Predictors of First Recurrence in *Clostridium difficile*-Associated Disease. A Study of 306 Patients Hospitalized in a Romanian Tertiary Referral Center

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**ABSTRACT**

**Background & Aims:** *Clostridium difficile* is recognized as the major cause of nosocomial gastroenteritis usually related to antibiotic treatment. Although treatable, *C. difficile*–associated disease (CDAD) tends to recur in many patients. The purpose of the study was to analyze the risk factors for recurrence in patients with CDAD after the first treatment with vancomycin, metronidazole or both.

**Method:** We conducted a retrospective study of all patients admitted to the Teaching Hospital of Infectious Diseases Cluj-Napoca, Romania, between January 2011 and October 2012 with the diagnosis of CDAD or who developed diarrhoea after admission. A clinical diagnosis was made and culture and toxin A and B detection were carried out. We performed a statistical analysis taking into consideration: age, gender, previous hospital exposure, previous antibiotic treatment, and treatment duration. The patients were followed-up for at least 60 days.

**Results:** We included 306 patients (177 women and 129 men) with a median age of 71 years; 208 patients (68%) had prior hospitalization and 195 (64%) had received prior antibiotic treatment. Actual treatment consisted of vancomycin in 76 (25%) patients, metronidazole in 132 (43%) and both combined in 98 (32%) patients. The average duration of treatment was 10 days. Sixty patients (20%) experienced 95 recurrences and 9 patients died (3%). Treatment with metronidazole, vancomycin or both for 10 or more days did not prevent recurrences. Age over 70 (RR 1.5, CI 95%: 1.055-2.71) and use of PPI (RR 1.3, CI 95%: 1.16-3.1) significantly increased the risk of first recurrence of CDAD.

**Conclusions:** CDAD recurrence rates were similar to those reported in the literature. The risk of first recurrence was significantly higher in patients older than 70 who also received PPI treatment.

**Key words:** *Clostridium difficile*-associated disease – nosocomial gastroenteritis – risk factors – recurrence.
of Infectious Diseases, Cluj-Napoca, Romania between January 2011 and October 2012 (22 months) with the diagnosis of CDAD or who developed diarrhoea after admission.

Inclusion criteria in the study were presence of clinical signs and symptoms of CDAD, positive stool culture for C. difficile and presence of C difficile toxins A/B (Vidas bioMerieux). C difficile-associated disease was considered to be hospital-acquired if the patient had been hospitalized when the inciting antibiotics were administered. Patients below the age of 2 years or those with an episode of CDAD in the previous three months were excluded.

The cohort of 306 subjects was divided into two groups: patients who were not readmitted with CDAD within 60 days (n = 246) and patients who required readmission for CDAD within 60 days after discharge from their last admission (n = 60). Although during the follow-up period some patients experienced up to 7 recurrences, we only analyzed the first recurrence.

Additional information was obtained from the patients’ charts regarding: age, gender, co-morbidities, place of onset of the disease (community, hospital), surgical history, antibiotics used, antacids such as proton-pump inhibitors (PPIs), symptoms (fever, abdominal pain, bowel movement, etc.) and laboratory findings (including white blood count, serum albumin level, and renal function tests).

At the onset of the first episode, patients were treated according to IDSA guidelines with metronidazole 500mg 3 times per day or with oral vancomycin 250-500mg 4 times per day [8]. Some patients with severe disease were treated with a combination of metronidazole and vancomycin, concomitantly or sequentially.

In this study, treatment response was defined as the complete resolution of all symptoms after treatment: bowel movement <3 times per day, fever <38°C, no abdominal pain and no positive toxin assay.

Two episodes of CDAD in the same patient were considered to be recurrent events if they occurred within 60 days after a prior episode. A recurrence could correspond either to a relapse with the same strain or to a re-infection with a different strain. It is not possible in clinical practice to differentiate between these two mechanisms, and the term “recurrence” was used as a designation for both.

A patient was considered to have a first recurrence if, within 60 days after the initial diagnosis: (1) there was recurrence of diarrhoea and (2) an additional specimen tested positive for C difficile toxin.

### Statistical analysis

Univariate analysis was conducted to determine the association between different clinical and laboratory variables and the development of recurrent CDAD. Categorical variables were compared using chi-square test. Continuous variables were compared using Student’s t-test or Wilcoxon Rank Sum test depending on whether the data were normally distributed [9]. Time from enrollment to recurrence was analyzed by Kaplan-Meier curves using the log rank test for comparison.

For all statistical analyses, the level of significance was 0.05 and confidence intervals were evaluated at 95%.

Multivariate analysis was based on the Cox model, which was built up sequentially, starting with the largest panel of variables selected after univariate analysis. The variables with the lowest level of significance were then rejected until all the remaining variables were significant [9].

### Ethics statement

This study was approved by the institutional review board of the Teaching Hospital of Infectious Diseases Cluj-Napoca (2/2013). Informed consent was signed by all patients upon admission.

### RESULTS

During the 22 month survey, 306 patients with CDAD were enrolled in our study.

There were 177 women and 129 men (ratio 1.4:1) and the average age was 67.1 ± 15.32 (range 15-91 years, median 71 years). Age distribution in groups of 10 years from 10 to 100 showed that 75% of our patients were over 60 and 50% over 70 (Fig. 1).

From 306 patients, 208 (68 %) had had prior hospitalization in medical or surgical wards; 74 (24%) had had surgery in the previous 3 months; 98 (32%) had community-acquired CDAD with no hospitalization in the previous 3 months [10].

Previous antibiotic treatment was revealed in 195 (64%) of our patients with CDAD, and 140 (46%) had received previous PPI treatment (Table I).

| Table I. Presence of risk factors for CDAD in our patients |
|---------------------------------|-----------|-----------|
| Risk factors                   | No        | %         |
| Previous hospitalization       | Yes       | 208       | 67.97     |
|                                | No        | 98        | 32.03     |
| Previous antibiotic treatment  | Yes       | 195       | 63.73     |
|                                | No        | 111       | 36.27     |
| Previous PPI treatment         | Yes       | 140       | 45.75     |
|                                | No        | 131       | 42.81     |
|                                | Unknown   | 35        | 11.44     |
| Previous surgery               | Yes       | 74        | 24.18     |
|                                | No        | 232       | 75.82     |

Treatment of the first episode of CDAD consisted of vancomycin in 76 (25%) patients and metronidazole in 132 (43%) patients. In 98 (32%) patients, both antibiotics were administered, simultaneously in 59 patients and sequentially in 39 patients. The average duration of treatment was 10 days (minimum 1 day and maximum 30 days).

From 306 patients included in our study, 60 patients (20%) experienced recurrences and 9 patients died (3%). The first recurrence occurred between 3 and 87 days with an average of 16.6 days.
Univariate analysis was used to evaluate the risk factors for the first recurrence according to: age, gender, prior hospitalization, surgery, previous antibiotic and PPI treatment, treatment and duration of therapy with metronidazole, vancomycin or both (Table II). Among these risk factors, only age over 70 was significantly associated with the risk of recurrence (recurrence rate 17% in patients under 70 and 28% in those over 70, p=0.02) (Fig. 2).

The use of antibiotics was associated with a predisposition to infection but the association with the recurrence rates was not statistically significant (p=0.07).

From 166 patients not treated with PPI, 135 patients (81%) had recurrence-free survival interval, while 101 (72%) of 140 patients treated with PPI had recurrence-free outcome (p=0.06). The difference was not statistically significant (Fig. 3).

**Table II. Risk factors for the first recurrence of CDAD (univariate analysis)**

<table>
<thead>
<tr>
<th>Categories</th>
<th>Recurrences +</th>
<th>Recurrences -</th>
<th>p</th>
</tr>
</thead>
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<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
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<td>123</td>
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<tr>
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<tr>
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<tr>
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<td>22</td>
<td>209</td>
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<tr>
<td>Metronidazole &amp; vancomycin no</td>
<td>11</td>
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</table>

*Fig. 1. Patient distribution in 10-year age groups*

*Fig. 2. Kaplan–Meier plot of the 60-day probability of a first recurrence of CDAD according to age (< or ≥70 years)*
There were no significant differences between patients with or without recurrences as far as gender and previous hospitalization were concerned (p>0.05).

The onset of the disease in community or in hospital (with or without previous surgery) were not predictors of recurrences (p>0.05).

Recurrences occurred in 24% of patients treated with metronidazole, 18% of those treated with vancomycin, 21% of those treated sequentially and 27% of those treated concomitantly with both antibiotics. Treatment with metronidazole, vancomycin or both did not prevent recurrences (29% vs. 22%, p=0.18) (Fig. 4). Duration of antibiotic treatment (if other than the recommended 10-14 days) did not significantly influence outcome (p>0.05) (Fig. 5).

The following variables which could influence the recurrence rate were introduced in the multivariate Cox regression model: age over 70, gender, prior hospitalization, prior antibiotic treatment, PPI treatment, use of vancomycin, use of metronidazole and vancomycin simultaneously, treatment for more than 10 days, treatment for more than 14 days.

Age over 70 and use of PPI were the risk factors independently associated with the risk of recurrence after the first favorable outcome (p<0.05).

When considering both risk factors, age over 70 and PPI treatment, we found that 75 of 148 patients (51%) under 70 and 65 of 158 patients (41%) over 70 years had been treated with PPI. The free survival interval was significantly lower when both risk factors were present (62% of patients over 70 who received PPI treatment versus 85% of patients under 70 without PPI treatment, p<0.01). Use of PPI in patients over 70 significantly increased the risk of recurrence. Age over 70 had a RR of 1.5 (CI 95%: 1.055-2.71) and previous use of PPI had a RR of 1.3 (CI 95%: 1.16-3.1) for the first recurrence of CDAD. The cumulative risk was 2.9 for both factors according to the multivariate analysis (Cox regression model) (Fig. 6).

We also compared the risk of the first recurrence in patients older or younger than 70 who had up to three risk factors such as previous antibiotic treatment, previous PPI treatment or hospitalization. According to the multivariate analysis, the risk for the first recurrence significantly increased when three or more risk factors were present both in patients younger and older than 70 (67% vs. 83% and 55% vs. 74%, p<0.01) (Fig. 7).

**DISCUSSION**

To our knowledge, this is the first study on CDAD epidemiology in Romania. We have observed an increase in CDAD cases in Romanian hospitals as well as worldwide, starting with 2005 [11-13]. A first episode of CDAD was followed by a symptomatic recurrence in approximately 5-35% of patients within 2 months, following the resolution of the initial infection. Episodes of recurrent *Cl. difficile* infection are difficult to treat for several reasons. Foremost, data are lacking to support any particular treatment strategy [14]. In addition, treatment of recurrent episodes is not always successful, and repeated, prolonged treatment is often necessary. Identification
Predictors of first recurrence in *Clostridium difficile*

of subgroups at risk for recurrent CDAD may help in diagnosing and treating these patients [15-17]. The incidence of recurrences is lower in Asia (between 5-15%) and higher in Europe, USA and Canada (15-40%) [2, 18-22]. Compared with European data, in our study the number of cases was very high (37 cases/10,000 patient days, 26/1,000 admissions) especially due to the fact that almost all cases of CDAD with onset in different hospitals were referred to our hospital. However, the 20% rate of recurrence is similar to that in other European countries [23].

Having one recurrence puts patients at a high risk of subsequent recurrences [5]. Recurrence typically occurs within 1 to 3 weeks after completion of treatment, but late recurrences of up to 2 months are not infrequent [5, 24]. According to one study, the mean time between the end of therapy for the prior episode and the relapse was 14.5 days, which is comparable with our interval of 16.6 days [25].

Recurrent CDAD occurs either due to relapse (endogenous persistence of the same strain of *C. difficile*) or re-infection (acquisition of a new strain of *C. difficile* from an exogenous source) [20]. The patient’s inability to develop an adequate immune response may be one of the major causes for the development of recurrent CDAD [26-29]. Another possible factor that increases the risk of recurrent CDAD is persistent disruption of the normal colonic flora [30].

The epidemiologic risk factors for recurrent CDAD have been described. Many authors have tried to identify the risk factors for *C. difficile* infection as risk factors for recurrences. In a retrospective study of hospitalized patients from Quebec, a group of Canadian investigators reported that independent predictors of recurrence included: age of 65 years or older, acquisition of CDAD during a hospital stay, and, to a lesser degree, length of hospital stay [3]. McFarland et al also found that increased age as well as decreased quality-of-life scores were associated with a higher risk of recurrent CDAD [5]. More recently, a meta-analysis of 12 studies that assessed risk factors for CDAD found that continued use of non-*C. difficile* antibiotics after diagnosis, concomitant antacid medication, and older age were significantly associated with recurrent CDAD [2]. Other risk factors for recurrence include: low serum albumin level [31], fecal incontinence [32], lower levels of immunoglobulin against toxin B or toxin A [28, 33], infection with the B1/NAP1/027 strain, hospital-acquired disease [3], history of surgery [34], concomitant treatment with antacid medication [2, 31, 32, 35], continued treatment with non-*C. difficile* adequate antibiotics after CDAD and fluoroquinolone use [36].

We attempted to confirm some of these findings but in our study only age higher than 70 and previous use of PPI were independent risk factors for recurrence. The reason why the elderly are more predisposed to recurrences could be the fact that they have more co-morbidities and are more prone to antibiotic treatment [37, 38]. Even if antibiotic treatment is a known risk factor for CDAD, it did not significantly influence recurrence rates in our study. Female gender, the place of onset (community vs. hospital) or surgery did also not influence the recurrence rate. Community-acquired infections did not seem to be less severe or less predisposed to recurrences.

According to multivariate regression analysis, correlation of PPI medication with age over 70 significantly increased the relative risk of recurrence. The mechanisms by which acid suppressive agents increase the risk of CDAD development and recurrence are poorly understood. However, although the spores of *C. difficile* are resistant to gastric acid, it has been suggested that the survival and germination of *C. difficile* spores are greater at lower acidity, and that higher gastric pH increases vegetative bacteria counts in the small and large intestine. Previous studies found that gastric acid suppression increases gastric and small bowel colonization by bacteria and *C. difficile* [31, 39].

All guidelines recommend metronidazole as the first line of treatment in non-severe CDAD and vancomycin only in severe cases [8, 40]. In recent years, studies regarding the increasing risk of failure or relapse after metronidazole treatment have been published. Pepin et al in a large retrospective study reported a recurrence rate of 28.9% after metronidazole treatment [3]. Moreover, another study reported a recurrence rate of 38% after metronidazole [41].

In our study, the recurrence rate after metronidazole treatment was comparable with data published in the Canadian study and lower than that published in the American study [3, 41]. The sustained response rates to metronidazole (74%) and vancomycin (82%) were higher compared to the above-mentioned reports but lower than results published in Korea (87.4%) [36-38].

Vancomycin was used only in severe cases, in association with or after metronidazole administration because of suboptimal response. The recurrence rate among patients treated with metronidazole was not different from that among patients treated with vancomycin (24% for metronidazole vs. 18% for vancomycin), which is comparable with data published by Pepin et al (21.2% vs. 16.7%) [42]. Such recurrence rates are similar to those observed for metronidazole (19%) and vancomycin (18%) in a trial of the toxin-binding agent televamer [43]. In another study published this year, the recurrence rates in patients treated with metronidazole or vancomycin were 7% and 14%, respectively (p<0.025) [44].

We could not confirm a significant difference regarding recurrences in patients treated with metronidazole, vancomycin
or both, even when we compared patients with the same severity of disease.

In our study, the recurrence rate in patients treated with vancomycin was 18%, significantly lower than that recorded in a fidaxomicin trial during a 30-day follow-up period (25%) or in the anti-toxin monoclonal antibody study (32%) [45, 46]. The newly approved antibiotic fidaxomicin had significantly lower recurrence rates compared to vancomycin, which indicates its usefulness in patients at high risk of recurrences [45, 47].

Using a combination of five simple clinical and laboratory variables measured at the time of CDAD diagnosis, Miller et al elaborated an 11-category scoring system (ATLAS), which seems to be able to accurately predict treatment response to CDAD therapy [48].

Introducing different clinical variables in the Cox model, we observed that if three or more factors are present at the same time, the risk of recurrence significantly increases regardless of age. Not all variables used in the Cox model had a significant influence in univariate or multivariate analysis; therefore, all factors could have a cumulative effect on the relative risk of recurrence.

Recurrences are a challenge for both physicians and patients. Awareness of the risk factors for recurrence may help the physician select appropriate treatment options.

Our study confirmed literature data regarding the risk of recurrence in patients over 70 treated with PPI or having multiple risk factors. However, it could not confirm the risk of CDAD recurrence in patients with hospital-acquired disease, a history of surgery or in female patients. Failure to select and compare patients according to disease severity constitutes one of the main study limitations.

**CONCLUSIONS**

In our patients, CDAD had a moderate risk of recurrence. The risk of first recurrence was significantly higher in patients over 70 years who had previously received PPI treatment. The presence of more than three risk factors increased the risk of recurrences. A treatment period longer than 14 days did not reduce the recurrence risk.

**Conflicts of interest:** No conflict to declare.

**REFERENCES**

Predictors of first recurrence in *Clostridium difficile*  


