Original Article

Clostridium difficile infection: a Serbian single-center experience

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Abstract

Introduction: *Clostridium difficile* infection (CDI) is the most common cause of hospital-acquired diarrhea. Severity of CDI is associated with advanced age and co-morbidities. The clinical spectrum varies from mild watery diarrhea to severe fulminant pseudomembranous colitis with complications.

Methodology: This study conducted over a six-year period (2008 to 2013) included 510 patients treated at the University Hospital for Infectious and Tropical Diseases in Belgrade, Serbia. In patients with a history of previous hospitalization and/or treatment with antimicrobial agents who developed diarrhea, the diagnosis was established with rapid tests for *C. difficile* toxin A and B and by stool culture for *C. difficile* (454 patients) or by endoscopic examination and histological analyses of the biopsy samples taken from the colonic mucosa (56 patients).

Results: The mean age of patients was 67.71± 13.34 years. A total of 67.8% patients were older than 65 years. Over half (58.7%) of the patients were female. 93% had been previously hospitalized and/or had surgical interventions, during which they had been treated with antibiotics. In the clinical presentation spectrum, pseudomembranous colitis occurred in 51.0% .The mean duration of illness after the introduction of specific antibiotic therapy was 7.10 ± 4.88 days. Complications developed in 14 patients. The disease relapsed in 43 (8.4%). Thirty-two (6.3%) patients died, mostly due to co-morbidities.

Conclusions: CDI is the most important cause of hospital-acquired diarrhea in Serbia. The disease mainly affects elderly patients with co-morbidities. The incidence of complications is low and prognosis is age dependent and related to pre-existing diseases.

Key words: *Clostridium difficile*; diarrhea; pseudomembranous colitis.

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Introduction

Clostridium difficile infection (CDI) is the most common hospital-accquired bacterial diarrhea in industrialized countries. The severity of CDI is influenced by advanced age and co-morbidities, disturbed intestinal microbiota as the result of antibiotic therapy, and exposure to *C. difficile* spores [1]. The clinical spectrum of the disease varies from mild acute diarrhea to severe fulminant disease such as pseudomembranous colitis, which may be complicated with toxic megacolon [2]. Rising incidence and higher mortality have been the two key characteristics of *C. difficile*-associated disease in the last decade [1]. The objective of this study was to determine the baseline characteristics, clinical presentation, prognostic factors, and outcome of CDI in patients treated at the University Hospital for Infectious and Tropical Diseases, Belgrade, Clinical Centre of Serbia.

Methodology

This was a retrospective study of a series of 510 patients treated at the University Hospital for Infectious and Tropical Diseases, Belgrade, Clinical Centre of Serbia, over a period of six years (2008–2013). In 454 patients with a history of previous hospitalization and/or treatment with antimicrobial agents who developed diarrhea, the diagnosis of CDI was established with rapid tests for *C. difficile* toxin A and B (RIDA QUICK Clostridium difficile Toxin A/B, R-Biopharm AG, An der neuen Bergstrase 17, 64297, Darmstadt, Germany) or by stool culture for *C. difficile* (medium Clo Agar, BioMerieux, 69280, Marcy l'Etoile, France). In 56 patients, the diagnosis was made by the endoscopic finding of pseudomembranous colitis with typical pseudomembranes on colonic mucosa and histological analyses of taken biopsy samples. Frequent watery stools with mucus and/or blood were considered to be due to colitis. Demographic characteristics, clinical

presentation, and duration of disease were analyzed, along with baseline laboratory analyses including inflammatory parameters, such as erythrocyte sedimentation rate (ESR), fibrinogen, C-reactive protein (CRP) and D-dimer in order to determine prognostic factors and final outcome. Treatment regimens included either the combination of metronidazole and vancomycin, or one of the drugs as monotherapy. Patients with severe colitis (abdominal distention, white blood cell [WBC] count > 25 x 10^9/L, albumin count < 30 g/L) were prescribed a dual regimen, while others were prescribed monotherapy [3,4].

All analyses were performed using an electronic database organized in SPSS version 16.0. The one-way analysis of variance (ANOVA) test was used to compare the means. The non-parametric variables were analyzed using the Chi-square or Fisher's exact test, as appropriate. Person's test was used for bivariate correlation. The level of significance was 0.05.

The consent for participation was obtained from all subjects, and the study was approved by the Clinical Centre of Serbia Ethics Committee.

Results

The patients' mean age was 67.71 ± 13.34 (range, 22–91) years, and most (67.8%) were older than 65 years of age. A total of 299 (58.7%) patients were female, while the majority (474; 93%) of patients had been previously hospitalized (Table 1) . Four hundred forty-six (87.4%) patients had been previously treated with antibiotics in hospital settings, while only 10 patients (1.9%) were outpatients. Three hundred seventeen (62.2%) examinees underwent major surgical interventions. Eighteen (3.5%) patients suffered from malignances and were on chemotherapy, while 11 (2.1%) had end-stage renal disease. For the remaining patients, the only risk factor was treatment with broad-spectrum antibiotics on an inpatient and/or outpatient basis. In most cases, diarrhea began after treatment with fluoroquinolones (57.9%) and third-generation cephalosporins (34.2%), while 7.9% of the patients took other antibiotics. The mean time between admission to hospital and occurrence of diarrhea was 15.15 ± 11.34 days (range, 3–63), and 60% of the patients developed diarrhea within 14 days. Fever, vomiting, and abdominal cramps occurred in 274 (53.8%), 107 (21%) and 211 (41.3%) patients, respectively (Table 1). Approximately half (51.0%) of the patients had clinical presentations suggestive of pseudomembranous colitis. Only eight patients (1.5%) had bloody stools. The mean number of stools was 5.53 ± 4.06 (1–20) per day; it was significantly higher among patients with colitis than among patients suffering diarrhea without colitis (6.5 ± 3.9 vs. 4.4 ± 3.9, p = 0.002). Recurrent disease was registered in 43 (8.4%) patients, and the mean time between hospital discharge and recurrence was 11.62 ± 10.75 (3–40) days. Complications (ileus, toxic megacolon) were registered in 14 (2.8%) patients, and they were referred to surgery. Thirty-two (6.3%) patients died in the thirty-day period, mostly due to co-morbidities (two patients died due to severe pseudomembranous colitis with complications) (Table 1).

Laboratory analyses, including inflammatory parameters such as ESR, fibrinogen, CRP, and WBC counts are presented in Table 2. In 175 (34.3%) patients, WBC count was above 15 x 10^9/L. Mean serum iron, total proteins, and albumin was below normal values (Table 2). Pleural effusion and ascites were registered in 14.7% and 9.8% of patients, respectively. Mean serum albumin in patients with ascites and pleural effusion was significantly lower than among the remaining patients (22.36 ± 4.46 g/L vs. 27.24 ± 6.12 g/L, p = 0.005, and 20.89 ± 4.29 g/L vs. 27.73 ± 5.85 g/L, p = 0.000, respectively). When the possible correlation of D-dimer concentrations with other inflammatory parameters, such as ESR, fibrinogen and WBC count, was analyzed, the only correlation recorded was with ESR (p = 0.032). Seventy-eight (15.3%) patients with severe colitis were treated with combined antibiotic therapy (metronidazol and vancomycin), 357 patients (70%) received vancomycin, and 75 (14.7%) received metronidazol alone. The mean duration of diarrhea was 7.10 ± 4.88 (1–25) days after specific antibiotic therapy for *C. difficile* was introduced. In 352 (69%) patients, diarrhea lasted not more than a week after the therapy was initiated. The analyses of factors that were associated with the duration of diarrhea showed that elevated WBC count (> 15 x 10^9/L), initial number of stools (> 5/day), elevated CRP (> 100 mg/L), and lower albumin count (< 30 g/L) were associated with prolonged duration of disease (Table 3). Diarrhea lasted longer in patients with colitis than in those who had diarrhea without colitis, but with no significant difference (7.79 ± 4.99 vs. 6.36 ± 4.69, p = 0.081). Being 65 years of age or older was significantly associated with poor outcome (OR 1.523, 95% CI 1.347–1.721; p = 0.027).

Table 1. Demographic and clinical characteristics of patients with *C. difficile* infection

Characteristic	Number of patients	Percent
Total number of patients	510	100%
Male	211	41.3%
Female	299	58.7%
Age > 65 years	346	67.8%
Prior hospitalization	474	93%
Prior antibiotic therapy	446	87.4%
Temperature > 38°C	274	53.8%
Vomiting	107	21%
Abdominal pain	211	41.3%
Diarrhea without colitis	250	49%
Colitis	260	51%
Recurrence	43	8.4%
Complications	14	2.8%
Death	32	6.3%

Table 2. Laboratory analyses of patients with *C. difficile* infection

Laboratory analyses	Mean (x± SD)
ESR	37.31 ± 22.83
Fibrinogen (g/L)	5.45 ± 1.60
CRP (mg/L)	99.20 ± 82.64
WBC x 10^9/L	14.51 ± 11.15
Procalcitonin (ng/mL)	1.81 ± 3.58
D-dimer (µg/L)	5.17 ± 3.92
Fe (µmol/L)	5.27 ± 4.21
Protein (g/L)	53.92 ± 9.76
Albumin (g/L)	26.69 ± 6.14

ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; WBC: white blood cell count

Table 3. Association of duration of diarrhea with inflammatory parameters

Factor	Duration of diarrhea (days)	p
WBC > 15 x 10^9/L	9.29 ± 6.11	0.000
WBC < 15 x 10^9/L	6.04 ± 3.72	
Number of stools > 5	8.29 ± 5.37	0.005
Number of stools < 5	6.00 ± 4.12	
CRP > 100 (mg/L)	9.28 ± 5.91	0.001
CRP < 100 (mg/L)	6.12 ± 3.84	
Albumin < 30 (g/L)	8.18 ± 5.43	0.001
Albumin > 30 (g/L)	4.97 ± 2.98	

WBC: white blood cell count; CRP: C-reactive protein

Discussion

In our series of patients with CDI, the majority were older than 65, had been previously treated with broad-spectrum antibiotics as inpatients, had co-morbidities such as malignancies, end-stage renal disease, and/or a history of major surgical interventions. The duration of symptoms was not associated with advanced age. Even though patients with colitis did not have prolonged diarrhea compared with those who had diarrhea without colitis, they suffered from more frequent stools.

Beginning as early as 2002 and extending through the following years, the outbreaks of unusually severe and recurrent CDI were noted in Canada and the United States [3]. The greater incidence of CDI was registered across hospitals in Europe [5]. It was recognized early that advanced age is one of the major factors predisposing CDI, since the disease incidence increased with age over 65 [3]. This is in concordance with our data, which showed that almost 68% of patients suffering CDI were older than 65 years of age. Most of our patients had been previously hospitalized and treated with broad-spectrum antibiotics. Similar results were recorded in other hospitals in Eastern Europe. Accordingly, at the Charles University in Prague, 76% patients with CDI were older than 60 years of age, and 92% had received antibiotics before the onset of the disease [6]. Exposure to antibiotics that disrupt the colonic microbial flora appears to be another important risk factor for CDI [4,7]. We demonstrated that fluoroquinolones and third-generation cephalosporins were mostly used among our patients. Until recently, fluoroquinolones were widely used for surgical prophylaxis in Serbia, which can explain why more than 50% of our patients received them before CDI occurred. Olson *et al*. reported that 96% of patients with CDI had received antibiotics within the 14 days (not more than three months) before the onset of diarrhea [8]. Similarly, we demonstrated that the mean time between the beginning of the antibiotic therapy and the occurrence of diarrhea was 15 days.

C. difficile usually affects the colon and causes diarrhea, often watery, sometimes with mucus (suggesting colitis), while bloody diarrhea is exceptional [4]. Fever, abdominal cramping, and peripheral leukocytosis are common, but found in less than half of patients [9]. Half of our patients had colitis, and less than 50% had vomiting, abdominal cramps, and peripheral leukocytosis. Among those with bloody diarrhea, one patient was a previously healthy person with a history of treatment with fluoroquinolones as an outpatient. He had serious CDI with fulminant pseudomembranous colitis and toxic megacolon. Serious outpatient infection probably occurred due to one of the hypervirulent strains of *C. difficile*, but unfortunately, we could not identify it [10,11]. Fulminant colitis was previously recorded in 3%–8% of patients [4]. The CDI mortality rate reported in a number of studies varies from 13%–27% [4,6,12]. However, in our patient series, only 2.8% had severe complicated CDI, while the mortality rate was 6.3%. The explanation for such a low mortality rate could be the usual practice of immediate referral of severe cases from other hospitals to surgery, bypassing our hospital.

The recently published CDI recurrence rate was about 20% [13]. In our patient series, the recurrence was registered in only 8.4%. Some of the patients with the recurrent disease were treated in regional hospitals and were not referred elsewhere; accordingly, the total numbers of recurrent CDI could have probably been much higher.

The laboratory analyses among our patients showed elevated inflammatory parameters. As a consequence of malabsorption, mean iron, protein, and albumin counts were lower than normal. Patients with pleural effusions and ascites had marked hypoalbuminemia. Manifestations of protein-losing enteropathy, including hypoalbuminemia, with peripheral edema, ascites, and pleural effusion were also previously described in patients with CDI [14]. We recorded increased D-dimer values in most of the patients, while procalcitonin, a marker of serious systemic infection, was mostly normal. There was also no correlation with other inflammatory parameters (except ESR). We thus speculate that increased D-dimer values were due to fibrinogen exudation and degradation in the bowel mucosa. Such a pathogenic mechanism had been previously reported among patients with inflammatory bowel diseases, since ulcerous colitis was associated with increased D-dimer. Furthermore, it proved to be a reliable marker of disease activity in ulcerous colitis [15]. We did not demonstrate the D-dimer level to be a factor that influenced the severity and the outcome of the disease.

In most of the patients, diarrhea lasted not more than a week after specific therapy was introduced. More aggressive disease is associated with hypoalbuminemia and leukocytosis or even leucopenia ($> 25 \times 10^9$/L or $< 1.5 \times 10^9$/L, respectively) [4]. We demonstrated that there are certain baseline clinical and laboratory factors associated with prolonged and more severe disease such as WBC count above 15 ×

10^9/L, initial number of stools more than five per day, increased CRP of above 100 mg/L, and hypoalbuminemia of below 30 g/L. It was previously shown that advanced age was associated with an increased death rate [5,16]. The analyses of prognostic factors for survival of patients with CDI revealed that the only factor that was significantly related to the increased death rate was age above 65 years.

Conclusions

C. difficile infection is a very important cause of hospital-acquired diarrhea in Serbia. The disease mainly affects elderly hospitalized patients with co-morbidities. The incidence of complications is low and the prognosis is age dependent and related to preexisting diseases.

References

1. DuPont HL (2011) The search for effective treatment of Clostridium difficile Infection. N Engl J Med 364: 473-475.
2. Gerding DN (2009) Clostridium difficile 30 years on: what has, or has not, changed and why? Int J Antimicrob Agent 33 Suppl 1: 2-8.
3. Cohen SH, Gerding DN, Johnson S, Kelly CP, Loo VG, McDonald LC, Pepin J, Wilcox MH; Society for Healthcare Epidemiology of America; Infectious Diseases Society of America (2010) Clinical practice guidelines for Clostridium difficile infection in adults: 2010 update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Disases Society of America (IDSA). Infect Contr Hosp Epidem 5:431-455.
4. Hookman P, Barkin JS (2009) Clostridium difficile associated infection, diarrhea and colitis. World J Gastroenterol 15: 1554-1580.
5. Bauer MP, Notermans DW, van Benthem BH, Brazier JS, Wilcox MH, Rupnik M, Monnet DL, van Dissel JT, Kuijper EJ; ECDIS Study Group (2011) Clostridium difficile infection in Europe: a hospital-based survey. Lancet 377: 63-73.
6. Polívková S, Sýkorová B, Džupová O, Reisingerová M, Beneš J (2010) Incidence and characteristics of Clostridium difficile infection in patients with diarrhea in a Prague teaching hospital. Klin Mikrobiol Infekc Lek 16: 206-210.
7. De Andres S, Ibanez M, Ballesteros A, Garcia B, Agud JL (2004) Clostridium difficile colitis associated with valaciclovir. Pharm World Sci 26: 8-9.
8. Olson MM, Shanholtzer CJ, Lee JT Jr, Gerding DN (1994) Ten years of prospective Clostridium difficile-associated disease surveillance and treatment at the Minneapolis VA Medical Center, 1982-1991. Infect Control Hosp Epidemiol 15: 371-381.
9. Bartlett JG (2002) Clinical practice. Antibiotic-associated diarrhea. N Engl J Med 346: 334-349.
10. McDonald LC, Killgore GE, Thompson A, Owens RC Jr, Kazakova SV, Sambol SP, Johnson S, Gerding DN (2005) An epidemic, toxin gene-variant strain of Clostridium difficile. N Engl J Med 353: 2433-2441.
11. Copt C, Christodoulou M, Friolet R, Berclaz R, Bettschart V, Troillet N (2010) Clostridium difficile: a serious outpatient infection? Rev Med Suisse 6: 1910-1913.
12. Arvand M, Hauri AM, Zaiss NH, Witte W, Bettge-Weller G (2010) Epidemiology of severe Clostridium difficile infections in Hesse, Germany in 2008-2009. Dtsch Med Wochenschr 135: 1963-1967.
13. Kelly CP, LaMont JT (2008) Clostridium difficile - more difficult than ever. N Engl J Med 359: 1932-1940.
14. Dansinger ML, Johnson S, Jansen PC, Opstad NL, Bettin KM, Gerding DN (1996) Protein –losing enteropathy is associated with Clostridium difficile diarrhea but not with asymptomatic colonization: a prospective, case control study. Clin Infect Dis 22: 932-937.
15. Xu G, Tian KL, Liu GP, Zhong XJ, Tang SL, Sun YP (2002) Clinical significance of plasma D-dimer and von Willebrand factor levels in patients with ulcer colitis. World J Gastroenterol 8: 575-576.
16. Lamontagne F, Labbe AC, Haeck O, Lesur O, Lalancette M, Patino C, Leblanc M, Laverdière M, Pépin (2007) Impact of emergency colectomy on survival of patients with fulminant Clostridium difficile colitis during an epidemic caused by a hypervirulent strain. Ann Surg 245: 267-272.

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