Chapter 7

All-cause and disease specific mortality in hospitalized patients with *Clostridium difficile* infections; a multicenter cohort study

Marjolein P.M. Hensgens[1], Abraham Goorhuis[2], Olaf M. Dekkers[3, 4], Birgit H.B. van Benthem[5], Ed J. Kuijper[1]

[1] Department of Medical Microbiology, LUMC, Albinusdreef 2, 2333ZA Leiden, the Netherlands; [2] Department of Infectious Diseases, AMC, Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands; [3] Department of Clinical Epidemiology, LUMC, Albinusdreef 2, 2333ZA Leiden, the Netherlands; [4] Department of Endocrinology and Metabolic diseases, LUMC, Albinusdreef 2, 2333ZA Leiden, the Netherlands; [5] Centrum Infectieziektebestrijding (Centre for Infectious Disease Control; Cib), Rijksinstituut voor Volksgezondheid en Milieu (National Institute for Public Health and the Environment; RIVM), Bilthoven, the Netherlands.

Clin Infect Dis 2013

Abstract

Background: Mortality among patients with *Clostridium difficile* infections (CDI) is high. Because of high age and multiple underlying diseases, CDI-related mortality is difficult to estimate. We estimated (CDI-related) mortality in an endemic situation, not influenced by outbreaks and consequently certain patients and *C. difficile* strains.

Methods: Between 2006 and 2009, 13 Dutch hospitals included all hospitalized CDI patients. Nine hospitals individually matched each CDI patient to two control patients, based on ward and time of CDI hospitalization. Survival status was obtained via the Dutch Civil Registration System. Kaplan Meier and Cox-regression were used for survival analysis.

Results: We identified 1,366 patients with CDI (1.33 per 1,000 admissions). All cause mortality risk was 13% after 30 days and 37% after 1 year. The highest mortality was seen among elderly patients and patients with PCR ribotype 027. 317 CDI patients were matched to 317 patients without diarrhea and 232 patients with diarrhea, with a 30-day mortality risk of 5.4% and 8.6% respectively. CDI patients had a 2.5 fold increased 30-day mortality rate compared to controls without diarrhea (Hazard ratio 2.5, 95% CI 1.4-4.3) when adjusted for age, sex and underlying diseases. CDI-related death occurred mainly within 30 days after diagnosis.

Conclusions: Mortality among CDI patients is high, even in an endemic situation. Our study shows that CDI leads to a 2.5 fold increase in 30-day mortality. This highlights the considerable disease burden and clinical impact of CDI, even in absence of an outbreak.

Introduction

Clostridium difficile infections (CDI) emerged in the beginning of the 21st century and are now the leading cause of antibiotic associated diarrhea[1, 2]. Outbreaks in the western world coincided with the emergence of a new type of *C. difficile*: PCR-ribotype 027[3]. This type was postulated to produce more toxin A and B in vitro, the major virulence factors of *C. difficile*[4, 5], and was frequently associated with severe disease in patients[3, 6, 7]. Within this new era of CDI, numerous studies focused on mortality rates among CDI patients. Studies were mainly conducted in outbreak situations or specific populations such as patients treated in Intensive Care Units or Surgical wards[3, 8, 9]. Mortality rates during outbreaks varied, due to the study population and the PCR-ribotype that was associated with the outbreak[8, 10, 11]. Studies in non-outbreak situations are less common. A Canadian surveillance study that identified 1430 CDI patients, of whom 282 were diagnosed during an outbreak, showed that all cause mortality in a setting of low incidence differed considerably from an outbreak situation (15% vs. 23% after 30 days)[12].

Similar to the all cause mortality, CDI-related death increased at least fourfold between 1999 and 2006[13, 14]. However, CDI-related death is difficult to objectify, because the existence of comorbidities is a risk factor for acquisition of the disease. Multiple outbreak investigations have concluded that CDI-related mortality frequently (14-19%) occurs within 30 days[3, 11, 15]. Surprisingly, an endemic study that matched cases and controls on the propensity to develop CDI, concluded that CDI had no direct effect on mortality in the first 60 days. After 3 months, however, the attributable mortality was 6%[16].

We performed a large multicenter cohort study in an endemic situation to estimate the mortality among CDI patients that is not influenced by outbreaks at certain wards or hospitals and consequently certain patient groups. Furthermore, we estimated the CDI-related mortality.

Methods

Study aims

The first aim of our study was to determine the absolute all cause mortality risk of CDI patients. The second aim was to determine CDI-related mortality (1) as the excess mortality when compared to 2 control groups and (2) according to the National Registration of Death certificates.

Patients and definitions

Between July 1st 2006 and April 30th 2009, 13 Dutch hospitals prospectively included CDI patients in the study. The total monthly number of admissions and patient days and type of hospital (university or local) were collected to study the incidence of CDI.

All unformed stool samples of patients who were hospitalized for ≥2 days were tested for *C. difficile* in addition to the patients for whom a *C. difficile* test was requested. The method to detect *C. difficile* toxins differed per hospital; 6 hospitals used the Immunocard (Meridian, bioMérieux), 4 used a cytotoxicity assay and 3 used the Vidas toxin A and/or B enzyme immunoassay (Meridian, bioMérieux). Hospitalized patients with unformed stool and a positive assay for *C. difficile* toxin were considered to have CDI. Patients were included only once.

To calculate the relative mortality rate CDI patients were individually matched to two hospitalized control patients: (1) without diarrhea, (2) with diarrhea and a negative test for the toxin of *C. difficile*. To maximize the feasibility of the study for the participating hospitals, matching was only requested during a pre-specified period of ≥6 consecutive months. Nine hospitals agreed to these terms, consisting of both academic and local hospitals (n=5 vs n=4) (Figure 1). Matching was based on ward of diagnosis and time of hospitalization (control patients were hospitalized within 14 days of the day on which CDI was diagnosed in the CDI patient). When several potential control patients were eligible, the first patient on the alphabetical ward list was chosen. A patient with non-CDI diarrhea fulfilling the matching criteria was not always available.

Demographic data and clinical information such as date of onset of diarrhea, prior underlying diseases, prior medication and prior abdominal surgery were collected for all patients. 'Prior' was defined as: within three months before the start of diarrhea. When symptom onset was unclear, the date of diagnosis was used as a proxy. For non-diarrheal patients 'prior' was defined as: within three months before the reference date. This date was calculated by adding the duration of hospitalization of the matched CDI patient (admission date to start of diarrhea) to the admission date of the patient without diarrhea. Matched CDI patients and controls without diarrhea therefore had a similar duration of hospitalization. In the matched cohort we additionally gathered data on prior admissions and the Charlson Comorbidity Index (CCI) before the current hospitalization[17]. Data were extracted by manual review of the electronic and paper patient chart and contact with the physician in charge. In each facility, data were collected on a standardized

questionnaire by trained research personnel. In patients with CDI, we requested the *C. difficile* strain for PCR-ribotyping[18]. Four hospitals responded well to this request (strains submitted in >75%), while five submitted 36-68% and four hospitals submitted <10%. The study protocol was approved by the Medical Review Ethics Committee of each participating hospital.

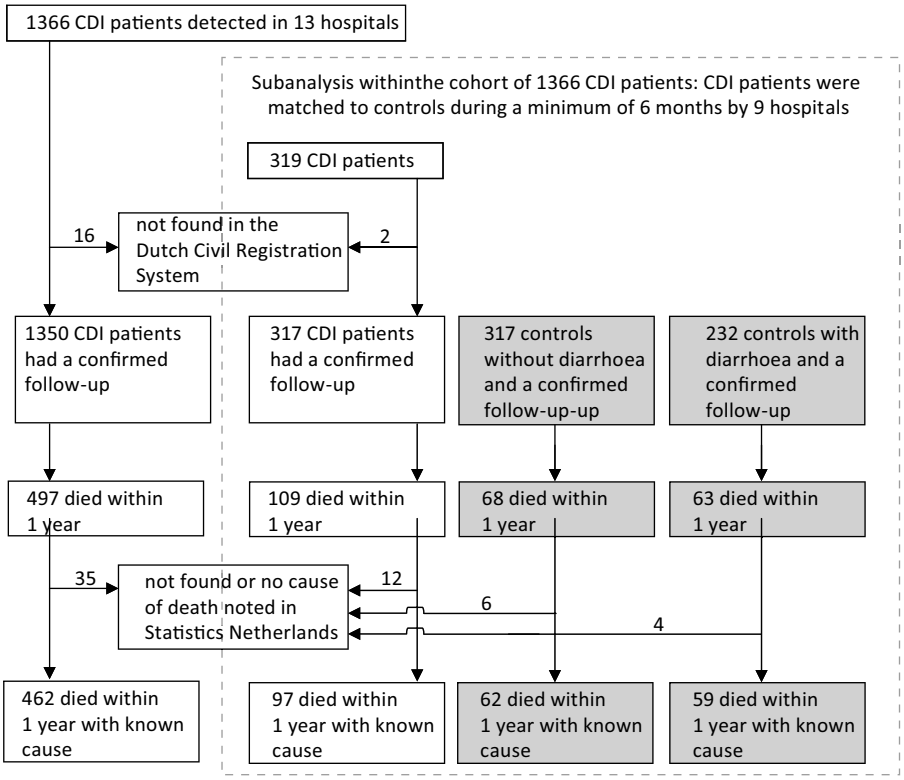

Figure 1. Study populations for analysis of CDI patients and CDI-related deaths.

Outcome measures

Follow-up started at diagnosis or the reference date. Dates of death were ascertained by searching the Dutch Civil Registration System in which dates of death or emigration of all Dutch residents are registered. Information on the cause of death was retrieved from the National Registry of Death certificates, where up to four different causes of death are registered per patient using the International Classification of Diseases, tenth revision (ICD-10)[19]. Patient data was linked to the registry of death certificates by the Netherlands Office of Statistics, thereby guaranteeing anonymity.

The cause of death was known for >90% of the patients that died within one year. We used the following ICD-10 codes for classification of CDI-related mortality:

A04.7 (*Clostridium difficile* enterocolitis); A04.8 and A48.8 (used in the Netherlands to indicate colitis due to *Clostridium* and *Clostridium* infection, not specified as *C. difficile*); the codes of a gastroenteritis of presumed infectious origin, septicaemia due to anaerobes and other bacterial infections of an unspecified site (A09, A41.4 and A49.8). These latter codes in combination with the mention of *C. difficile* in the text of the death certificate are used in England and Wales to select patients with CDI as a cause of death[20]. In the Netherlands the text of death certificates is not available, which might have introduced misclassification.

Statistical analysis

Proportions were compared using the chi-square test. Kaplan Meier was used to calculate the mortality risk and rate and show the 1 year mortality. Proportional hazards modeling (Cox regression) was used to adjust for the effects of age, sex and CCI. To limit confounding by underlying diseases, we additionally adjusted for six ICD-10 Chapters (Method 1), and for medication, admission and abdominal surgery in the three months prior to the onset of diarrhea and admission to an Intensive Care Unit (Method 2). Results are presented as hazard ratios (HR) with accompanying 95% confidence interval (95% CI). Statistical significance was considered to have been reached if a 2-sided p-value was ≤0.05. We used PASW Statistics version 18.0 (SPSS Inc., Chicago) and STATA software package 10.1 (StataCorp, College Station) for our analyses.

Results

Incidence

In the 34-months study period 1,030,202 hospital admissions and 1,366 patients with CDI occurred. The mean incidence was 1.33 per 1,000 admissions (2.1 per 10,000 patient days), varying between 0.74 and 2.30 per 1,000 admissions among the 13 participating hospitals. The monthly variation of CDI incidence within hospitals was small; however, in two hospitals the incidence exceeded 6.00 per 1,000 admissions during one month. No seasonal variation was observed (data not shown).

Table 1. Characteristics of CDI patients and matched controls.

Characteristics	CDI patients (n=1366)		Matched CDI patients (n=317)		Controls without diarrhoea (n=317)		Controls with diarrhoea (n=232)	
Mean age, yr (±SD)	62.6	(±21.6)	61.9	(±21.1)	59.6	(±21.2)	58.4	(±21.6)
Age > 65 years	778 / 1366	57.0%	175 / 317	55.2%	151 / 317	47.6%	100 / 232	43.1%
Male sex, no. (%)	692 / 1366	50.7%	168 / 317	53.0%	165 / 317	52.1%	111 / 232	47.8%
Hospital service, no. (%)								
Internal medicine	777 / 1242	62.6%	196 / 317	61.8%	192 / 317	60.6%	159 / 232	68.5%
Surgery	255 / 1242	20.5%	70 / 317	22.1%	76 / 317	24.0%	44 / 232	19.0%
Health-care association, no. (%)	919 / 1069	86.0%	261 / 303	86.1%	-	-	-	-
Underlying diseases, no. (%)								
Neoplasms	307 / 1113	27.6%	95 / 314	30.3%	91 / 316	28.8%	71 / 232	30.6%
Respiratory system diseases	335 / 1117	30.0%	79 / 315	25.1%	64 / 316	20.3%	41 / 232	17.7%
Digestive system diseases	336 / 1105	30.4%	95 / 316	30.1%	66 / 316	20.9%	81 / 232	34.9%
Circulatory system diseases	488 / 1111	43.9%	172 / 316	54.4%	160 / 316	50.6%	115 / 232	49.6%
Genitourinary system diseases	362 / 1105	32.8%	111 / 316	35.1%	78 / 315	24.8%	59 / 232	25.4%
Endocrine diseases	245 / 1104	22.2%	79 / 316	25.0%	83 / 316	26.3%	63 / 232	27.2%
Antibiotic therapy, no. (%)	953 / 1157	82.4%	264 / 316	83.5%	184 / 314	58.6%	138 / 228	60.5%
Cytostatic agents, no. (%)	165 / 1063	15.5%	51 / 315	16.2%	35 / 316	11.1%	34 / 232	14.7%
Immunosuppressive agents, no. (%)	374 / 1055	35.5%	136 / 312	43.6%	104 / 314	33.1%	90 / 230	39.1%
Abdominal surgery, no. (%)	227 / 1118	20.3%	83 / 314	26.4%	47 / 304	15.5%	46 / 229	20.1%
Admission, no. (%)	-	-	167 / 308	54.2%	93 / 307	30.3%	77 / 229	33.6%
ICU admission, no. (%)	-	-	73 / 313	23.3%	34 / 315	10.8%	36 / 232	15.5%
Most common PCR ribotypes, no. (%)								
014	112 / 689	16.3%	32 / 172	18.6%	-	-	-	-
078	76 / 689	11.0%	21 / 172	12.2%	-	-	-	-
001	57 / 689	8.3%	8 / 172	4.7%	-	-	-	-
027	55 / 689	8.0%	13 / 172	7.6%	-	-	-	-
Charlson Comorbidity Index, no. (%)								
0	-	-	51 / 315	16.2%	65 / 317	20.5%	47 / 232	20.3%
1-2	-	-	118 / 315	37.5%	133 / 317	42.0%	88 / 232	37.9%
3-4	-	-	95 / 315	30.2%	79 / 317	24.9%	60 / 232	25.9%
5+	-	-	51 / 315	16.2%	40 / 317	12.6%	37 / 232	15.9%

Medication use and abdominal surgery were positive when the patient used/experienced this in the 3 months prior to the start of diarrhea. Admission was positive when the patient was admitted to a healthcare facility in the 3 months prior to the start of diarrhea, excluding the current hospitalization. ICU admission (admission to the intensive care unit) was positive when a patient was admitted to an ICU in the 3 months prior to the start of diarrhea or reference date (could be during the current hospitalization).

CDI cohort

The mean age of the 1,366 CDI patients was 63 years, half of them were male (50.7%). Eighty-six percent had healthcare-associated CDI (development of diarrhea >48 hours after admission or <12 weeks after discharge). Underlying diseases were common and 82.4% received antibiotic therapy in the three months prior to diarrhea (Table 1). The most frequently found PCR-ribotype among CDI patients was type 014 (112/689; 16.3%). Other frequently found types were 078 (11.0%), 001 (8.3%) and 027 (8.0%). Patients with a typing result resembled those without, with respect to age (mean 62.4 vs 62.8), underlying diseases (mean CCI 2.6 vs 2.8), medication use and outcome.

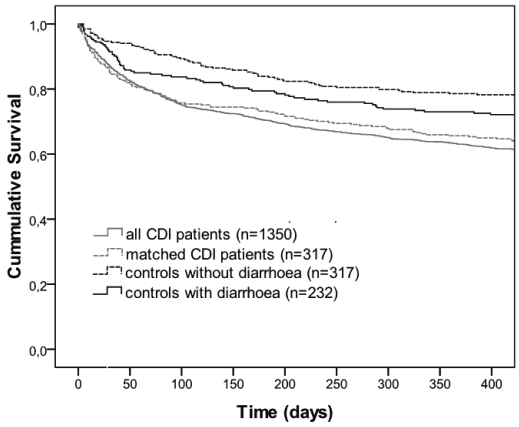

Figure 2. Mortality rate of all CDI patients and the matched cohort (CDI patients and matched control patients) during the first year of inclusion.

	< 30 days		< 3 months		< 6 months		< 1 year	
Death, no. (%)								
all CDI patients	177/1350	13.1%	319/1350	23.6%	401/1350	29.7%	497/1350	36.8%
matched CDI patients	47/317	14.8%	74/317	23.3%	85/317	26.8%	109/317	34.4%
controls without diarrhoea	17/317	5.4%	31/317	9.8%	51/317	16.1%	68/317	21.5%
controls with diarrhoea	20/232	8.6%	38/232	16.4%	48/232	20.7%	63/232	27.2%

Outcome Of 1350 CDI patients with known follow-up, 177 patients died within 30 days, accounting for a mortality risk of 13.1% (47.3 per 10,000 person years). One year after diagnosis 497 patients had died (36.8%) (Figure 2) and in 1% (10/1145) a colectomy was performed. The 30-day mortality increased with age (Table 3), with the highest case fatality observed for persons between 80 and 89 (52/244; 21.3%) and above 90 years (8/39; 20.5%). PCR-ribotype 027 was associated with the highest

30-day mortality (12/55; 21.8%). Compared to patients with CDI due to other PCR-ribotypes, patients with type 027 had a significantly higher mortality risk (21.8% vs 11.3%; Cox regression analysis: p=0.02; HR 2.1, 95% CI 1.1-3.8). The mortality among patients with type 027 remained significantly higher after adjustment for age and sex (p=0.04; HR 1.9, 95% CI 1.0-3.5).

Table 2. Mortality risk stratified by PCR-ribotype and age.

		Deaths		
	Total *n=1350*	**<30 days** *n=177*	**<3 months** *n=319*	**<1 year** *n=497*
Age (decades)				
≤9	58	0,0%	1,7%	6,9%
10-19	40	2,5%	7,5%	15,0%
20-29	33	6,1%	9,1%	12,1%
30-39	52	1,9%	3,8%	15,4%
40-49	90	10,0%	14,4%	28,9%
50-59	191	12,0%	18,8%	28,3%
60-69	252	11,9%	23,0%	34,5%
70-79	351	14,5%	29,9%	45,6%
80-89	244	21,3%	34,8%	51,2%
≥90	39	20,5%	33,3%	59,0%
PCR ribotype				
014	111	10,8%	20,7%	32,4%
078	76	14,5%	23,7%	38,2%
001	57	15,8%	22,8%	33,3%
027	55	21,8%	32,7%	40,0%
other	387	10,1%	20,2%	34,9%
no type result	664	14,2%	25,5%	38,6%

Mortality risk stratified by age decades. Additionally, stratification displayed the four most frequently found PCR ribotypes, a group of all other types combined (n=387) and a group of patients with CDI but without a PCR ribotype result.

Matched cohort

The 317 CDI patients that were matched to controls without diarrhea (n=317) and, if available, controls with diarrhea (n=232) resembled the total cohort of CDI patients (Table 1). Statistically significant differences between matched CDI patients and the total population were: lower frequency of respiratory diseases (25% vs 30%), higher frequency of circulatory diseases (54% vs 44%) and more frequent use of immunosuppressive agents (43% vs 36%). Treatment for CDI consisted of metronidazole (234/309; 76%), vancomycin (2%) or a combination of both (11%). Eleven percent was not treated for CDI.

The mean age of controls without and with diarrhea was 60 and 58 years, respectively, compared to 62 years in matched CDI patients (p=0.17 and p=0.03). Underlying diseases were more prevalent in CDI patients, except for endocrine diseases. The mean CCI was higher among CDI patients than among controls patients without and with diarrhea: 2.68, 2.28 and 2.42, respectively (p=0.01 and p=0.04). Sixteen percent of CDI patients (51/317) had an index of five or above.

Outcome Mortality of CDI patients and controls is displayed in Figure 2 and Table 3. Among matched CDI patients, 14.8% died within 30 days (53.9 per 10,000 person years), which was similar to the cohort of CDI patients (percentage: p=0.21). The 30-day mortality among control patients without and with diarrhea was considerably lower: 5.4% and 8.6% (p<0.01 and p=0.01), respectively.

Within the first 30 days, mortality among CDI patients was 2.9 times higher than among non-diarrheal controls (HR 2.9, 95% CI 1.7-5.1). After adjustment for baseline differences in age, sex and CCI, CDI was still associated with a 2.5 fold increased 30-days mortality rate (95% CI 1.4-4.3). The hazard ratio decreased to 1.8 (95% CI 0.9-3.5) and 0.9 (95% CI 0.6-1.4) within 3 months and one year, respectively. Overall, CDI was associated with a 1.5 times increased mortality (95% CI 1.1-2.0) in the first year. Results were similar when additional adjustments for underlying diseases or medication and admissions were made (Method 2 and 3 in Table 3), or when the in-hospital mortality was assessed in stead of the 30-day mortality (HR according to Method 1: 2.3, 95% CI 1.1-4.7). Post-discharge mortality (a proxy for long-term mortality) with up to one year follow-up was not significantly different in matched CDI patients and non-diarrheal controls (HR according to Method 1: 1.0, 95% CI 0.7-1.5).

When CDI patients were compared to controls with diarrhea, the hazard ratio for 30-day mortality was 1.9, 95% CI 1.1-3.3 (58.0 vs 29.9 per 10,000 patient years). In multivariable analysis, this mortality rate was 1.6 (95% CI 0.9-2.8).

PCR-ribotypes were known for 25 patients with CDI-related codes on their death certificates. Type 078 (6/25; 24%), 045 and 001 (both 3/25; 12%) were the most common. The primary cause of death was a neoplasm or a disease of the respiratory or circulatory tract in most CDI patients as well as controls (Table 4).

Table 3. Absolute and relative mortality rates in the matched cohort.

	Absolute mortality rate				Rate Ratio, HR (95% CI)			
	deaths / py at risk	*deaths per 10,000 py*	*deaths / py at risk*	*deaths per 10,000 py*	*Unadjusted*	**Method 1** *Adjusted for age, sex and Charlson Index*	**Method 2** *Method 1 + underlying diseases (ICD-10)*	**Method 3** *Method 1 + medication + admission*
	Matched CDI patients		**Controls without diarrhea**					
< 30 days	47 / 8717	53.9	17 / 9211	18.5	2.9 (1.7-5.1)	2.5 (1.4-4.3)	2.4 (1.3-4.2)	2.6 (1.4-4.9)
30 days - 3 months	27 / 15337	17.6	14 / 17609	8.0	2.2 (1.2-4.2)	1.8 (0.9-3.5)	1.9 (1.0-3.7)	2.1 (1.0-4.4)
3 months - 1 year	35 / 61758	5.7	37 / 72016	5.1	1.1 (0.7-1.7)	0.9 (0.6-1.4)	0.9 (0.6-1.5)	1.0 (0.6-1.6)
Overall	109 / 85812	12.7	68 / 98836	6.9	1.8 (1.3-2.4)	1.5 (1.1-2.0)	1.5 (1.1-2.0)	1.6 (1.1-2.3)
	Matched CDI patients		**Controls with diarrhea**					
< 30 days	37 / 6379	58.0	20 / 6681	29.9	1.9 (1.1-3.3)	1.6 (0.9-2.8)	1.6 (0.9-2.7)	1.5 (0.9-2.7)
30 days - 3 months	19 / 11121	17.1	18 / 12013	15.0	1.1 (0.6-2.2)	0.9 (0.5-1.8)	0.9 (0.5-1.8)	0.9 (0.4-1.7)
3 months - 1 year	25 / 44577	5.6	25 / 49650	5.0	1.1 (0.6-1.9)	1.0 (0.5-1.7)	1.0 (0.5-1.7)	0.9 (0.5-1.6)
Overall	81 / 62077	13.0	63 / 68344	9.2	1.4 (1.0-1.9)	1.2 (0.8-1.6)	1.2 (0.8-1.6)	1.1 (0.8-1.6)

The Rate Ratio was calculated using Cox regression analysis.
HR: Hazard ratio.
py: patient years.

In Method 1 we used age (continuous variable), sex and Charlson Comorbidity index (continuous variable) as adjusting variables. Method 2 adjusted for these 3 variables, supplemented with adjustment for the six most frequently found ICD-10 Chapters among CDI patients and controls (dichotomous variables: Chapter II Neoplasms; Chapter IV Endocrine, nutritional and metabolic diseases; Chapter IX Diseases of the circulatory system; Chapter X Diseases of the respiratory system; Chapter XI Diseases of the digestive system; Chapter XIV Diseases of the genitourinary system). In Method 3 we adjusted for age, sex, Charlson Comorbidity index, medication in the previous 3 months (antibiotics, immunosuppressives, chemotherapeutic agents), prior admission, prior abdominal surgery and admission to an Intensive Care Unit in the 3 months prior to diarrhea (see Table 1 for exact information on the occurrence of these variables in CDI patients and controls).

CDI-related mortality according to death certificates

Of the 497 patients that died within 1 year, death certificates could be accessed in 93% (462/497). According these certificates (Table 4), the cause of death was related to CDI in 46 patients (10.0% of all deaths; 3.7% of all CDI patients). Three certificates specifically coded death due to *C. difficile* enterocolitis, 36 had a code for *Clostridium* infection/colitis and 7 had one of the unspecific codes possibly related to CDI (2x A09; 5x A41.4). Most (72%; 33/46) CDI-related deaths occurred within 30 days. Eleven other patients died within 3 months, only two patients died thereafter (105 and 193 days, respectively). In contrast, no control patients had a primary or secondary cause of death related to CDI.

Table 4. Primary cause of death according to death certificate data of CDI and control patients that died within one year.

	CDI patients (n=1350)		**Controls without diarrhea (n=317)**		**Controls with diarrhea (n=232)**	
	n	*%*	*n*	*%*	*n*	*%*
Death within one year	497		68		63	
Known cause of death	462	93.0	62	91.2	59	93.7
Primary cause of death						
Infectious and parasitic diseases	36	7.8	0	0.0	3	5.1
Neoplasms	170	36.8	27	43.5	28	47.5
Digestive organs	38	8.2	10	16.1	8	13.6
Lung / bronchus	26	5.6	2	3.2	1	1.7
Lymphoid / haematopoietic tissue	48	10.4	8	12.9	12	20.3
Endocrine, nutritional and metabolic diseases	14	3.0	5	8.1	0	0.0
Circulatory system	88	19.0	16	25.8	8	13.6
Ischaemic heart disease	18	3.9	4	6.5	1	1.7
Respiratory system	54	11.7	5	8.1	9	15.3
Digestive system	37	8.0	6	9.7	4	6.8
Genitourinary system	25	5.4	1	1.6	2	3.4
Other	38	8.2	2	3.2	5	8.5
C. difficile-related death (primary or secondary cause)	46	10.0 #	0	0.0	0	0.0

Causes of death that were noted as primary cause of death. Causes of death are listed by ICD-10 Chapter and then by ICD-10 Block: e.g. Chapter II is referred to as "Neoplasms", Block C15-C26 is referred to as "digestive organs" (nomenclature as displayed in the ICD-10) (19). *C. difficile*-related death was determined by selecting those patients who had ICD-10 code A04.7, A04.8, A48.8, A09, A41.4 or A49.8 as a cause of death. Up to four different causes of death are registered.
10% of all deaths is equal to 3.7% of all CDI patients.

Discussion

During the study period, we experienced a stable, low incidence of CDI in hospitals. Even in this endemic setting mortality among hospitalized CDI patients was high: 13% within 30 days. This percentage was only slightly lower than observed during outbreaks (15% to 25% within 30 days)[3, 8, 11, 12]. Compared to matched patients without diarrhea, the 30-day mortality rate of CDI patients in our study was increased2.5 fold; a rate estimate consistent with a Canadian study with a ten-fold higher incidence[21]. This highlights CDI as a serious healthcare problem, even in absence of an outbreak.

Our study showed that CDI-related mortality occurred mainly within 30 days; long-term consequences of CDI (mortality after 90 days) were small. As the one-year mortality in CDI patients was 50% times higher (adjusted Hazard ratio) than in controls (1-year mortality risk: 21.5%), we estimate that CDI-related mortality risk is about 10% (50% of 21.5%). Given a yearly incidence of 2700 CDI patients in the Netherlands[22], about 270 deaths annually (10%) are estimated to occur as a consequence of the infection. This corresponds to 0.2% of all deaths in the Netherlands. Although this number is lower than the number of CDI related deaths in England and Wales (1.1% of all deaths; derived from death certificates)[20], it underscores the importance of CDI as a cause of death in the Netherlands.

According to the death certificates, 3.7% of the CDI patients died as a consequence of CDI. This is clearly less than our estimated CDI-related mortality risk (10%). Although we used relatively non-specific ICD-10 codes, in addition to a specific code (A04.7), to estimate CDI-related death, a majority (72%) of the patients with CDI-related ICD-10 codes died within 30 days of their diagnosis. Furthermore, non-specific ICD-10 codes were not observed in control patients. Therefore, we believe that we did not overestimate CDI-related mortality. Rather, our study indicates that death certificates lack sensitivity to provide a correct estimate of the CDI-related mortality, which is in accordance with studies from the UK[25] and Canada[26], who report that death certificates may be inaccurate to investigate CDI-related death[14, 27].

A large number of *C. difficile* strains were available for further typing (n=689; 50.4%) and we were able to relate these types to patient characteristics. Since patients without typing results resembled typed patients with respect to clinical characteristics and outcome, and most hospitals either responded well or did not respond at all to the typing request, we believe selection bias based on severity of disease is limited. The PCR-ribotypes found in our study are also common in Europe[28]. The finding that type 027 was significantly associated with a higher 30-

day mortality rate, adds evidence to the hypothesis that type 027 has hypervirulent characteristics[3, 11, 29].

The multicenter approach and large timeframe of our study resulted in 1,366 CDI patients during more than a million hospital admissions. This design enabled us to analyze CDI in a low incidence environment with numerous different PCR-ribotypes, which ensured us that our conclusions were not substantially influenced by outbreaks with specific types among specific groups of patients. Another strength of our study is that data were complete and carefully obtained since they were extracted by manual review of patient charts and contact with the treating physician after which outcome data was checked using national registries.

Our study has few limitations. First, we had access to two control groups that were selected by the criterion of presence vs absence of diarrhea. A control group without considering this criterion would have been more representative[31, 32]. Because only ten percent of the hospitalized patients experience diarrhea during their hospitalization, the comparison of CDI patients to controls without diarrhea was considered the most reliable. However, when we analyzed both control groups in one group, we found a similar one-year CDI-related mortality, which therefore did not influence our conclusions. A second limitation is the possibly that we failed to identify all CDI patients due to the low sensitivity of enzyme immunoassays (EIA)[33]. Including EIA-negative patients as diarrheal controls could have led to under-estimation of the CDI-related mortality rate, which is a second motive to report the comparison of CDI patients to controls without diarrhea as the most reliable. Finally, as with any observational study we cannot rule out residual confounding due to underlying diseases in the estimation of the CDI-related mortality. In our analysis we adjusted for age, sex and underlying diseases (using three methods). Matching accounted for hospital and ward of admission. Additionally, CDI patients and controls without diarrhea had a similar duration of hospitalization. By taking into account parameters for chronic underlying diseases (e.g. Charlson Comorbidity index) and acute disease (duration of hospitalization), we think we provided a good estimate of the true excess mortality in CDI patients.

In conclusion, our large multicenter study shows that all cause mortality rates among CDI patients are high and that CDI increases mortality 2.5 fold, even in an endemic situation. This highlights the considerable disease burden and clinical impact of CDI, even in absence of an outbreak and emphasizes the need for preventive strategies and novel therapeutic approaches.

Reference List

1 Pepin J, Valiquette L, Alary ME et al. Clostridium difficile-associated diarrhea in a region of Quebec from 1991 to 2003: a changing pattern of disease severity. CMAJ 2004;171(5):466-72.

2 Bartlett JG, Gerding DN. Clinical recognition and diagnosis of Clostridium difficile infection. Clin Infect Dis 2008;46 Suppl 1:S12-S18.

3 Loo VG, Poirier L, Miller MA et al. A predominantly clonal multi-institutional outbreak of Clostridium difficile-associated diarrhea with high morbidity and mortality. N Engl J Med 2005;353(23):2442-9.

4 McDonald LC, Killgore GE, Thompson A et al. An epidemic, toxin gene-variant strain of Clostridium difficile. N Engl J Med 2005;353(23):2433-41.

5 Kuijper EJ, van den Berg RJ, Debast S et al. Clostridium difficile ribotype 027, toxinotype III, the Netherlands. Emerg Infect Dis 2006;12(5):827-30.

6 Goorhuis A, van der KT, Vaessen N et al. Spread and epidemiology of Clostridium difficile polymerase chain reaction ribotype 027/toxinotype III in The Netherlands. Clin Infect Dis 2007;45(6):695-703.

7 Freeman J, Bauer MP, Baines SD et al. The changing epidemiology of Clostridium difficile infections. Clin Microbiol Rev 2010;23(3):529-49.

8 Hubert B, Loo VG, Bourgault AM et al. A portrait of the geographic dissemination of the Clostridium difficile North American pulsed-field type 1 strain and the epidemiology of C. difficile-associated disease in Quebec. Clin Infect Dis 2007;44(2):238-44.

9 Kenneally C, Rosini JM, Skrupky LP et al. Analysis of 30-day mortality for clostridium difficile-associated disease in the ICU setting. Chest 2007;132(2):418-24.

10 Goorhuis A, Bakker D, Corver J et al. Emergence of Clostridium difficile infection due to a new hypervirulent strain, polymerase chain reaction ribotype 078. Clin Infect Dis 2008;47(9):1162-70.

11 Pepin J, Valiquette L, Cossette B. Mortality attributable to nosocomial Clostridium difficile-associated disease during an epidemic caused by a hypervirulent strain in Quebec. CMAJ 2005;173(9):1037-42.

12 Gravel D, Miller M, Simor A et al. Health care-associated Clostridium difficile infection in adults admitted to acute care hospitals in Canada: a Canadian Nosocomial Infection Surveillance Program Study. Clin Infect Dis 2009;48(5):568-76.

13 Office for National Statistics (2008): Deaths involving Clostridium difficile: England and Wales, 1999 and 2001-06. Health Stat Q. 2007; (33):71-5. 2012.

14 Redelings MD, Sorvillo F, Mascola L. Increase in Clostridium difficile-related mortality rates, United States, 1999-2004. Emerg Infect Dis 2007;13(9):1417-9.

15 Labbe AC, Poirier L, Maccannell D et al. Clostridium difficile infections in a Canadian tertiary care hospital before and during a regional epidemic associated with the BI/NAP1/027 strain. Antimicrob Agents Chemother 2008;52(9):3180-7.

16 Dubberke ER, Butler AM, Reske KA et al. Attributable outcomes of endemic Clostridium difficile-associated disease in nonsurgical patients. Emerg Infect Dis 2008;14(7):1031-8.

17 Charlson ME, Pompei P, Ales KL et al. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 1987;40(5):373-83.

18 Bidet P, Lalande V, Salauze B et al. Comparison of PCR-ribotyping, arbitrarily primed PCR, and pulsed-field gel electrophoresis for typing Clostridium difficile. J Clin Microbiol 2000;38(7):2484-7.

19 World Health Organisation (WHO). International Statistical Classification of Diseases and Related Health Problems, 10th Revision (ICD-10). 1992.

20 Office for National Statistics (2010): Deaths involving Clostridium difficile: England and Wales, 2006 to 2010, accessed 20 December 2011, available at: www.ons.gov.uk/ons/rel/subnational-health2/deaths-involving-clostridium-difficile/2006-to-2010/statistical-bulletin.html. 2011.

21 Oake N, Taljaard M, van WC et al. The effect of hospital-acquired Clostridium difficile infection on in-hospital mortality. Arch Intern Med 2010;170(20):1804-10.

22 Fifth Annual Report of the National Reference Laboratory for Clostridium difficile (May 2010 to May 2011) and results of the sentinel surveillance. Accessed 1-3-2012, available at: www.rivm.nl/Bibliotheek/Algemeen_Actueel/Uitgaven/Infectieziekten/Fifth_Annual_Report_of_the_National_Reference_Laboratory_for_Clostridium_difficile_May_2010_to_May_2011_and_results_of_the_sentinel_surveillance. 2012.

23 Dubberke ER, Reske KA, McDonald LC et al. ICD-9 codes and surveillance for Clostridium difficile-associated disease. Emerg Infect Dis 2006;12(10):1576-9.

24 Dubberke ER, Butler AM, Yokoe DS et al. Multicenter study of surveillance for hospital-onset Clostridium difficile infection by the use of ICD-9-CM diagnosis codes. Infect Control Hosp Epidemiol 2010;31(3):262-8.

25 Mlangeni DA, Harris MD, Franklin L et al. Death certificates provide a poor estimation of attributable mortality due to Clostridium difficile when compared to a death review panel using defined criteria. J Hosp Infect 2011;77(4):370-1.

26 Hota SS, Achonu C, Crowcroft NS et al. Determining Mortality Rates Attributable to Clostridium difficile Infection. Emerg Infect Dis 2012;18(2):305-7.

27 Shears P, Prtak L, Duckworth R. Hospital-based epidemiology: a strategy for 'dealing with Clostridium difficile'. J Hosp Infect 2010;74(4):319-25.

28 Bauer MP, Notermans DW, van Benthem BH et al. Clostridium difficile infection in Europe: a hospital-based survey. Lancet 2011;377(9759):63-73.

29 Morgan OW, Rodrigues B, Elston T et al. Clinical severity of Clostridium difficile PCR ribotype 027: a case-case study. PLoS One 2008;3(3):e1812.

30 Knetsch CW, Terveer EM, Lauber C et al. Comparative analysis of an expanded Clostridium difficile reference strain collection reveals genetic diversity and evolution through six lineages. Infect Genet Evol 2012;12(7):1577-85.

31 Garey KW, Graham G, Gerard L et al. Prevalence of diarrhea at a university hospital and association with modifiable risk factors. Ann Pharmacother 2006;40(6):1030-4.

32 Thomas C, Stevenson M, Riley TV. Antibiotics and hospital-acquired Clostridium difficile-associated diarrhoea: a systematic review. J Antimicrob Chemother 2003;51(6):1339-50.

33 Eastwood K, Else P, Charlett A et al. Comparison of nine commercially available Clostridium difficile toxin detection assays, a real-time PCR assay for C. difficile tcdB, and a glutamate dehydrogenase detection assay to cytotoxin testing and cytotoxigenic culture methods. J Clin Microbiol 2009;47(10):3211-7.

34 Guerrero DM, Chou C, Jury LA et al. Clinical and infection control implications of Clostridium difficile infection with negative enzyme immunoassay for toxin. Clin Infect Dis 2011;53(3):287-90.