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Nosocomial *Clostridium difficile*-associated disease in pediatric cancer patients – results from a prospective German surveillance study

Summary

Background: The role of *Clostridium difficile* as nosocomial pathogen and the management of *C. difficile*-associated disease (CDAD) in terms of hospital hygiene and antimicrobial management is still controversial in pediatric oncology.

Methods: Prospective surveillance (Oncopaed 2001) of all CD-toxin positive patients with symptoms of an abdominal infection (onset > 48 hours after hospital admission) in whom the attending physicians started an antimicrobial treatment against *C. difficile*.

Results: In 209 months (54'824 inpatient days) 24 CDAD cases were documented in 5 centers (cumulative incidence density 0.48 / 1000 inpatient days; 8 % of all 263 prospectively documented nosocomial infections in this study). The median age was 12 years; only one was younger than 12 months. The majority of patients suffered from leukemia or lymphoma (54 %), 46 % had solid tumors (including CNS). About 50 % of all patients had received broad spectrum antibiotics before the onset of CDAD, 33 % were neutropenic at the time of diagnosis (neutrophils $< 0.5 \times 10^9$/L). In 75 % an objective inflammatory reaction of the colonic wall (ultrasound, radiography, CT) led to the diagnosis of enterocolitis. One patient showed severe lower gastrointestinal bleeding; in 3 patients (12 %) a surgical intervention had to be performed, no patent died related to the CDAD. Treatment consisted of metronidazol (83 %; monotherapy: 54 %), 46 % (monotherapy: 17 %) received vancomycin. A probable outbreak in one center was detected by the module and could be contained with a multifaceted intervention.

Conclusion: Although at a low overall incidence density, symptomatic nosocomial CDAD was associated with significant morbidity in affected patients. Considering the possibility of outbreaks and the threat of hypervirulent isolates, it seems mandatory to continue the prospective surveillance. The next generation Oncoped 2006 module is a software tool, which can easily be used in this clinical context.

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Introduction

Clostridium difficile-associated disease [CDAD] a communicable infection [1] and an important cause of nosocomial diarrhea in pediatric patients except in premature infants and in neonates, where symptom-free carriage of toxinogenic and non-toxinogenic strains is frequent [2,3].

In a retrospective survey on nosocomial diarrhea (defined as diarrhea occurring more than 48 hours after admission, and no likely non-infectious cause) Langley *et al.* identified *C. difficile* as the single most common cause of nosocomial diarrhea in pediatric patients [2]. They determined a median age of 3.9 years for children with nosocomial CDAD, and 49 % of the patients were incontinent (diapered) at the time of their first episode. Morinville *et al.* recently reported on 200 pediatric patients with a diagnosis of CDAD (cell culture cytotoxin assay) between February 2000 and November 2003 [4]. There were 107 males and 93 females (median age 2.6 years). Underlying factors were identified in 19 % of all patients (of these, 12 patients underwent chemotherapy, 6 were transplantation recipients, and 7 had an immunodeficiency), 149 (75 %) had received antimicrobials in the previous 2 months, and 111 (56 %) had been hospitalized in the previous month. Recurrence occurred in a high proportion (31 %) of those treated with metronidazole and re-treatment consisted of vancomycin (15 %), probiotics (15 %) and cholestyramine (6 %).

In 1988, after the analysis of an outbreak in a pediatric oncology unit, Brunetto *et al.* [1] recommended investigations to detect *C. difficile* in all children with malignant disease who have diarrhea (3 ore more

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loose stools per day). In their retrospective study of trends in infection morbidity in a pediatric oncology ward from 1986 to 1995 Wehl *et al.* [5] detected an increasing frequency of *C. difficile*-associated enterocolitis in the pediatric oncology unit since 1993. Schuller *et al.* found 28 (13 %) of 214 prospectively investigated pediatric oncology patents to be infected (at least one positive toxin assay) but Pulse-field-gel electrophoresis (PFGE) typing identified several different types of *Clostridium difficile* [6].

Pediatric cancer patients often receive broad spectrum antibiotics [7,8,9] as a consequence of febrile neutropenia after intensive antineoplastic chemotherapy [10]. Antibiotics and the gastrointestinal toxicity of chemotherapy predispose oncology patients to colonization and subsequent infection with *C. difficile* [11], but so far the role of this pathogen in pediatric oncology patients is poorly defined. For example, Burgner *et al.* tested prospectively 149 fecal samples from symptomatic pediatric oncology patients and 58 samples from asymptomatic patients for C.difficile toxins A and B. In 8.7 % of the symptomatic samples and 19 % of the asymptomatic samples toxigenic *Clostridium difficile* was found. No association was found between the use of antibiotics, the administration of chemotherapy and the presence of toxigenic *C. difficile*. The authors concluded that in the absence of a defined outbreak, *C. difficile* does not appear to be an important pathogen in pediatric oncology patients [12]. A prominent part in immune defense against CDAD is the capacity of the patient to produce an effective humoral response against toxin A. Patients receiving intensive chemotherapy are certainly not able to mount such an immune response [13]. The part of the prospective multicenter non-interventional surveillance study presented here was undertaken to further elucidate the role of *C. difficile* as a nosocomial pathogen in pediatric cancer patients.

Materials and Methods

The Oncopaed 2001 Module was a specific software tool developed at our institution for the prospective surveillance of nosocomial infections in pediatric cancer patients; it used CDC-methodology [14,15] with adjusted definitions [16] for this particular risk group [17,18]. The overall results of this prospective surveillance will be presented elsewhere. This article focuses on the results on CDAD.

CDAD in pediatric cancer patients is not so easy to define, since there is a substantial overlap in the clinical presentation of gastrointestinal mucositis/neutropenic colitis (without detection of *Clostridium difficile* (CD)- toxin) [19] and CDAD in pediatric cancer patients [20].

Neutropenia makes the assessment of clinical severity difficult, since the patient is unlikely to show specific signs of intraabdominal/peritoneal inflammation below 0.5×10^9 neutrophils /L in his peripheral WBC-count. Diarrhea and abdominal pain may be symptoms of chemotherapy induced mucositis and the treatment of high grade mucositis with continuous opiate infusion may mask the clinical symptoms of CDAD. After treatment with high dose methotrexate patients may develop a severe enterocolitis without neutropenia. In severe cases of CDAD diarrhea is not an obligatory symptom [21].

A case with nosocomial CDAD was defined as a patient with or without confirmation of colonic involvement by ultrasound, plain radiography or computer tomography of the abdomen

- with symptoms of an abdominal infection starting at least 48 hours after hospital admission;
- with a positive CD-toxin cell culture assay (detecting CD toxin B) from at least one stool specimen.

In addition, the attending physicians had to initiate an antimicrobial treatment directed against CDAD for at least 5 days duration. Additional testing with immunofluorescence or ELISA for the presence of CD-toxin A was not routinely performed in the attending microbiology laboratories [22,23].

The diagnosis of severe enterocolitis was made [24] if the patient presented with fever (temperature, >38.5 °C), clinical symptoms suggestive of an inflammatory reaction in the abdomen (e.g., abdominal pain, tenderness on palpation, subileus, vomiting, diarrhea), and radiographic or ultrasound confirmation of involvement of the colonic wall (constant thickening ≥ 4 mm after recovery of the leukocytes).

Neutropenia at the time of diagnosis was defined as a decrease in WBC count to $< 1 \times 10^9$ /L or a decrease in neutrophils to $< 0.5 \times 10^9$ /L at the time of first symptoms related to CDAD. Patients were included irrespective of neutropenia at the time of diagnosis.

All participating institutions had direct access to a tertiary care diagnostic microbiologic laboratory (university clinics). From all inpatients who showed 3 or more loose stools per day (patients with diarrhea), stool specimens were routinely investigated for CD-toxin by means of cell culture assay. Stool filtrates were inoculated onto Vero cells in tissue culture. The cell cultures were screened for cytopathic effects by means of microscopic inspection after incubation periods of 24 h and 48 h. Specimens that induced a cytopathic effect compatible with that typically caused by *C. difficile* toxin were inoculated onto Vero cells supplemented with antitoxin against Clostridium sordellii toxin (toxin neutralization test) [25]. At least in some centers the internal standard recommends the reevaluation of all patients at the end of a 7 days course of antimicrobial therapy directed against CD for persistence of toxin. Although not recommended as a routine procedure by international guidelines and other experts [26,27], the control supports the assessment of treatment success.

Informed consent was requested from the patient or his/her legal guardian to allow the storage and analysis of anonymized data sets in the reference database. The study was approved by the ethic committee of the University of Bonn, Germany.

Results

Seven pediatric oncology centers, all located at tertiary care university facilities, and outlined as C1 to C7 in the course of the manuscript, participated in this study for at least 6 consecutive months from April 01, 2001 to August 31, 2005. While C3 was a specialized unit for allogenic and autologous SCT and BMT, all other units offered conventional chemotherapy and radiotherapy, as well as myeloablative chemotherapy and autologous SCT to their patients. The study covered 204 months of prospective surveillance in the 7 centers. They took part for 14, 53, 30, 6, 32, 41 and 28 months (C1 to C7). In total, information on 54'824 days (150.1 years) of inpatient surveillance was collected in this study.

Twenty-four patients with nosocomial CDAD were identified in 5 of 7 participating centers. Thus, the cumulative incidence density was 0.48 events / 1000 in-

patient days. CDAD accounted for 9 % of 263 prospectively documented nosocomial infections.

The basic clinical data of the patients are shown in Table 1. Only one patient (4.2 % of all patients with CDAD) was younger than 12 months, 46 % of all patients had solid tumors, 54 % leukemia or lymphoma as underlying malignancy, 3 (12.5 %) patients suffered from a relapsed malignancy. Eight (33 %) of 24 patients showed neutropenia ($< 0.5 \times 10^9$ /L) at the time of the diagnosis (duration of neutropenia before the onset of the clinical symptoms 3–28 days, median 9 days).

Table 2 describes the symptoms, the different treatment approaches and the outcome of the infection. Three quarters of the patients (75 %) showed objective criteria of enterocolitis in ultrasound, plain radiography or CT examinations. One of the patients (4 %) developed severe lower intestinal bleeding, which necessitated blood product transfusions. None of the patients died as a result of the enterocolitis but in 3 patients (12.5 %), surgical interventions were necessary during the clinical course of the acute intra-abdominal infection.

In one center (C6), an outbreak was suspected by the attending physicians, since 9 consecutive cases were detected from July 2001 to January 2002 (Figure 1) with an incidence density of 3.79 cases/1000 inpatient days. After a couple of interventions, which were suggested by the principal investigator and by other experts on request (Table 3), the incidence density declined to 0.44 cases/1000 inpatient days.

Table 1: Basic clinical data in 24 patients with Clostridium difficile infection.*

Age in years: median (range)	12 (0.9–2.7)
Inpatients days before the onset of symptoms: median (range)	12 (2–77)
Item	**No of patients: n=24 (100%)**
Solid tumor (outside CNS) Acute lymphoblastic leukemia Acute myeloic leukemia Non Hodgkin lymphoma Central nervous system (CNS) tumor	8 (33) 5 (21) 4 (17) 4 (17) 3 (13)
Conventional chemotherapy High dose chemotherapy and autologous stem cell transplantation Radiation therapy	23 (96) 1 (4) 3 (12)
Neutropenia ($< 0{,}5 \times 10^9$ / L)	8 (33)
Systemic antibiotics preceding CD-associated disease	11 (46)
Chemotherapy induced mucositis (WHO grade III or IV)	3 (12)
Relapse of CD-associated enterocolitis **	6 (25)

CD = *Clostridium difficile.* * The gender of the patients was not documented. ** Normalization of clinical symptoms and a negative stool toxin assay between the events. Note: eventually, only those cases were included, in which the attending physicians decided to treat the patient with antimicrobial therapy directed against *C. difficile.*

Table 2: Symptoms, treatment and outcome of the CD-infection.

Item	**No of patients: n=24 (100%)**
Diarrhea; abdominal pain and tenderness	24 (100)
Blood in stool specimen	8 (33)
Abdominal sonography yields thickening of colonic wall*	18 (75)
Metronidazole po. / iv. Metronidazol monotherapy	20 (83) 13 (54)
Vancomycin po. Vancomycin po. monotherapy	11 (46) 4 (17)
Combination treatment (metronidazole iv/ vancomycin po.)	7 (29)
Surgical intervention required during the clinical course of enterocolitis	3 (12)
Attributable mortality	0 (0)

* Diameter ≥ 4 mm

Discussion

The results from this first multicenter surveillance study of nosocomial infections in pediatric cancer patients confirm that symptomatic nosocomial CDAD – although at a low overall incidence density – was associated with significant morbidity in affected patients. In addition, the Onkopaed 2001 module detected a possible outbreak in one participating center, which eventually ceased after a multifaceted intervention. However, a nosocomial outbreak was not confirmed using the necessary discriminating typing techniques [13,28].

Our results confirm the observation of Dettenkofer *et al.* [29] in adult patients after allogenic stem cell transplantation that a remarkable proportion of all symptomatic patients with CDAD does not have neutropenia at the time of diagnosis. This can be explained from a clinical perspective, as the empiric antibiotic treatment of febrile neutropenia [30,31,32] does not cover *C. difficile* and fosters the production of CD-toxins due to interference with intestinal colonization resistance [27,33]. As soon as the bone marrow function recovers, the patient displays clinical symptoms of intraabdominal inflammation due to leukocyte infiltration of the colonic mucosa [24]. Nosocomial CDAD justifies the surveillance of NI in cancer patients beyond the period of neutropenia.

Patients with CDAD normally excrete larger numbers of organisms in faeces, and bacterial spores have been found in abun-

dance in the environment of individuals with disease [34]. The organism has also been found on the hands of healthcare workers dealing with affected patients [27]. Thus, the risk of nosocomial transmission [26,27] of *C. difficile* may be higher in pediatric patients. First, the contamination of the environment [35,36,37] as well as transmission through patient-to-patient or patient-to-healthcare worker contacts [38] may be fostered by the age-related imperfectness of hand hygiene. In addition, understaffing in particular during the night shift [39] and safety issues in younger children may hamper the implementation of strict barrier precautions [40,41,42].

Particularly during outbreak management, the evaluation of the efficacy of complex infection control strategies is difficult. In most cases, several uncontrolled interventions are implemented simultaneously and analyzed in retrospect by the outbreak management team. This was the case in case in Center 6. The incidence density of CDAD sharply decreased after a complex intervention in response to the feedback to a particular high incidence density through the regular monthly reports from the reference database. Since this was only an observational study without a control group, we are not able to address the question, which particular intervention resulted in the lower CDAD incidence density after February, 2002. In addition, the *C. difficile* isolates were not typed with pulsed-field gel electrophoresis and thus may have represented a pseudoepidemic as in the study of Hernandez *et al.* [13]. Regardless of the presumed source of a case, rapid diagnosis, isolation, and disinfection of equipment and room surfaces with a sporicidal agent are necessary to limit the risk of spread [34,43]. Discontinuation of the offending antibiotic is a general treatment principle in patients with CDAD [44]. However, this is difficult or even impossible for most patients with neutropenia because of a high risk of septic complications [24]. Gorschlüter and coworkers performed a retrospective chart review of all adult patients treated in the leukemia ward of a university medical center during 1991–2000. CDAD occurred in 7.0 % of all chemotherapy cycles. In 8.2 % of the patients, severe enterocolitis developed. *C. difficile* infection was not clinically considered to be the primary cause of death in any of the patients. The response rate to oral metronidazole was 91 %. The authors concluded that *C. difficile* infection is not rare

Table 3: Multifactorial intervention in center 3 to control a suspected outbreak of CDAD in pediatric oncology patients.*

Item	Intervention
Diagnostic approach	Immediate testing of all symptomatic patients for *C. difficile* toxin (at least 2 stool specimens)
Isolation ** (single room or cohorting, single use gloves and gowns; mobile toilet chairs)	Strict contact precautions for all symptomatic patients even if the results of CD-toxin testing are pending.
Hand hygiene	After contact with the patient or to potentially contaminated surfaces hands were washed with an antimicrobial soap and water and dried with paper towels from closed dispensers. Afterwards hands were disinfected with an approved hand disinfectant.
Non-critical items and fomites	Workflows for disinfection / sterilization of non-critical items were reevaluated. Patient-related items were used for symptomatic patients.
Disinfection of surfaces	All hand contact surfaces were disinfected at least once a day with an approved surface disinfectant. After discharge, all hand contact surfaces and the floor were disinfected with a sporicidal agent [71].
Treatment	Metronidazole iv. (Broviac, Port) as first line treatment; Vancomycin p.o. in case of relapse or persistent symptoms (> 5 days). Any case of severe enterocolitis (in particular in a patient with neutropenia) got early combination treatment with Vancomycin po. In most of these patients, a nasogastric tube was in place.

* The outbreak was not confirmed by PFGE typing or any other reliable method to authenticate clonality of the isolates [47].
** Strict isolation could only be implemented after translocation of the unit into a new ward with a higher number of rooms and opportunities to isolate symptomatic patients.

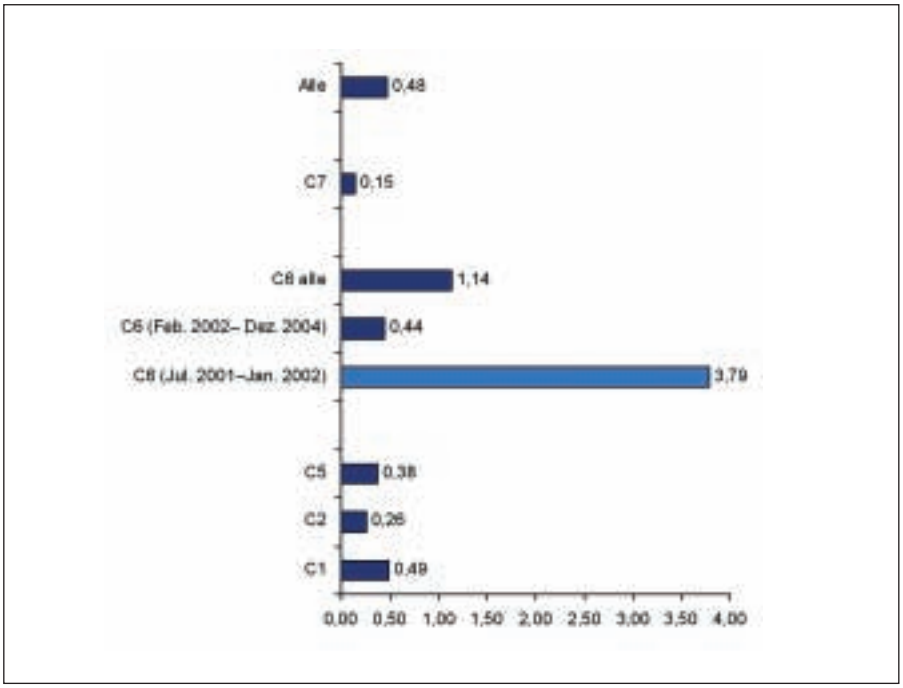

Figure 1: Incidence density (documented cases /1000 inpatient days) of Clostridium difficile -associated infections in 5 centers, which reported at least one event (209 months of prospective surveillance; 50.121 inpatient days). Nine of 13 cases documented in center 6 were diagnosed from July, 2001 to January 2002 (see text for details).

and should be suspected whenever a hospitalized patient with neutropenia develops diarrhea.

Oral (or intravenous) metronidazole can be recommended as initial drug of choice for treatment of patients with neutropenia who have hematologic malignancies and CDAD. Our experiences and the data presented here underline these conclusions.

The reduction of the uncritical use of proton pump inhibitors may be an additional way to decrease the probability of CDAD [45], but no controlled study has been published to confirm this risk factor in pediatric cancer patients. It is not known, whether the avoidance of third generation cephalosporines and the preferred use of piperacillin-tazobactam results in a lower incidence of CDAD in pediatric oncology [46].

There is still no international standard available for the diagnosis of CDAD and the best diagnostic approach is still a matter of debate [22,23,47,48,49]. Specific assays for the detection of CD-toxin A were not routinely used in the attending microbiology laboratories of the participating study centers. Thus, up to 20 % of cases (positive for CD-toxin A but negative for CD-toxin B) may have been missed in our study. In recent studies real-time PCR amplifying the *tcd*B gene showed the highest concordance with toxinogenic culture and may therefore become preferred method for diagnosing CDAD in faecal samples. It was also concluded that diagnosis of adult patients with diarrhoea who have been hospitalized for more than 72 h should focus mainly on the detection of *C. difficile*, irrespective of the physician's request [50]. Penders *C. difficile* [51) developed real-time, quantitative PCR assays for the detection of *Bifidobacterium spp.* and *Clostridium difficile* to determine the influence of either exclusive breast-feeding or formula feeding on both composition and quantity of the gut microbiota in infants. *C. difficile* counts were significantly lower in breast-fed infants than in the formula-fed group (median values of 3.28 log10 and 7.43 log10 CFU g(-1), respectively; p=0.03). Using the same PCR assays, the same group investigated fecal samples from 1032 infants at 1 month of age, who were recruited from the KOALA Birth Cohort Study in the Netherlands [52]. Hospitalization and prematurity were associated with higher prevalence and counts of *C. difficile*. Antibiotic use in the infant was associated with decreased numbers of bifidobacteria and *Bacteroides spp.* Although the detection of *C. difficil*e with PCR based methods does not confirm the presence of a pathogen in particular not in newborns and infants [44], children with cancer should be breastfed as long as possible in particular during periods of hospitalization and antibiotic treatment.

In a systematic metaanalysis of the available literature, Dendukuri *C. difficile* [53] came to the conclusion, that studies conducted up to 2005 did not provide sufficient evidence for the routine clinical use of probiotics to prevent or treat CDAD. In this context, it has to be considered, that *Lactobacillus spp.* have been described as a relevant pathogen in bloodstream infection in severely immunocompromised patients [54,55,56]. In addition, pediatric oncologists are reluctant to use *Saccharomyces boulardii* in the prevention of CDAD, since there have been a growing number of reports about its implication as an etiologic agent of invasive infections which were often catheter-related [57,58,59,60,61]. Randomized controlled studies which investigate the use of probiotics in the prevention of nosocomial diarrhoea in pediatric cancer patients are missing [62]. Promising studies of a vaccination against *C. difficile* Toxin A in healthy volunteers [63] and in a small number of patients with multiple recurrences of CDAD [64] do not offer a new perspective for severely immunocompromised patients, who are not able to mount a relevant immune response in response to vaccination [65].

Recently, hyper virulent nosocomially transmitted *C. difficile* isolates have been described in several countries causing increased morbidity, hospital stay and mortality in adult patients [35,66,67,68]. Probably, pediatric oncologists and infectious disease specialists as well as infection control personnel in Germany are facing a great future challenge [69] in terms of hospital hygiene, infection control and judicious use of the offending antibiotics [70] to prevent and control CDAD. It seems mandatory to continue the prospective surveillance of all inpatients with symptomatic CDAD in pediatric cancer units and to report back incidence densities and objective outcome parameters regularly and timely to the treatment team. The next generation Oncoped 2006 software module will be available for the prospective surveillance of NI in pediatric cancer patients in 15 pediatric oncology centers in near future.

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