Keywords
Clostridium difficile
Enterocolitis
Neutropenia
Children with cancer

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 × 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 Oncopaed 2006 module is a software tool, which can easily be used in this clinical context.

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 toxicogenic and non-toxicogenic 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
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 pa-tients to colonization and subsequent infection with C. difficile [11], but so far the role of this pathogen in pediatric oncology pa-tients is poorly defined. For example, Burgner et al. tested prospectively 149 fecal samples from symptomatic pediatric oncology patients and 58 samples from asymptom-atic patients for C.difícile toxins A and B. In 8.7 % of the symptomatic samples and 19 % of the asymptomatic samples toxigenic Clostridium difficile was found. No asso-ciation was found between the use of antibiotics, the administration of chemo-therapy and the presence of toxigenic C. difícile. The authors concluded that in the ab-sence of a defined outbreak, C. difficile does not appear to be an important pathogen in pediatric oncology patients [12]. A promi-nent 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 im-mune response [13]. The part of the prospective multicenter non-interventional surveillance study presented here was un-dertaken to further elucidate the role of C. difficile as a nosocomial pathogen in pediat-ric cancer patients.

### Materials and Methods

The Oncopaed 2001 Module was a specific software tool developed at our institution for the prospective surveillance of nosoco-mial infections in pediatric cancer patients; it used CDC-methodology [14,15] with ad-justed 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 re-sults on CDAD.

CDAD in pediatric cancer patients is not so easy to define, since there is a substan-tial overlap in the clinical presentation of gastrointestinal mucositis/neutropenic col-itis (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 intra-abdominal/peritoneal inflammation below 0.5 × 10⁹ 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 pa-tients may develop a severe enterocolitis without neutropenia. In severe cases of CDAD diarrhea is not an obligatory symp-tom [21].

A case with nosocomial CDAD was de-fined as a patient with or without confir-mation of colonic involvement by ultra-sound, plain radiography or computer tomography of the abdomen

-- with symptoms of an abdominal infection starting at least 48 hours after hospital ad-mission;

-- with a positive CD-toxin cell culture as-say (detecting CD toxin B) from at least one stool specimen.

In addition, the attending physicians had to initiate an antimicrobial treatment di-rec ted against CDAD for at least 5 days du-ration. Additional testing with immuno-fluorescence 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 2-4 mm after recovery of the leukocytes).

Neutropenia at the time of diagnosis was de-fined as a decrease in WBC count to < 1 × 10⁹/L or a decrease in neutrophils to < 0.5 × 10⁹/L at the time of first symp-toms related to CDAD. Patients were in-cluded irrespective of neutropenia at the time of diagnosis.

All participating institutions had direct access to a tertiary care diagnostic microbio-logic 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 pe-riods of 24 h and 48 h. Specimens that in-duced a cytopathic effect compatible with that typically caused by C. difficile toxin were inoculated onto Vero cells supplemented with antitoxin against Clostridium sordel-li toxin (toxin neutralization test) [25]. At least in some centers the internal standard recommends the reevaluation of all pa-tients at the end of a 7 days course of anti-microbial therapy directed against CD for persistence of toxin. Although not recom-mended as a routine procedure by inter-national guidelines and other experts [26,27], the control supports the assess-ment of treatment success.

Informed consent was requested from the patient or his/her legal guardian to al-low the storage and analysis of anonymized data sets in the reference database. The study was approved by the ethic commit-tee 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 man-ucript, participated in this study for at least 6 consecutive months from April 01, 2001 to August 31, 2005. While C3 was a special-ized unit for allogenic and autologous SCT and BMT, all other units offered con-ventional chemotherapy and radiotherapy, as well as myeloablative chemotherapy and autologous SCT to their patients. The study covered 204 months of prospective sur-veillance 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 participat-ting centers. Thus, the cumulative incidence density was 0.48 events / 1000 in-
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 ineffectiveness 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.

<table>
<thead>
<tr>
<th>Item</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostic approach</td>
<td>Immediate testing of all symptomatic patients for <em>C. difficile</em> toxin (at least 2 stool specimens)</td>
</tr>
<tr>
<td>Isolation **</td>
<td>Strict contact precautions for all symptomatic patients even if the results of CD-toxin testing are pending.</td>
</tr>
<tr>
<td>(single room or cohorting, single use gloves and gowns; mobile toilet chairs)</td>
<td></td>
</tr>
<tr>
<td>Hand hygiene</td>
<td>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.</td>
</tr>
<tr>
<td>Non-critical items and fomites</td>
<td>Workflows for disinfection / sterilization of non-critical items were reevaluated. Patient-related items were used for symptomatic patients.</td>
</tr>
<tr>
<td>Disinfection of surfaces</td>
<td>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].</td>
</tr>
<tr>
<td>Treatment</td>
<td>Metronidazole iv. (Broviac, Port) as first line treatment; Vancomycin p.o. in case of relapse or persistent symptoms (&gt; 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.</td>
</tr>
</tbody>
</table>

* 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).](image)
and should be suspected when a hospitalised 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 tcdB 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 diarrhea 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 influence of either exclusive breast-feeding or formula feeding on both composition and microbiota in infants. The quantity of the gut microbiota in infants differs between breast-fed and formula-fed infants than in the formula-fed infants, lactobacillus spp. and Bifidobacterium spp. have been described as a relevant probiotic in breast-dripped blood-stream 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 diarrhea 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 immuno-compromised 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.

References


Originalia

35. Owens RC. Clostridium difficile-Associated Disease:  
2005 May;41 Suppl C:59–66.

26. McFarland LV, Brandmarker SA, Guandalini S. Pedia-


37. Hota B. Contamination, disinfection, and cross-co-

36. Kramer A, Schwebke I, Kampf G. How long do noso-

23. Bentley AH, Patel NB, Sidorczuk M, Loy P, Fulcher J,  

22. Barbut F, Delmee M, Brazier JS, Petit JC, Poxton IR,  

28. van den Berg RJ, Schaap I, Templeton KE, Klaassen


39. Stegenga J, Bell E, Matlow A. The role of nurse un-

36. Kramer A, Schwebke I, Kampf G. How long do noso-

28. van den Berg RJ, Schaap I, Templeton KE, Klaassen


34. Worsley MA. Infection control and prevention of Clo-

33. Donskey CJ. Antibiotic regimens and intestinal colo-

32. Simon A, Beutel K, Marklein G, Fleischhack G.

40. Gerdinger DN, Johnson S, Peterson LR, Mulligan ME,  

18. van den Berg RJ, Schaap I, Templeton KE, Klaassen

35. Owens RC. Clostridium difficile-Associated Disease:  


36. Kramer A, Schwebke I, Kampf G. How long do noso-

23. Bentley AH, Patel NB, Sidorczuk M, Loy P, Fulcher J,  

22. Barbut F, Delmee M, Brazier JS, Petit JC, Poxton IR,  

28. van den Berg RJ, Schaap I, Templeton KE, Klaassen


35. Owens RC. Clostridium difficile-Associated Disease:  
