CLOSTRIDIUM DIFFICILE – AETIOLOGICAL AGENT FOR ANTIBIOTIC-ASSOCIATED DIARRHEA

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Keywords: *Clostridium difficile, Antibiotic-Associated Diarrhea, aetiology*

Abstract: *Antibiotic-associated diarrhea is defined as the diarrhea that occurs in association with the administration of antibiotics. The frequency of this complication varies among antibacterial agents. The aetiology of antibiotic-associated diarrhoea (AAD) varies. The disruption of the normal enteric flora caused by antibiotics may lead to overgrowth of pathogens and functional disturbances of the intestinal carbohydrate and bile acid metabolism, resulting in osmotic diarrhoea. Allergic, toxic and pharmacological effects of antibiotics may also affect the intestinal mucosa and motility. Cytotoxin-producing Clostridium difficile is held to be the causative agent of approximately 20% of AAD and of nearly all cases of pseudomembranous colitis, the most severe manifestation of AAD. In hospitals, Clostridium difficile is an increasing problem, especially among the elderly patients with serious underlying diseases. AAD has been associated with increases in mortality, length of hospitalization and cost of medical care.*

Cuvinte cheie: *Clostridium difficile, diareea postantibioterapie, etiologie*

Rezumat: *Diareea postantibioterapie este definită ca diareea care apare în asociere cu administrarea de antibiotice. Frecven□a acestei complica□ii variază în func□ie de agentul antibacterian administrat, etiologia fiind variabilă. Distrugerea florei normale intestinale cauzată de antibiotice poate duce la cre□terea necontrolată a bacteriilor cu poten□ial patogen □i la dereglarea metabolismului intestinal al carbohidra□ilor □i al bilei, cauzând astfel diaree osmotică. Efectele toxice, alergice □i farmacologice ale antibioticelor pot afecta de asemenea mucoasa □i motilitatea intestinală. Tulpinile de Clostridium difficile producătoare de citotoxine sunt considerate a fi responsabile de 20% din sindroamele diareice postantibioterapie □i aproape a tuturor cazurilor de pseudocolită membranoasă, cea mai severă cauză a diareei postantibioterapie. În spitale, infec□ia cauzată de Clostridium difficile este o problemă tot mai acută, în special între pacien□ii vârstnici care prezintă □i alte tare ale organismului, diareea postantibioterapie fiind asociată cu o cre□tere a mortalită□ii, a perioadei de internare □i implicit a costurilor*

The first reported case of pseudomembranous enterocolitis was reported by J. M. Finney in association with William Osler in 1893. They described a 22-year-old woman who underwent resection of gastric tumour and developed postoperative diarrhoea. She died on the 15th postoperative day, and at autopsy, the small bowel revealed diphtheritic membranes.

During the dawn of the antibiotic era, PMC became a common complication of antibiotics use. Staphylococcus aureus, the principal nosocomial pathogen at that time, was implicated as the agent responsible for this condition due to its identification by Gram stains and cultures of stools. Thus, vancomycin became the standard treatment.

Because vancomycin therapy worked, the causative agent was not questioned until the middle to late 1970s. The use of clindamycin had become widespread during this period.(1)

In the 1970s, important clinical observations of clindamycin-associated pseudomembranous colitis and the demonstration of the potent cytopathic effects of *Clostridium difficile*–derived toxin in animal models established the cause and pathogenesis of this condition.(2)

Antibiotic-associated diarrhoea is defined as the diarrhoea that occurs in association with the administration of antibiotics. The direct toxic effects of antibiotics on the intestine can alter digestive functions secondary to reduced concentrations of the normal gut bacteria, or may cause pathogenic bacterial overgrowth.(3)

The frequency of this complication varies among the antibacterial agents. Diarrhea occurs in approximately 5-10% of the patients who are treated with ampicillin, 10-25% of those who are treated with amoxicillin–clavulanate, 15-20% of those who receive cefixime, and 2-5% of those who are treated with other cephalosporins, fluoroquinolones, azithromycin, clarithromycin, erythromycin, and tetracycline. The rates of diarrhoea associated with parenterally administered antibiotics, especially those with enterohepatic circulation, are similar to the rates associated with orally administered agents.(4)

The aetiology of antibiotic-associated diarrhoea (AAD) varies. The disruption of the normal enteric flora caused by antibiotics may lead to the overgrowth of pathogens and functional disturbances of the intestinal carbohydrate and bile

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Article received on 28.10.2011 and accepted for publication on 31.01.2012
ACTA MEDICA TRANSILVANICA March 2012;2(1):219-221

acid metabolism, resulting in osmotic diarrhoea. Allergic, toxic and pharmacological effects of antibiotics may also affect the intestinal mucosa and motility. Cytotoxin-producing *Clostridium difficile* is held to be the causative agent of approximately 20% of AAD and of nearly all cases of pseudomembranous colitis, the most severe manifestation of AAD.

In hospitals, *Clostridium difficile* is an increasing problem, especially among the elderly patients with serious underlying diseases.(5) AAD has been associated with increases in mortality, length of stay and cost of medical care. Other infectious agents with less convincing correlations with AAD include *Clostridium perfringens*, (6), *Staphylococcus aureus*, (7), *Klebsiella* spp., (8), *Salmonella* spp., *Candida* spp. i *Pseudomonas aeruginosa*.(9)

CDAD (*Clostridium difficile* associated disease)

Clostridium difficile is a gram-positive, spore-forming anaerobic bacillus that was first linked to disease in 1978, when it was identified as the causative agent of pseudomembranous colitis. It has been associated with gastrointestinal infections ranging in severity from asymptomatic colonization to severe diarrhea, pseudomembranous colitis, toxic megacolon, intestinal perforation, and death.(10)

Clostridium difficile is widespread in the environment and colonizes up to 3-5% of adults without causing illness. Some strains are producing toxins: toxin A, which is responsible mainly for diarrhea and toxin B - a cytotoxin. A recently identified strain of *Clostridium difficile*, designated North American pulsed-field gel electrophoresis type 1 (NAP 1), has caused numerous outbreaks of clinically severe disease in North America and Europe. NAP 1 produces 16 times more toxin A and 23 times more toxin B than other strains. In addition, NAP 1 produces a third toxin, known as binary toxin.(11)

Clostridium difficile toxins can be found in the stool of 15% to 25% of patients with antibiotic-associated diarrhoea and more than 95% of patients with pseudomembranous colitis.

Data from the US Centers for Disease Control and Prevention (CDC) reveal that hospitalizations with a discharge diagnosis of CDAD have significantly increased from 31 per 100,000 population in 1996 to 61 per 100,000 in 2003.(12) The rate of mortality associated with *Clostridium difficile* has been increasing between 1999-2002 in the United States and between 2001-2005 in England and Wales (13), being between 6%-30% when pseudomembranous colitis is present, but maintaining high even in the absence of the colitis.(14) Furthermore, a study conducted in Canada in 2005 presented a mortality rate of 17% at one year.(15)

Information on the incidence of CDAD in Europe is available from two surveys which were performed by the ESCMID Study Group for *Clostridium difficile* (ESGCD). The aims of ESGCD are to determine the prevalence of nosocomial *Clostridium difficile* infection in the European hospitals. The first survey involved 212 hospitals in UK, France, Belgium, Denmark, Germany, Italy and Spain in 2002. The incidence was 11 per 10,000 admissions. In contrast, data from studies in the USA showed that the incidence among hospitalized patients is much higher, ranging from10 and 200 per10,000 admissions. In 2005, a second European surveillance was performed in 38 hospitals from 14 different European countries. Data from this study indicated wide variations in the incidence of CDAD ranging from 0.13 to 7.1 per 10,000 patients-days (mean 2.45 per 10,000 patients-days). The incidence was higher in countries that experienced recent *Clostridium difficile* 027 outbreaks (Netherland, Belgium and France). The prevalence of the 027 epidemic strain was 5.7%.(16)

A study performed in the Microbiology Departement from UMF Târgu-Mure, from 2008 until 2009, showed a 22.7 percentage of positive samples for *Clostridium difficile*, being statistically significant higher than the 1.7 percentage identified for comunitary enteric pathogens (Escherichia coli, Salmonella spp., Shigella spp., Campylobacter coli/jejuni, Yersinia enterocolitica). The rigorous selection of the stool samples processed for C. difficile has an important role in obtaining an increased percentage of positive samples forCDAD. The low percentage identified for comunitary enteric pathogens ernphasizes the importance of the CDAD diagnosis.(17)

With the recent emergence of hypervirulent strains, the incidence of C. difficile-associated diarrhoea and intestinal inflammatory disease has increased significantly in both North America and Europe, causing lengthy hospitalization, substantial morbidity and mortality. Of further concern is the recent emergence of hypervirulent strains that are resistant to antibiotics. (18) The epidemic strain is resistant to fluoroquinolones in vitro, a characteristic which was an infrequent observation in *Clostridium difficile* strains prior to 2001. The epidemic strain produces a binary toxin, an additional toxin that is not present in other *Clostridium difficile* strains. Binary toxin is related to the iota-toxin found in *Clostridium perfringens*, and its role in CDAD pathogenesis is not fully understood. The epidemic strain produces substantially larger quantities of toxins A and B in vitro than other *Clostridium difficile* strains. The epidemic strain is toxinotype III; most other *Clostridium difficile* strains are toxinotype 0. Toxinotyping is based on analysis of the pathogenic locus (PaLoc) of the *Clostridium difficile* genome, the region that includes the genes for toxin A (tcdA), toxin B (tcdB), and neighbouring regulatory genes. The epidemic strain has a partial deletion of tcdC, a gene in PaLoc that is responsible for down-regulation of toxin production.

Outbreaks of CDAD due to the new, highly-virulent strain of *Clostridium difficile* have been recognized throughout European health care facilities, including 75 hospitals in England, 16 hospitals in the Netherlands, 13 healthcare facilities in Belgium, and nine healthcare facilities in France. In Germany, the first cases of the highly-virulent *Clostridium difficile* strain, reported in 2007 and characterized as PCR ribotype 027, were associated with high mortality. Larger outbreaks of *Clostridium difficile* have been reported in northern France in particular. These outbreaks are very difficult to control, and preliminary results from case-control studies indicate a correlation with the administration of fluoroquinolones and cephalosporins.

In Dublin, Ireland, *Clostridium difficile* is a major cause of infectious diarrhea in hospitalized patients. Between August 2003 and January 2004, there was an appreciable increase in the incidence of CDAD, peaking at 21 cases per 1000 patient admissions. Of the *Clostridium difficile* isolates recovered, 85 (95%) were identical toxin A-negative and toxin B-positive strains, corresponding to toxinotype VIII and PCR ribotype 017. All clonal isolates were resistant to multiple antibiotics, including ofloxacin, ciprofloxacin, levofloxacin, moxifloxacin and gatifloxacin [minimum inhibitory concentrations (MICs) > 32 μg/mL] and erythromycin, clarithromycin and clindamycin (MICs > 256 μg/mL). Recurrent CDAD occurred in 26 (36%) of the patients.

Reported mortality rates from CDAD in the United States increased from 5.7 per million population in 1999 to 23.7 per million in 2004. These increased rates also may be caused by the emergence of a highly virulent strain of *Clostridium difficile*.(19) CDAD is now responsible for approximately 3 million cases of diarrhoea and colitis annually in the United States, and has a mortality rate of 1%-2.5% (20). Zilberberg et

alhave reviewed a sample of more than 36 million annual discharges from non-governmental US hospitals, and have concluded that 2.3% of the cases of CDAD were fatal, amounting for roughly 5500 deaths. That was nearly double the percentage that resulted in death in 2000.(21)

Due to the lack of a national diagnostic protocol for CDAD and that only a few laboratory diagnosis this infection, at the moment the incidence of this infection in our country is underestimated and cannot be compared to the multitude of existing data at European level and worldwide.

Unfounded use of antibiotics has led to a significant increase in microorganism's drug resistance. This led to the necessity of using some broad spectrum antimicrobial agents more often, thus maintaining a vicious circle. A major side effect of the unfounded antibiotherapy is a significant increase in the incidence of antibiotic induced diarrhoea, but its aetiology is not sufficiently studied in our country, yet.(22)

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