Clinical and Laboratory Aspects of Clostridium difficile Toxin Associated Colitis

Background

Clostridium difficile, a motile, gram positive rod, is the major recognized agent of antibiotic associated diarrhea. Penicillin, ampicillin, amoxicillin, ticarcillin, nafcillin, lincomycin, clindamycin, cephalosporins, tetracycline, metronidazole, vancomycin, moxalactam, cotrimoxazole, imipenem, ciprofloxacin, trimethoprim-sulfamethoxazole, rifampin, spiramycin, erythromycin, and oral aminoglycosides have all been reported to result in C. difficile associated diarrhea(25,38). It has also been reported in chemotherapy patients(28) and other patients without antecedent antibiotic usage(23). The watery diarrhea is toxigenic in origin(13). Cytotoxicity assays are positive for C. difficile toxin in 15-25% of patients with antibiotic associated diarrhea, 50-75% of patients with antibiotic associated colitis and 90-100% of patients with pseudomembranous colitis(1).

The pathophysiology of C. difficile associated diarrhea is related to four factors(1): 1. disturbance in the mechanism for population control in the colonic lumen, 2. source of C. difficile-endogenous or exogenous, 3. potential for producing toxin, 4. age-related susceptibility. C. difficile will not produce enteric disease following simple oral challenge but may cause lethal disease in combination with antibiotics(1). Thus, antibiotics must first disrupt the normal colonic flora. The three main antibiotics resulting in C. difficile associated diarrhea are clindamycin, ampicillin, and the cephalosporins(1). Vancomycin is effective against almost all strains of C. difficile, but the disease may recur presumably due to sporulation with regeneration of the vegetative forms when the antibiotic levels decrease(1).

3% of healthy adults and 10-20% of hospitalized patients harbor C. difficile as a part of their colonic flora(20,23,40). The disease is toxin mediated, with no evidence of microbial invasion into the gut epithelium(5). There are two toxins, the 440-500 kd toxin A and the 360-470 kd toxin B(8,36), both of which are lethal when injected into animals(5). Toxin A is more weakly cytopathic in cell culture(27) but binds to the brush border membrane(5) and
reproduces enteric disease, including infiltration of neutrophils, alteration of membrane permeability and hemorrhagic necrosis, in the hamster model(5,8). Toxin A has been shown to be a chemotactic factor for granulocytes, which may result to a significant degree in its toxicity(18). Its enterotoxicity may also result from its ability to cause a disorganization of cells in the brush border, allowing serum proteins and fluids to pass into the lumen(27). Intestinal myoelectric response and peristalsis are also increased(38). Some researchers now report the possible existence of a second enterotoxin distinct from toxin A, but this has yet to be uniformly accepted(36).

Toxin B is cytopathic to virtually all cell lines and is detectable in standard cell culture assays, but fails to produce intestinal disease in experimental models(8,27). Toxin B may have no biological importance in gastrointestinal disease. However, when given systemically, it is lethal(1). Toxin B is less stable than toxin A and is more susceptible to pH extremes and proteases(8). Cell culture is used to detect the cytopathic toxin, the identity of which is confirmed by its ability to be neutralized by C. sordelli or C. difficile antitoxin(There is a great similarity in the toxins of the two organisms)(1). Both toxins produce similar types of cytopathic effects, but Toxin B is 1000-10,000 times more potent(1,27). As little as .2-1 pg of toxin B produces cytopathic effect(1,27), suggesting that even a single molecule may cause cell death(1). It is important to note that the amount of toxin produced by C. difficile varies not only with number of bacteria but with the specific isolate, culture medium and, in some cases, the antibiotic treatment received(5). Virulence factors other than toxins have also been suggested in C. difficile associated disease(36).

Clinical Manifestations

The clinical manifestation are highly variable and may be represented by anything from asymptomatic carriage to life-threatening colitis. There is a high carriage rate of C. difficile and its toxin in healthy neonates. The toxin is reported to be found in about 25% of patients with antibiotic associated diarrhea(5). Clinical disease may be manifested by abdominal cramps, fever and leukocytosis and, sometimes, nausea and vomiting(20). There may be a leukomoid reaction with white blood counts exceeding 50,000/ml, and the patient's temperature may exceed 106 F(1,14,38). Severe/late complication include toxic megacolon,
severe electrolyte imbalance, colonic perforation, and hypoalbuminemia with anasarca (1,5,38). Systemic manifestations, such as polyarthitis are rarely reported (1).

Patients who are more likely to develop C. difficile associated diarrhea are those who are older than 65 years of age, those who are ICU residents, those who have had GI procedures, and those who have had >10 days of antibiotic therapy (17). A slight female predominance exists (38). Increases in the incidence of C. difficile associated diarrhea have been associated with increased use of cephalosporins (17,22).

There is uncertainty as to why neonates have such a high rate of C. difficile toxin carriage without clinical disease (33). It has been estimated that 50% or higher [in some studies as high as 95% (10)] of infants are colonized with toxigenic C. difficile and are asymptomatic (5). Most infants acquire the infection nosocomially (10,38). There are many hypotheses why infants are not effected. Colostrum contains substances which neutralize toxins A and B (5). More importantly, fetal intestinal cells are reported to be much less sensitive to the toxins than adult intestinal cells, possibly because the toxin A receptor on infant intestinal cells exists in an immature form which is not able to bind by toxin A (5,33,38). The receptor is thought to be a branched carbohydrate, and branched carbohydrate receptors are developmentally regulated and unlikely to be developed in infants and young children (33). However, although C. difficile associated diarrhea does not occur in neonates, rare cases of invasive C. difficile infections with systemic circulation of toxin and fatal consequences can occur (21).

C. difficile toxin is reported to occur in 20-30% of patients with otherwise unexplained diarrhea and antibiotic usage (1). The probability of C. difficile related disease is increased if diarrhea is prolonged, symptoms persist after the antibiotic is stopped, hypoalbuminemia is present, or pseudomembranous colitis is present (1). Fecal leukocytes are found in 38% of cases of C. difficile associated diarrhea and a predominance of gram positive rods is seen in 41% of fecal gram stains, which is not significantly different from patients who have diarrhea unassociated with C. difficile (35). Bloody diarrhea is relatively uncommon, occurring in only 10% of patients (20). Although pseudomembranous colitis is the classic finding, colonoscopy findings are often limited to erythema, edema and friability of the colonic mucosa (1).

C. difficile associated diarrhea may be epidemic or endemic within hospitals or nursing homes, often restricted to wards (1,34). This is partly responsible for the wide range in the reported
incidence of disease (0.01-10%) (5). The bacterium may be cultured from clothing and room fixtures in contaminated areas and may be transmitted by hospital personnel (5, 20, 34, 38). For this reason, efforts should be made to isolate patients with C. difficile associated diarrhea and minimize cross-contamination between patients. Detection of toxin is not sufficient for epidemiologic studies; the bacterium must be isolated so that it may be subtyped for such studies (1, 34).

Atypical presentations of C. difficile infections also occur. C. difficile has occasionally been found in sites outside the colon, including abscesses, wound infection, osteomyelitis, pleuritis, peritonitis, septicemia and urogenital tract infections (1, 5, 24, 25). Fatal invasive toxigenic systemic infections have occurred in pediatric patients (21, 24) and adults (24).

Nonantibiotic associated pseudomembranous colitis may comprise up to 10% of the total number of cases of C. difficile associated diarrhea (1, 20, 31). Determining what perturbations of the colonic environment allow C. difficile proliferation, leading to the development of toxin associated colitis in patients without antibiotic therapy, may be difficult to ascertain in many cases (22, 23). C. difficile associated colitis has occurred in concert with chronic inflammatory bowel disease (31) and cystic fibrosis (5), which are known to alter the colonic environment. Indeed, C. difficile toxin has been identified in 2.5% of diarrheal infections not associated with antibiotic therapy (23). In the pre-antibiotic era, such cases were reported to be associated with abdominal surgery, bowel obstruction, vascular insufficiency and uremia (20). C. difficile has also been implicated as causing recurrent bouts of idiopathic inflammatory bowel disease (1), but follow-up studies have failed to confirm these findings (2, 3), and, as was mentioned earlier, C. difficile associated colitis may follow rather than result in bouts of inflammatory bowel disease (31).

Treatment

Most patients with antibiotic associated diarrhea have no evidence of pseudomembranous colitis or C. difficile toxin in their stools and have disease resulting from a change in bowel flora and treatable by discontinuing the offending antibiotic therapy (38). Indeed often even C. difficile associated diarrhea resolves when antibiotics are discontinued (5). However, symptoms may persist for weeks or months. There is little evidence to support that treatment with the same antibiotic therapy that caused the disease results in
recurrence of the disease(1). The rare recurrences with antibiotic treatment are most often associated with different antibiotics(1).

After withdrawal of the offending antibiotic, the standard treatment for C. difficile associated diarrhea is vancomycin(19,38), although metronidazole and bacitracin have been used successfully(5,38). There is only a 3% failure rate to oral vancomycin, attributed to ileus, toxic megacolon and colonic perforation(1). There is usually a rapid decrease in fever and systemic manifestations within 24-48 hours, with a gradual reduction in diarrhea, returning to normal in 5-7 days(1,38). Relapses occur in 5-55% (average about 20%(5,22,38)] an average of 7 days after discontinuing vancomycin(1,38) and occur more rapidly in severe cases(22). Problems with oral vancomycin include the inability to use it in cases of ileus, the high cost (At $200-$600/course, vancomycin is 4X more expensive than gold on a gram per gram basis.), and the high frequency of relapse(1). For this reason, oral metronidazole has been suggested as a less expensive alternative to vancomycin therapy, although it has a similar relapse rate(22) and metronidazole resistant organisms have been reported(38). The organism presumably survives by sporulation with return of toxin producing vegetative state with the antibiotic is discontinued(1). To prevent recurrence, vancomycin or metronidazole can be used for 10-14 days, followed by a three week course of an alternative regimen, such as cholestyramine or cholestipol, which bind the toxin of C. difficile although they have somewhat variable results(5,38), administration of lactobacillus, or pulse doses of vancomycin(125 mg. q.o.d.)(4). Inoculation of the GI tract with nontoxigenic strains of C. difficile has been successfully used to treat the disease in hamsters and is now being tried in patients(5,38). Certain prostaglandins have also been used to inhibit gastrointestinal disease in experimental animals(5).

Laboratory Detection

The most definitive diagnosis is by the endoscopic detection of pseudomembranes in antibiotic treated patients with diarrhea who have C. difficile toxin in their stools. Classic pseudomembranes are composed of mucin, neutrophils, sloughed epithelial cells and entrapped colonic flora(20). Often the microabcesses or plaques are isolated in certain parts of the colonic wall with other areas remaining normal in appearance(5). In as many as 1/3 of patients, only the proximal colon may be involved(1). However, as the disease
is now often detected early in its course, the classic presentation of pseudomembranous colitis is more rarely seen(5,14).

Direct examination of fecal specimens has little utility in the diagnosis of C. difficile associated diarrhea. Obviously, the patient must have watery diarrhea; the presence of formed stool is inconsistent with C. difficile associated diarrhea. As has been mentioned previously, the presence of fecal leukocytes, a predominance of gram positive rods and the presence or lack of blood in the specimen are all nonspecific findings(35,37,38). Direct visualization of C. difficile organisms by immunofluorescence techniques has poor specificity due to cross-reactivity with other Clostridial species(20,37). Thus, direct examination of diarrheal specimens contributes little to the diagnosis of C. difficile associated disease.

The isolation of toxigenic C. difficile from the stool has been used for the diagnosis of C. difficile associated colitis(5). Cycloserine-cefoxitin-fructose-egg yolk medium serves as a selective and differential medium for C. difficile(5,20,23). As few as 2000 organisms/gm of fecal specimen may be isolated(30). Once the organism has been isolated, identification may be confirmed using the API and similar system(5,20). Numerous additional studies may be performed on isolated C. difficile for epidemiologic purposes(34). Positive cultures for C. difficile have been obtained in 96% of toxin positive stools(23). Although nontoxigenic C. difficile may result in diarrhea(15,23), the severe diarrhea classically seen is toxin mediated, and culture for C. difficile would pick up numerous nontoxigenic C. difficile strains(23). A gene probe is now available for rapid differentiation of toxigenic and nontoxigenic C. difficile strains(26). However, culture for and isolation of C. difficile is not routinely performed in the work-up of possible C. difficile mediated toxigenic diarrhea.

The various clinical assays used in the detection of the toxins of C. difficile are described below:

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<tr>
<th>Test</th>
<th>Antigen detected</th>
<th>Sensitivity</th>
<th>Evaluation</th>
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<tr>
<td>Tissue culture assay</td>
<td>Toxin B</td>
<td>1 pg (50 pg/ml)</td>
<td>The test is based on the detection of cytotoxic activity in stool specimens as noted by rounding of the tissue culture cells and neutralization of the activity by C. difficile of C. sordelli antitoxin. The assay shows a good correlation with the disease and serves as the &quot;gold standard&quot; by which to measure other tests for the disease. The test is extremely sensitive, with its primary disadvantages being the assay time and expense.</td>
</tr>
<tr>
<td>Counterimmunoelectrophoresis</td>
<td>Not specific for Tox* isolates</td>
<td>10 ng of antigen (0.5 µg/ml)</td>
<td>The test is not recommended as a clinical assay for C. difficile or its toxins. The C. difficile antiserum which has been used in the assay in clinical trials cross-reacts with C. sordelli and C. bifermentum and gives false-positive reactions.</td>
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<tr>
<td>ELISA</td>
<td>Toxin A or B depending on specific antibody</td>
<td>0.1 to 1 ng (1-10 ng/ml)</td>
<td>The ELISA is still in the research and development stage. The toxin A ELISA is close to the sensitivity needed for the test but it needs to be shortened to &lt;1 h to make it more suitable as a clinical test.</td>
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<tr>
<td>Latex agglutination test</td>
<td>Not specific for Tox* isolates</td>
<td>Not reported</td>
<td>The Culturette Brand Rapid Latex Test for C. difficile has created much excitement because it is rapid (ca. 3 min) and simple. The assay is not specific for Tox* isolates; it reacts with Tox* isolates as well as C. sporogenes, P. anaerobius, and B. ovatus.</td>
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The tissue culture assay is used most extensively, and >90% of patients with pseudomembranous colitis have cytotoxic activity in their stools(5). The cytotoxic titer does not usually correlate well the severity of disease(5,20,30) although there is some indication that the titer is higher in severe cases of pseudomembranous colitis(5). Dilutions of stool are added to tissue culture cells, and a positive reaction is noted by the rounding up of cells. A variety of cells including Vero cells, rhesus monkey kidney cells and human foreskin fibroblasts(UCSF) may be used in this assay(9). Many stool specimens contain other toxins which may cause rounding of the cells, so the identity of the toxin must be confirmed by neutralizing the cytotoxic activity with antiserum against C. difficile or C. sordellii toxin(5,20,37,38), the cytotoxicity detected being due to the presence of toxin B.

There are problems with cytotoxicity assays, however. As toxin A cases the gastrointestinal disease, the level of toxin B may not accurately indicated toxin A associated disease. Fortunately, no strain producing toxin B has been isolated that does not also produce toxin A(27). Toxin A can be tested for by selective neutralization of toxin B(27), but this remains an experimental procedure. Clinical laboratories also use different cell lines, making it difficult to compare clinical studies, and the assay procedures have not been standardized with variations in the stool specimens tested, the antiserum used and the time to recording results(5,20). The sensitivity of the cell culture to the toxin decreases as the age of the cell culture increases with the possible exception of human foreskin fibroblasts(32). Cell culture also requires specialized equipment and personnel(37), making the assay inconvenient and expensive. The use of microtiter cytotoxicity assays may allow cytotoxicity assays to be performed in more conventional laboratories(11,12). Results are not rapidly available, a 1-2 day incubation being required in the standard cytotoxicity assay at UCSF. Also, as the toxin is quite labile, specimens greater than 12 hours old may yield false negative results, with less sensitivity for older specimens(7). This is a particular problem at the SF VAH since transport to the laboratory then transport to UC for processing may result in delays. In the period of 1/91-3/91, over 50% of specimens sent for C. difficile toxin assays were rejected, the majority of these due to the age of the specimen.

Some authors feel that the cytotoxicity assay is the only valid assay(1). However, most laboratories do not perform cell culture. Other alternatives include counterimmunofluorescence, ELISA and latex particle agglutination. Counterimmunofluorescence was the first of these tests developed and is considerably faster with a 1.5
hour turn-around time(5). However, the antiserum used is prepared against cultured filtrates of C. difficile, and the immunoprecipitation bands detected in most stool specimens represent proteins other than the toxins(5). The concentration of toxin necessary to give a band is much greater than that needed for the cytotoxicity assay, 10-100 ng., and many stool specimens do not have this concentration of toxin(5,38). Also, nontoxigenic strains of C. difficile, C. sordelli and C. bifermentans react with the antiserum to give false positive reactions(5), resulting in poor specificity(37,38). Some studies also describe poor sensitivity with this technique(37).

ELISA, using antibodies to toxins A and B has been found to have >90% sensitivity and extremely high specificity when compared to cell culture(1,30). "False positive" specimens have been shown to be culture positive for C. difficile(1). False negatives can occur at low titers(1). However, specificity is very high(12). About 1 ng/ml of toxin B is necessary for detection(5,30). High specificity and sensitivity, perhaps higher than that described by the toxin B ELISA(37), have been demonstrated in the ELISA to toxin A alone(5,37), with false negatives occurring only in patients with very low titers(6). One study described a positive predictive value of 96% for the ELISA for toxin A(20). Toxin A has the further advantages that it is stable for a longer period of time, and it is easier to purify, making it easier to obtain antibodies to toxin A(5). The turn-around time for the ELISA is also more rapid than the cell culture technique.

Latex agglutination tests are rapid and simple. However, despite early reports to the contrary(37,39), the antibody in the commercially available test does not react with toxin A and gives false positive results with nontoxigenic C. difficile, C. botulinum, C. sporogenes, Peptostreptococcus anaeribius, and Bacteroides assacharolyticus(16,29,38). Another latex agglutination assay was shown to be only 68% sensitive(20). Thus, the latex agglutination assays for C. difficile toxin presently available are not recommended for diagnostic purposes(5,29,38).

Bibliography

2. LaMont JT., Trnka YM. Therapeutic Implications of Clostridium difficile Toxin During Relapse of Chronic Inflammatory Bowel Disease. Lancet. 1980. 1:381-3.


