Severity factors of *Clostridium difficile*-associated colitis with attention to at-risk conditions and immunosuppressive states

Ph.D. thesis

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1. INTRODUCTION

Today, the treatment of hospital-acquired *Clostridium difficile*-associated colitis (CDAC) represents a significant challenge to patients, the health care system, and the national economy alike. In a methodology guideline document jointly issued in 2011 by Hungary's National Centre for Epidemiology, the Professional Association for Medical Microbiology, and the Professional Association for Infectology, the drugs metronidazole and vancomycin are recommended for the treatment of the disease (Epinfo, 2011). Choice between the two is recommended to be based on clinical severity: mild or medium-severity cases require oral metronidazole, and severe cases, oral vancomycin treatment.

Symptoms and signs listed by Hungarian methodological guidelines as indicative of a severe infection include: fever, cold chills, symptoms of peritonitis, signs of ileus, alarming laboratory findings (white blood cell count $> 15\times10^9$/l, left shift, elevation of serum creatinine > 50%, anaemia, hypoproteinaemia, elevated serum lactate), intestinal wall thickening confirmed via imaging, accumulation of lipid tissue around intestinal walls, ascites, bowel distension > 6 cm, and pseudomembranous colitis confirmed via colonoscopy.

Clinical manifestations may vary in high-risk patients: immunosuppressive treatment, diabetes, tumorous disease, haematological disease, chronic liver disease – i.e. conditions of secondary immunodeficiency – may modify the disease's classic set of symptoms towards a subdued clinical presentation. These patients will greatly benefit from appropriately chosen initial treatment through more rapid recovery, improved control of diarrhoea-related dehydration symptoms, as well as prevention of severe complications and premature mortality.

2. OBJECTIVES

1. Daily follow-up and consultation services for CDAC patients at inpatient departments of Kenézy Gyula Hospital and Outpatient Facility.
2. Severity classification of patients, assessment of risk factors and immunosuppressive states, and identification and initiation of antibiotic treatment based on severity classification and in accordance with guidelines.
3. Evaluation of the association between risk factors and immunosuppressed states present in severe cases, and therapeutic efficiency; identification of independent predictive factors, if any, the presence of which can be a standalone alarming indication of a high case severity.
4. Assessment of how severity scores, risk factors, immunosuppressive states, and treatment modalities affect case mortality.

3. METHODS (PATIENT GROUPS, EXAMINATION SPECIMENS)

3.1 Case definitions

Based on 2014 European and Hungarian guidelines, we applied the following case definitions (Debast, 2014):

Nosocomial infection – onset of symptoms is at least 48 hours after hospital admission and/or within four weeks of discharge.

Therapeutic response – antibiotic treatment is regarded efficacious if there is a reduction of bowel movement frequency, or an improvement of stool consistency, a reduction of severity parameters, and there are no emerging symptoms indicative of deterioration.

Immunosuppressed state – patients with the following conditions are considered to be secondarily immunosuppressed: diabetes mellitus, autoimmune disease, chronic hepatitis, and reception of immunosuppressive therapy within one month prior to onset of diarrhoea.

Mild, medium-severity CDAC – watery diarrhoea is usually the only symptom.

Severe CDAC – in addition to clinical symptoms of high-frequency diarrhoea (bowel movements > 6 times a day), other accompanying clinical symptoms of severity emerge, such as fever, dehydration symptoms, as well as certain laboratory deviations and severe radiological signs (as detailed below).

Relapse – a re-emergence of infection subsequently to a completed antibiotic course, after two symptom-free days, within eight weeks of the onset of the primary event.

Fatality – we considered the relationship between CDAC infection and death to be causal when the patient died in relation to CDAC treatment within 30 days counting from the first day of treatment, or after 30 days if PMC was unquestionably confirmed via autopsy and the death occurred within the patient's CDAC-related nursing period. Deaths *after 30 days* were not considered to be a direct consequence of CDAC if pathological signs of CDAC were ruled out by autopsy, or if the treating physician declared a different cause of death in cases without autopsy.

3.2 Operation of the Clostridium difficile surveillance service

This thesis is a data assessment of all adult patients presenting with and treated for CD infection by inpatient departments of Kenézy Gyula Hospital and Outpatient Facility in the period January 1, 2012 through June 30, 2013. Patients were treated on the premises of the department where they acquired the infection. Most cases presented at an internal medicine department, and also at departments of

pulmonology, chronic nursing, urology, infectious diseases, surgery including limb surgery, neurology, rheumatology, and rehabilitation.

Departments in charge of treatment maintained a practice of patient isolation in line with infection control guidelines and regulations. Upon observation of diarrhoea and confirmation of infection (*Clostridium difficile* antigen and toxin positivity), the microbiological laboratory notified the consultant physician and an infectology resident or candidate consultant member of the surveillance service, who then made contact with the patient at the treating department.

3.3 Severity score – assessment points, classification

Formulated by our surveillance service on the basis of a system in use in England, the severity scoring algorithm included: severe increase in the frequency of daily bowel movements (> 6) as monitored using the Bristol stool scale, white blood cell count (WBC) elevation (> 15 G/l), serum albumin deficiency (< 25 g/L), acute elevation of serum creatinine (creat), onset of fever (> 38.3 C), and severe radiological abnormalities. Each parameter present was assigned a score of one point; points were then summed up (**Table 1)**.

Table 1. *C. difficile* surveillance service daily evaluation chart (Kenézy Gyula Hospital)

Severity score points		**Date**			
WBC > 15 G/l	1 point	Days of treatment	0th	1st	2nd
Albumin < 25 g/L	1 point	WBC (G/l)			
Acute elevation of creatinine level	1 point	Serum albumin			
Stool count > 6/day	1 point	CRP			
Temperature > 38.3C	1 point	Serum potassium			
Severe radiological signs	1 point	Serum creatinine			
		Stool count/day			
Total maximum: 6 point		Bristol scale			
Score evaluation:		**Initial therapy**			
Mild, medium-severity cases: 0-2 points		3×500 mg metronidazole orally			
Severe cases: 3-6 points		4×125 mg vancomycin orally.			

Bristol scale: assessment chart for an objective evaluation of stool consistency, with grades 1 to 7 for progressive levels of softness. Grades 1 and 2 represent constipation, 3 and 4 are for normal stool forms, and grades 5 to 7 indicate diarrhoeal stool.

Radiological abnormalities: these indicate a severe case if there is bowel distension (> 6 cm) confirmed via imaging, intestinal wall thickening, accumulation of lipid tissue around intestinal walls, or ascites not attributable to any other cause.

Based on the severity score classification, we defined two distinct disease forms: patients scoring 0 to 2 points were classified as mild/medium severity, and those scoring 3 to 6 points, as severe cases. We recommended a switch from

metronidazole used in the first group to vancomycin if the patient's condition and clinical symptoms did not change within three days, the patient failed to respond to first-choice antibiotic treatment, or their condition was observed to deteriorate. For analysis, patients were classified into three categories based on their antibiotic treatment history: group 1, metronidazole treatment (M); group 2, treatment switch (from metronidazole to vancomycin, M/V); and group 3, patients initially started on vancomycin (V). We consulted with the treating physician upon each patient on a daily basis, and for cases with deteriorating or persistent symptoms, we recommended a treatment switch.

3.4 At-risk groups, susceptibility factors

Highlights of risk factors representing a susceptibility for CDAC include age (> 65 years), co-morbidities, PPI (proton pump inhibitor) use within one month prior to CDAC, antibiotic (AB) use within two months prior to CDAC, abdominal surgery immediately preceding the infection, prior prolonged (> 21 nap) hospital treatment, and chronic renal insufficiency or dialysis. Based on the presence of these risk factors, we classified patients into risk groups with 1, 2, 3, 4, 5, or 6 risk conditions present. We made a distinction between patients treated and not treated with PPI; the PPI-untreated group was a pool of patients receiving no acidity regulator treatment at all and those receiving H2-blockers. Patients were considered immunosuppressed if diabetes mellitus, autoimmune disease, or chronic hepatitis was confirmed, and/or when the patient had received immunosuppressive therapy within one month prior to the onset of diarrhoea.

3.5 Microbiological examination

Microbiological examination of the stool of CDAC patients was based on rapid enzyme immunoassay (Techlab®), with a parallel assessment of the presence of *C. difficile* glutamate dehydrogenase antigen, toxin "A", and toxin "B". For antigen positive, toxin negative results, toxin assessment was repeated using a Wampole® immunoassay rapid test for the detection of toxins "A" and "B". Cases that continued to be toxin negative upon the repeat test were examined further by toxin "A" and "B" assay on a stool culture. All toxin positive samples prompted the starting of a stool culture for detection of *C. difficile* infection.

3.6 Statistical methods

For descriptive purposes, categorical variables were described using absolute and relative frequencies of categories, and continuous variables, using count, mean, and standard deviation statistics. Unadjusted comparisons between patient groups were made using Fisher's exact tests for categorical variables; for continuous variables, Student's two-sample t tests or Wilcoxon's rank sum tests were used, subject to distributional normality assumptions for t tests being satisfied or not. The effects of available clinical factors on case fatality, a binary outcome, were analysed using multiple logistic regression modelling; results were expressed as odds ratios with 95% confidence intervals. The analysis included Charlson's index, a validated prognostic indicator derived from age and presence or absence of 16 distinct co-morbidity states, with higher index values indicating poorer mortality outlooks (Charlson 1987).

4. RESULTS

4.1 Epidemiological data

Of our 164 patients, 56.1% were female, whose mean age (75.6 years) was significantly higher than that of male subjects (72.4 years;**Table 2**). The at-risk group of those older than 65 years included 79.27% of patients.

4.2 Immunosuppressed state

A state of secondary immunosuppression was confirmable in a total of 98 subjects. There were 18 autoimmune disease cases, 34 subjects with a tumorous disease, 50 diabetics, and 13 patients were identified as having received prolonged immunosuppressive therapy. In 33 patients, an accumulated (> 2) presence of immunosuppressed states was observed. Prolonged immunosuppressive therapy was present in 7.93% of all patients; the condition was more frequent in women (10.87%). Of immunosuppressed patients, 66 (67.35%) had received prior PPI therapy, a significantly greater proportion ($p = 0.03$) than in immunocompetent subjects. The three treatment groups did not differ with respect to PPI and AB use (64.29%, 58.62%, 47.22%, $p = 0.244$).

4.3 Cases of recurrent disease

Patients affected by CDAC recurrence were younger on average (72.4 years) than subjects without recurrent episodes (74.2 years). As to the frequency of immunosuppressed states, these patients did not differ from subjects without recurrence ($p = 0.999$). In our sample, the treatment group receiving vancomycin

only showed an elevated incidence of recurrent cases; however, the difference was not significant (p = 0.934).

Table 2. Epidemiological data of patients with *Clostridium difficile* infection

	NUMBER (%) OF PATIENTS		TOTAL (%)	P
	2012	2013		
All patients	98	66	164	0.649
Age				
≤ 65 years	22 (22.5)	12 (18.2)	34 (20.7)	0.560
> 65 years	76 (77.6)	54 (81.8)	130 (79.3)	
Sex				
Male	44 (44.9)	28 (42.4)	72 (43.9)	0.873
Female	54 (55.1)	38 (57.6)	92 (56.1)	
Risk factors				
Prior PPI treatment	62 (63.3)	34 (51.5)	96 (58.5)	0.148
Prior antibiotic treatment	95 (96.9)	59 (89.4)	154 (93.9)	0.091
Initial antibiotic treatment				
metronidazole (oral)	44 (44.9)	26 (39.4)	70 (42.7)	0.140
switch to vancomycin (oral)	29 (29.6)	29 (43.9)	58 (35.4)	
vancomycin only (oral)	25 (25.5)	11 (16.7)	36 (22.0)	
Recurrence	19 (19.4)	7 (10.6)	26 (15.9)	0.190

4.4 Mortality data

General characteristics

The observed lethality figure in our sample was 23.2% (38 out of 164 patients, **Table 3**). In the year 2012, 24 patients (24.49%) died, of whom four had developed a relapse; in 2013, 14 patients (21.21%) died, none of which were relapse cases. A total of 21 patients of the 38 fatalities underwent an autopsy, which confirmed

pseudomembranous colitis in seven of those cases; in one patient, the causal relationship between CDI and the fatality could not be fully ascertained. In the 17 patients without autopsy, the treating physician declared a presumable causal association between CDI and the death judging by clinical progress.

Table 3. Mortality data - outcome

Patients	**Number (%) of patients**		**Total (%) subjects**	**p**
Outcome	2012	2013, 1st half		
Recovery	59 (60.2)	45 (68.1)	104 (63.4)	0.288
Recurrence	19 (19.39)	7 (10.61)	26 (15.85)	0.190
Deaths in recurrent cases	4	0	4 (10.53)	0.447
Deaths total	24 (24.49)	14 (21.21)	38 (23.2)	

Risk factors, immunosuppressed state

The overall mortality figure in our patients was 23.17%, reaching 24.64% in the subgroup without recurrent infections, and 15.38% in cases with recurrent CDAC. The figure was 24.49% in 2012, to decrease to 21.21% in 2013.

Within the first half of 2013, no deaths were observed in patients of recurrent disease. Men were observed to have a greater incidence of death (26.93%). In patients with prior antibiotic treatment, there was a 23.38% mortality, which did not constitute a significant difference relative to those without such treatment history. A substantial proportion of patients had used a PPI before the infection (96 cases); mortality in this group was higher, with the difference approaching

significance (28.13%; vs 16.18%; p = 0.091). In patients in an immunosuppressed state, mortality was higher than in those not having received such treatment (27.55% vs 18.03%, p = 0.180). When there were multiple immunosuppressed conditions present at the same time, a tendency of increasing lethality was observable (0 = 17.74%, 2-4 = 32.43%, p = 0.244).

Of the various immunosuppressed states, a history of prolonged immunosuppressive therapy (13 patients) was identified to be associated with a significantly elevated mortality relative to those not having received similar treatment (53.85% vs 20.53%, p = 0.012). The female to male ratio of these 13 patients was 10 to 3, and their mean age was 72.7 years. Their most common primary disease was chronic obstructive pulmonary disease (7 patients, 54%). The treatment agent was a steroid in all these cases, with the additional risk factor of prior antibiotic therapy being also present. A single case of recurrence was observed in this patient group. No primary immunodeficiency patients were observed in the examined population.

Severity score and mortality figures

In 2013, more patients were classified into the severe score group than in 2012 (24.24% vs 16.67%); overall, 19.75% of patients were in this group. All three treatment groups showed a closely similar occurrence of pseudomembranous colitis. Of the patients in the high score group, 7.35% received metronidazole treatment (5 cases); 32.76% of patients had their treatment switched to vancomycin. There was no significant mortality difference between the three treatment groups (overall, 23.17%; p = 0.607); the highest level of lethality was observed in treatment switch patients (27.59%). Higher score values showed no significant association with the presence of an immunosuppressed condition with respect to case outcome. Metronidazole-only therapy was given to 42.68% of

patients, 35.37% had their treatment switched, and 21.95% were started initially on vancomycin treatment. Increasing score was confirmed to be associated with a tendency of steadily increasing mortality, with a significant difference ($p = 0.045$).

Characteristics of cases with recurrent disease

Patients with a recurrent course had a lower mortality than those without (24.6% vs 15.38%); there was no significant difference between males and females. No deaths were observed in patients with recurrent infection in the first half of 2013.

4.5 Regression analysis findings

When added to the initial model, no extra variables showed other than a neutral effect or behaved as confounding factors, and were therefore not included in the final model. Upon exploring further interactions, no evidence of significant effect modification phenomena between the explanatory variables was found.

The findings of the final multiple regression model are summarized in **Table 4.** The interaction between treatment scheme and case severity was significant (3-5 point severity category × M/V treatment switch group: $p = 0.045$; 3-5 point severity category × vancomycin treatment group: $p = 0.051$). The main effects of treatment scheme were themselves not significant, which is presumably explained by confounding by indication. In patients of the metronidazole treatment group, cases classified as severe had close to 25 times greater odds for a fatal outcome than non-severe cases (OR = 24.8; 95% CI: 2.6 – 232.8, $p = 0.0049$). This effect was reduced in the treatment switch group, and practically failed to manifest in patients treated with vancomycin as a first choice.

The adjusted effect of PPI treatment, although not significant, indicated an approximate double odds for death relative to the pooled reference group of

patients receiving no such treatment or using a H2-blocker only (OR = 1.9; 95% CI: 0.7 – 5.0, p = 0.18). Prolonged immunosuppressive treatment was estimated to be associated with a greater than fourfold increase in lethality odds (OR = 4.7; 95% CI: 1.2 – 18.0, p = 0.025). Averaging at 6.8 points (SD = 2.97) across the whole sample, a single-point increase in Charlson's index led to a strongly significant 44% increase of the odds of death (p < 0.0001). Model checking revealed no indication of insufficient fit (Hosmer–Lemeshow test: p = 0.3458).

Table 4. Effects of clinical factors on odds of death in CD infected patients as estimated by multiple logistic regression modelling. Abbreviations: CI, confidence interval; M, metronidazole; M/V, switch from metronidazole to vancomycin; PPI, proton pump inhibitor; T, therapy; V, vancomycin; vs, versus

Factor	Contrast	Stratum	OR	95%CI	p
Therapy	M/V vs M	Score = 0-2	1.876	0.589; 5.969	0.2869
Therapy	M/V vs M	Score = 3-6	0.123	0.012; 1.306	0.0821
Therapy	V vs M	Score = 0-2	2.040	0.537; 7.756	0.2954
Therapy	V vs M	Score = 3-6	0.103	0.007; 1.449	0.0920
Score	3-6 vs 0-2	T = M	24.830	2.648; 232.841	0.0049
Score	3-6 vs 0-2	T = M/V	1.625	0.389; 6.792	0.5058
Score	3-6 vs 0-2	T = V	1.257	0.171; 9.256	0.8221
Charlson index	+1 point	all patients	1.438	1.220; 1.694	< 0.0001
Age	+1 year	all patients	1.000	0.953; 1.049	0.9958
Acidity regulator therapy	PPI vs none or H2 blocker only	all patients	1.916	0.738; 4.975	0.1818
Prolonged immuno-suppressive therapy	yes vs no	all patients	4.659	1.205; 18.021	0.0258
Sex	male vs female	all patients	1.463	0.588; 3.641	0.4132

5. CONCLUSIONS

In our analysis sample, patients receiving prolonged immunosuppressive therapy had a significant 4.7 times greater odds for fatality than those without such treatment. This finding raises the possibility that immunosuppressive treatment might be a useful addition to the severity scoring algorithm as a seventh factor, extending the scoring range to 0 to 7 points. By including this assessment point in

the severity scoring system as a seventh risk entity, we could facilitate the immediate classification of affected patients as severe cases (subject to the presence of another two severity points), then start them on vancomycin as a first choice, thereby reducing their risk of mortality.

Recently introduced European guidelines list immunodeficiency conditions as a risk factor for the development of severe CDAC, which corroborate our study findings (Debast 2014). In light of the above, further research is still required to assess independent predictive risk factors for their effect on case lethality in CDI, with special emphasis on secondary immunosuppressed states; understanding these relationships better has the potential to improve choice algorithms for optimum treatment.

Performance assessment studies on a number of CDI severity scoring systems are available in the literature (Velazquez-Gomez 2008, Fujitani 2011). A comparison of severity score systems was undertaken by Shigeki et al in 2011; eight different scoring systems were compared. The authors highlighted the Hines VA index, which includes onset of fever, radiological abnormalities, blood pressure readings, and pathological white blood cell counts; a score of 3 or greater classifies a case as severe. In overall assessment, the authors found this score system to be the best predictor of the severity of CD infection. Our local adaptation efforts of the system as used in the UK should be expanded to a nationwide level in Hungary to establish a practice of advanced and rapid professional therapeutic decision making in the management of CDI patients.

Due to its severity and associated high mortality, CD infection is a crucial public health challenge, placing a substantial burden on our inpatient departments; timely and appropriately chosen treatment is quintessential as it is known to improve outcomes.

The quality of a hospital's advanced antibiotic treatment policy is truly attested by the incidence and reduction of CD infection. Efforts to achieve strict adherence to antibiotic treatment guidelines introduced at Kenézy Gyula Hospital and Outpatient Facility as of the second half of 2013 have the potential to reduce the incidence of CD infection.

Adequate and more reserved use of PPI treatment in line with modern guidelines may further improve these indicators.

The quality of the assessment of our prospective study is reduced by the presence of five patients whose first choice treatment by their departments was out of line with their case severity status due to failure to seek consultancy with our surveillance service.

6. SUMMARY

Our *Clostridium difficile* surveillance service for inpatient departments of Kenézy Gyula Hospital and Outpatient Facility has been operational since January 1st 2012. During the period covered, we have followed up the cases of 164 patients on a daily basis. A severity score system had been implemented to assist therapeutic decision making: mild or moderately severe cases (score: 0 to 2) were to receive metronidazole, while severe (score: 3 to 5) cases were to be started on vancomycin. We assessed patients in terms of presence of risk factors and immunosuppressed states, and analyzed whether these factors had an effect on treatment efficacy and outcomes.

1. Our surveillance service initiative has shown to be successful: the hospital's departments have come to accept the system's existence, resulting in adjustments to their continued treatment management procedures to comply with national guidelines. The severity classification system has made therapy decisions more straightforward than before.
2. When it comes to evaluating immunosuppressed states, immunosuppressive therapy prior to the infection is a red flag entity that, if included in the group of risk factors underlying the severity score, has the potential to further improve professional therapeutic judgement in these high risk cases.
3. The adjusted estimate of the effect of antacid therapy indicated more than a doubling of the odds for death in patients treated with proton pump inhibitors, relative to a pooled group of those not on such therapy or only taking H2 blockers for acidity neutralization. Our statistical analysis suggests that the effects of multiple target proton pump inhibitors – as opposed to those of single-target H2 blockers – represent an increased level of risk during CD infection, and may change prognosis for the worse, which is why we recommend general restrictions on continued PPI therapy in patients with CD infection, except when thorough deliberation identifies such treatment to be indispensable.

7. LIST OF PUBLICATIONS AUTHORED BY CANDIDATE

Candidate's publications of relevance to the content of the PhD thesis

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