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Case fatality associated with a hypervirulent strain in patients with culture-positive *Clostridium difficile* infection: a retrospective population-based study

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SUMMARY

Background: Clostridium difficile is a major infectious cause of healthcare-associated diarrhea. The epidemiology of *C. difficile* infection (CDI) is changing, with evidence of increased incidence and severity. The first patient with a hypervirulent strain type in Pirkanmaa Hospital District, Finland was reported in September 2008.
Methods: We reviewed all culture-positive *C. difficile* episodes that occurred in Pirkanmaa Hospital District during the period September 2008 to May 2010.
Results: A total of 780 episodes of *C. difficile* occurred in 622 patients. A hypervirulent strain caused 14.2% of all episodes. The day 30 case fatality associated with CDI was 8.5% in episodes with a non-hypervirulent strain and 20.7% in episodes with a hypervirulent strain type ($p < 0.001$, odds ratio 2.8, 95% confidence interval 1.6–4.8). The median age among those infected by a hypervirulent strain was higher than among those infected by a non-hypervirulent strain (83 vs. 75 years, $p < 0.001$). Hypervirulent strain type remained a significant factor associated with case fatality in a logistic regression model. Blood leukocytes were significantly higher in episodes due to a hypervirulent strain (11.0 vs. 9.4 $\times 10^9$/l, $p = 0.007$). Blood leukocytes and C-reactive protein (CRP) on the day of diagnosis were significantly higher in non-survivors compared to survivors in CDI (13.2 vs. 9.6 $\times 10^9$/l, $p = 0.009$, and 106.0 vs. 79.4 mg/l, $p < 0.001$, respectively).
Conclusions: Infection due to a hypervirulent strain is a factor associated with increased case fatality in CDI. Blood leukocytes are significantly higher in CDI caused by a hypervirulent strain. Leukocyte count and CRP are useful prognostic biomarkers in patients with CDI.

1. Introduction

Clostridium difficile is a leading infectious cause of healthcare-associated diarrhea. Several countries have reported increased *C. difficile* infections (CDI) and outbreaks, which have been attributed to the emergence of hypervirulent strains.[1–3] CDI ranges from mild diarrhea to severe diarrhea, shock, paralytic ileus, pseudomembranous colitis, toxic megacolon, and death. The treatment options for severe CDI have been limited so far. However, several novel therapies for CDI are now at different stages of development.[4]

There has been an increase in the morbidity, mortality, and economic burden associated with CDI.[1] A few studies have examined the role of hypervirulent *C. difficile* strain type as a predictor of outcome in this condition. In some studies, CDI due to hypervirulent strain types has been associated with a significantly increased case fatality rate,[5,6] although not all investigations have confirmed this.[2,7] The emergence of toxigenic strains is a major concern in healthcare facilities, and affects elderly and immunocompromised patients in particular.[6] Advanced age has been shown to be a major risk factor for fatal CDI.[6,8] However, Miller et al. indicated that infection due to a hypervirulent strain would constitute an independent risk factor for mortality after adjusting for patient age.[6] Early prognostic stratification of patients with infectious diseases is a major challenge, and is necessary in order to target therapeutic strategies efficiently. Thus, biomarkers may offer important information to clinicians.

We retrospectively reviewed all culture-positive episodes of *C. difficile* after the emergence of the first case of a hypervirulent

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strain in our hospital district in Finland. We compared the outcomes in patients infected with a hypervirulent strain to those of patients infected with a non-hypervirulent one. We also assessed the prognostic value of basic laboratory markers in CDI. We determined that hypervirulent strain and advanced age were factors associated with increased case fatality in CDI, and that leukocyte count and C-reactive protein (CRP) are useful prognostic biomarkers in patients with CDI.

2. Methods

2.1. Pirkanmaa Hospital District

The Pirkanmaa Hospital District is a joint municipal authority comprising 22 municipalities and is responsible for the healthcare services of about 477 600 inhabitants (population in year 2009). The overall population of Finland in 2009 was 5 351 427. Thus, Pirkanmaa Hospital District covers the health care of approximately 9% of the total Finnish population.[9]

2.2. *Culture-positive* Clostridium difficile *episodes*

We searched the laboratory database of the Centre for Laboratory Medicine, Pirkanmaa Hospital District, Tampere, for all patients with cultures positive for *C. difficile* detected in Pirkanmaa Hospital District during the period September 10, 2008 to May 26, 2010. In Pirkanmaa Hospital District *C. difficile* cultures are performed only for those patients with signs and/or symptoms of *C. difficile* (diarrhea and/or pseudomembranous colitis or toxic megacolon). Asymptomatic carriers are not sought or tested. Demographic characteristics including age and sex were recorded. Underlying conditions were also recorded; data were available for 157 episodes. Only patients aged ≥16 years were included. Patients with multiple positive cultures for *C. difficile* were also recorded, and episodes were considered as separate episodes if the subsequent culture positivity was detected ≥15 days after the previous one. Day 7, day 14, day 30, and day 90 case fatality following positive culture for *C. difficile* was studied using the Finnish Population Register Centre database.

2.3. Laboratory tests

Stool samples were cultured on selective cycloserine–cefoxitin–egg yolk–fructose agar (CCFA) broth medium plates, and typical colonies for *C. difficile* were identified 2 days later. Antimicrobial sensitivity testing to penicillin, clindamycin, vancomycin, metronidazole, and moxifloxacin was done for all strains. Since resistance to moxifloxacin is typical for *C. difficile* hypervirulent strain ribotype 027,[10] which was the prevailing hypervirulent strain in Finland during the study period,[8] the hypervirulence test was carried out for all moxifloxacin-resistant strains, either by in-house PCR[11] or by GeneXpert test (Cepheid, Sunnyvale, CA, USA).

Other laboratory tests included were plasma CRP (mg/l; data available for 399 patients), plasma creatinine (μmol/l; data available for 319 patients), blood platelets ($\times 10^9$/l; data available for 382 patients), and leukocytes ($\times 10^9$/l; data available for 382 patients). Day 0 (culture day) values were recorded.

2.4. Statistical methods

SPSS statistical software package (version 7.5/10) was used for the statistical analyses, and a two-sided p-value of <0.05 was taken as the cut-off for statistical significance. Categorical data were analyzed by Chi-square test or Fisher's exact test as appropriate, and non-parametric data by Mann–Whitney *U*-test or Kruskal–Wallis test. A logistic regression model was used to study the independent effect of hypervirulent strain type on mortality models adjusted for potential confounders. Odds ratios (ORs) were expressed with their 95% confidence intervals (CI).

3. Results

Seven hundred and eighty episodes in 622 patients were detected during the study period. One hundred and eleven episodes (14.2% of all isolates) were caused by a hypervirulent strain and 669 episodes

Table 1
Characteristics of the study population. All culture-positive episodes of *Clostridium difficile* in Pirkanmaa Hospital District from September 10, 2008 to May 26, 2010 were included in the study. Results are *n* (%) or median (quartiles).

Characteristic	All episodes ($N=780$)	Non-hypervirulent strain ($n=669$)	Hypervirulent strain ($n=111$)	OR (95% CI)	p-Value[a]
Demographic characteristic/underlying diseases					
Male sex	283 (36.3%)	243 (36.3%)	40 (36.0%)	1.0 (0.7–1.5)	0.938
Age, years	77 (63–86)	75 (61–85)	83 (77–88)		<0.001
Age >65 years	559 (71.7%)	457 (68.3%)	102 (91.9%)	5.3 (2.6–10.6)	<0.001
Atherosclerosis[b]	45 (28.7%)	31 (22.8%)	14 (66.7%)	6.8 (2.5–18.3)	<0.001
Coronary artery disease[b]	43 (27.4%)	35 (25.7%)	8 (38.1%)	1.8 (0.7–4.6)	0.237
Diabetes[b]	31 (19.7%)	25 (18.4%)	6 (28.6%)	1.8 (0.6–5.0)	0.275
Cancer[b]	36 (22.9%)	34 (25%)	2 (9.5%)	0.3 (0.1–1.4)	0.116
Dementia[b]	30 (19.1%)	25 (18.4%)	5 (23.8%)	1.4 (0.5–4.1)	0.556
COPD[b]	12 (7.6%)	11 (8.1%)	1 (4.8%)	0.6 (0.1–4.6)	1.0
At least one chronic disease[b]	146 (93.0%)	125 (91.9%)	21 (100%)	1.2 (1.1–1.2)	0.362
Case fatality					
Age in deceased patients (day 30 case fatality)	82 (75.7–8.8)	82 (76.0–88.9)	82 (77.0–88.1)		0.811
Day 7 case fatality	34 (4.4%)	24 (3.6%)	10 (9%)	2.7 (1.2–5.7)	0.009
Day 14 case fatality	53 (6.8%)	41 (6.1%)	12 (10.8%)	1.9 (0.9–3.7)	0.069
Day 30 case fatality	80 (10.2%)	57 (8.5%)	23 (20.7%)	2.8 (1.6–4.8)	<0.001
Day 90 case fatality	145 (18.6%)	112 (16.7%)	33 (29.7%)	2.1 (1.3–3.3)	0.01
Laboratory parameters (day 0)[c]					
CRP, mg/l ($n=399$)	82.2 (3.0–132.3)	82.4 (31.5–137.5)	79.3 (38.8–126.7)		0.561
Blood leukocytes, $\times 10^9$/l ($n=382$)	9.8 (7.0–14.7)	9.4 (6.7–14.3)	11.0 (8.8–16.9)		0.007
Plasma creatinine, μmol/l ($n=319$)	79 (61–117)	83 (61–117)	79 (57.5–108)		0.691
Blood platelets, $\times 10^9$/l ($n=382$)	269 (199–353)	270 (195–350)	265 (209–370)		0.231

OR, odds ratio; 95% CI, 95% confidence interval; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein.
[a] The p-value indicates the difference between groups of patients infected with a non-hypervirulent strain type compared to a hypervirulent strain type.
[b] Data available for 157 patients; 136 with a non-hypervirulent strain and 21 with a hypervirulent strain.
[c] Measured on the day of positive culture for *Clostridium difficile*.

Table 2
Age-specific all-cause day 30 case fatality in *Clostridium difficile* infection.

Age group, years	Hypervirulent strain ($n=111$)	Non-hypervirulent strain ($n=669$)	OR (95% CI)	p-Value[a]
<65	2 (22.2%)	5 (2.4%)	11.9 (1.9–72.0)	0.028
65 to 74	4 (25%)	6 (5.8%)	5.4 (1.3–22.1)	0.028
>74 to 86	9 (19.6%)	26 (12.4%)	1.7 (0.7–4.0)	0.236
>86	8 (20%)	20 (13.9%)	1.6 (0.6–3.8)	0.341

OR, odds ratio; 95% CI, 95% confidence interval.
[a] p-Value indicates the difference between groups of patients infected with a non-hypervirulent strain type compared to a hypervirulent strain type.

Table 3
Logistic regression model of the factors associated with day 30 case fatality in *Clostridium difficile* infection.

Variable	OR (95% CI)	p-Value
Hypervirulent strain	4.6 (1.4–15.0)	0.01
Age >75 years[a]	1.5 (0.5–4.9)	0.496
Male sex	1.7 (0.6–4.9)	0.361
Atherosclerosis[b]	2.4 (0.8–7.2)	0.134

OR, odds ratio; 95% CI, 95% confidence interval.
[a] Age remained a significant factor associated with mortality if atherosclerosis variable was not included in the model (continuous variable or age >75 years).
[b] Data available for 157 patients; 136 with a non-hypervirulent strain and 21 with a hypervirulent strain.

by a non-hypervirulent strain (85.8% of all isolates). The median age of patients was 77 years (range 17–100 years). Age among those infected with a hypervirulent strain was higher than in those infected with a non-hypervirulent strain (median 83 vs. 75 years, $p < 0.001$). The majority of patients (>60%) were women (Table 1).

Death occurred within 30 days of culture in 80 episodes (10.3%). Infection due to a hypervirulent strain was associated with a higher case fatality rate at day 30 as compared to infection caused by a non-hypervirulent strain (20.7% vs. 8.5%; OR 2.8, 95% CI 1.6–4.8) (Table 1). Those who died of CDI were significantly older as compared to those who survived (median 82 vs. 75 years, $p < 0.001$). With regard to chronic conditions, atherosclerosis was found to be associated with an increased case fatality in CDI (11/45 vs. 8/112; OR 4.2, 95% CI 1.6–11.3, $p = 0.003$).

Infection due to a hypervirulent strain was associated with an increased case fatality in patients aged <65 years and in those aged 65–74 years, but not in those aged >74 years (Table 2). Hypervirulent strain type remained a significant factor associated with case fatality in the logistic regression model adjusted for gender (Table 3).

3.1. Laboratory markers

Blood leukocytes, but not CRP or creatinine level predicted infection due to a hypervirulent strain type. Plasma CRP level and blood leukocyte count on the day of diagnosis (day of culture) predicted case fatality (at day 30) in patients with CDI (Table 4).

Table 4
Laboratory markers on the day *Clostridium difficile* was detected (cultured) in relation to case fatality at day 30. Results are median (quartiles).

Laboratory marker	Died ($n=80$)	Survived ($n=700$)	p-Value
CRP, mg/l ($n=399$)	106.0 (75–170.9)	79.4 (29.5–130.3)	<0.001
Leukocytes, $\times 10^9$/l ($n=382$)	13.2 (8.5–22.0)	9.6 (6.9–14.3)	0.009
Creatinine, μmol/l ($n=319$)	74 (54–165)	60 (62–116)	0.629
Platelets, $\times 10^9$/l ($n=382$)	262 (166–359)	270 (201–353)	0.759

CRP, C-reactive protein.

4. Discussion

The findings presented here show a significantly higher case fatality associated with CDI due to a hypervirulent strain compared to CDI associated with a non-hypervirulent strain. Although the median age in those infected with a hypervirulent strain was higher, the association between increased case fatality and hypervirulent strain type retained its significance when adjusted for the effect of age in a logistic regression model. This study showed that simple laboratory tests – CRP and leukocyte count – are useful prognostic markers in CDI; blood leukocytes on the day of diagnosis (positive culture) were significantly higher in patients infected with a hypervirulent strain type compared to those with an infection due to a non-hypervirulent strain.

The case fatality rate in CDI found in the present study is in accord with a recent report from Finland.[8] It has previously been shown that infection due to a hypervirulent *C. difficile* strain type is associated with increased mortality,[5,6] although not all studies have confirmed this.[2,7] Infection and colonization due to hypervirulent strain types have been shown to be strongly healthcare-associated.[12] Miller et al. showed that infection due to a hypervirulent strain type was significantly associated with mortality in patients aged 60–90 years but not younger,[6] and that patients >90 years of age experience high rates of severe CDI, regardless of strain type. Consistent with previous investigations,[2] the present study showed that CDI is an infection predominantly affecting the elderly. The present study indicated that patients aged >74 years have a high case fatality rate in CDI regardless of the strain type (Table 2).

Blood leukocytes were significantly higher in patients infected with a hypervirulent strain compared to patients infected with a non-hypervirulent strain, whereas CRP, blood platelet count, and creatinine levels did not differ between the types of infecting strains. It has been shown that surface layer proteins from *C. difficile* induce inflammatory and regulatory cytokines in human leukocytes.[13] Recent data indicate that the surface layer protein A variant of *C. difficile* PCR ribotype 027 might contribute to its clinical characteristics.[14] The simple biological markers blood leukocytes and CRP were valuable in predicting the outcome in patients with *C. difficile* infection. This finding is in accord with a previous investigation showing the prognostic value of blood leukocytes in CDI.[15]

Some limitations should be conceded here. Although there is a diagnosis number for *C. difficile* listed in the International Classification of Diseases 10th Revision (ICD-10), no specific number for pseudomembranous colitis exists. This limits the ability to retrospectively record all patients with CDI and those with negative culture results despite obvious *C. difficile*-associated disease. The study was based solely on positive culture results and we did not study hospital records regarding the clinical severity in patients with a positive culture result. However, the present study used the definition for *C. difficile*-associated disease (CDAD) introduced by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA).[16] In

Finland *C. difficile* cultures are only examined in the case of diarrhea, pseudomembranous colitis, or toxic megacolon, and no asymptomatic patients are examined. This approach is in accord with recently published practice guidelines for *C. difficile* infection in adults.[16] The present findings represent the crude mortality associated with culture-positive *C. difficile* infection. Given the low prevalence of asymptomatic *C. difficile* carriers in the adult population (3–4%),[17] the potential number of those with no *C. difficile*-associated clinical disease in this material is low. As the clinical significance of *C. difficile* in children is less certain,[18] only adult patients were included in the study. Cause-specific mortality is difficult to assess in patients with CDAD, and all-cause mortality was taken as an endpoint in the present study. Due to the fact that all patients with a positive *C. difficile* culture in Pirkanmaa Hospital District during the study period were included, there was no possibility for selection bias and this study was population-based by design. As CDI is predominantly an infection affecting the elderly population, it may be difficult to assess the independent role of multiple comorbidities in patients with this condition. A clear limitation of the present study was that the data on underlying diseases were not available for all patients.

In conclusion, infection due to a hypervirulent strain and advanced age are factors associated with increased case fatality in CDI. Blood leukocytes were significantly higher in episodes associated with a hypervirulent strain, and leukocyte count and CRP were found to be useful prognostic biomarkers in patients with CDI.

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Ethics: The study was a register-based retrospective study. Study subjects were not contacted in this study. The laboratory data were collected due to routine patient care and no additional samples were taken from the study subjects for the study. The study was approved by the medical director of Pirkanmaa Hospital District. Neither patient consent nor the approval of the hospital ethics board was required.

Conflict of interest: We declare that we do not have any conflicts of interest.

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