIGN (CD 209) for *C. albicans* on dendritic cells in dialysate fluids with high concentrations of lactate, glucose and its products. In recent years, the new dialysis solutions with bicarbonate, low lactate concentrations, and without the glucose products that do not inhibit the receptor, seem to have improved the peritoneal response (2, 17).

Peritonitis by *N. hiratsukae* occurred in the only patient with no history of recent antibiotic use, but with a clear epidemiologic cause that explained the exogenous origin of the intraluminal infection. The episode developed while the patient was building her house, and the exchanges were done in an inappropriate environment.

In our patients, the clinical findings were similar to those described for bacterial peritonitis, and included abdominal pain and cloudy dialysates, but with no fever or catheter malfunction. The peritoneal cell counts varied widely. We found a clear association between antibiotic exposure and the development of all but one episodes of fungal peritonitis, as had been reported in a range of 34 to 87.3% (1, 5).

We found a statistically non-significant difference between rates of peritonitis in patients who developed fungal peritonitis and those who did not, as was reported by Johnson *et al.* (19) and Tapson *et al.* (20).

In conclusion, the careful and continuous training of patients, especially in handwashing technique and in doing the connection, are critical for preventing exogenous related peritonitis with rare environmental microorganisms, and those colonizing the skin.

We found that recent exposure to antibiotics was strongly associated with the development of fungal peritonitis by yeasts. The peritonitis caused by the environmental filamentous fungus did not require antibiotic pressure.

There was no statistically significant difference in peritonitis rates.

Direct microscopy of the dialysate pellet could be available a few hours following the diagnosis of the infectious episode, which is extremely useful for the prompt diagnosis and management of the fungal episode. Treatment requires proper antifungal therapy and early catheter removal in order to eliminate the main colonization site.

Candida albicans accounts for the majority of fungal isolates.

Although we had few episodes to draw definitive conclusions, in our patients, fungal peritonitis preceded by multiple episodes of bacterial peritonitis always determined the definitive dropout of the patient from the peritoneal dialysis program. Patients with *de novo* yeast-related peritonitis could continue on CAPD. We believe that further large-scale, prospective and randomized studies are needed to investigate the difference in outcome and the progressive loss of peritoneal membrane ultrafiltration capacity between patients with *de novo* fungal peritonitis and those developing fungal peritonitis secondary to bacterial peritonitis.

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