Clostridioides difficile Infection (CDI) and Inflammation

The role of *C. difficile* in intestinal disease was discovered over 40 years ago

Last year, there were 450,000 cases of CDI reported in the US, killing close to 30,000 people. These numbers do not include co-morbidities. The healthcare costs approached $5-6 billion, more than the costs associated with methicillin-resistant *Staphylococcus aureas*. CDI is now the most common hospital-acquired infection and it is recognized as a global disease. New strains that exhibit increased virulence continue to be identified, and in some countries who are only now beginning to look for the disease, CDI is considered an emerging disease.

Challenges of diagnosing CDI

- Not all people who are colonized with *C. difficile* have CDI. The presence of a toxigenic strain does not mean that toxin is present.
- Mild cases may resolve on their own and perhaps do not require treatment with antibiotics.
- There can be co-infections with other pathogens that complicate the diagnosis.
- Even so, CDI can become debilitating and life-threatening, and moderate to severe cases require antibiotics, and perhaps a fecal transplant.
- The disease continues to be particularly problematic in patients over the age of 65, but has spread to the community.
- Outbreak strains due to genetic mutations continue to be a problem. Mutations occur not only in the toxin genes but also in core genes as well.
- Measurement of blood and fecal biomarkers can help assess the patient's condition.

Three guidelines for diagnosing CDI

ESCMID Guideline

CDI is "a clinical picture compatible with CDI such as diarrhea, ileus and toxic megacolon in combination with either microbiologic evidence of free toxins in stool or the presence of toxigenic *C. difficile* in stool without reasonable evidence for an alternative cause of diarrhea; or pseudomembranous colitis diagnoses during endoscopy, after colectomy or at autopsy."

IDSA/SHEA Guideline

CDI is defined "by the presence of symptoms (usually diarrhea) and either a stool test positive for *C. difficile* toxins or detection of toxigenic *C. difficile*, or colonoscopic or histopathologic findings revealing pseudomembranous colitis."

American Society for Microbiology Guideline

Focused on the detection of the organism in fecal specimens.

CDI often is characterized by an intense neutrophil infiltration, and in the majority of cases, can be described as an inflammatory diarrhea.

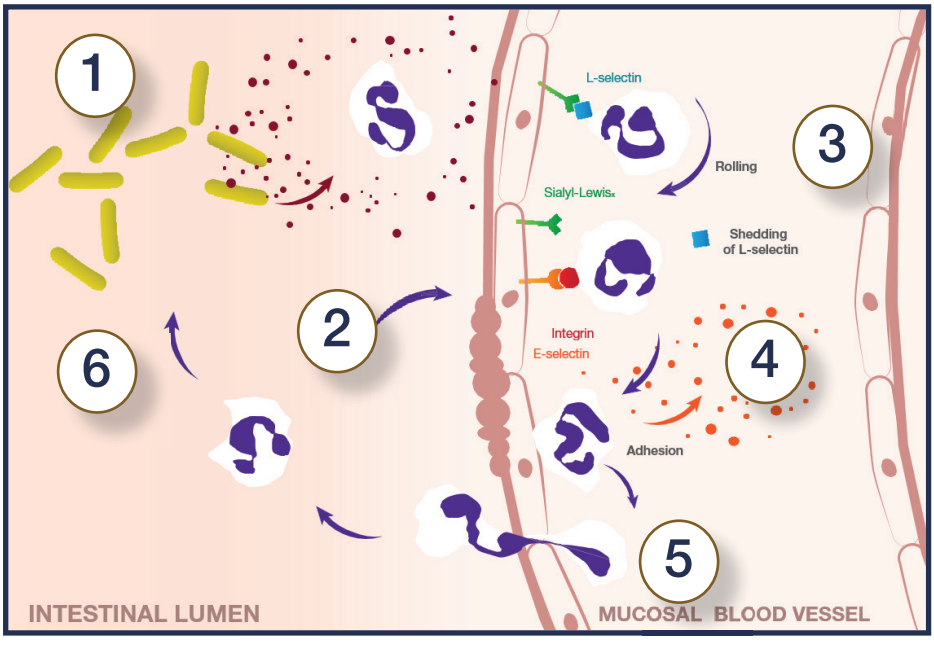
CDI and Inflammation

The initial infection with toxigenic *C. difficile* spores occurs via the fecal-oral route, with growth in the large intestine resulting in the release of toxins A and B. The toxins damage mucosal cells, triggering an influx of neutrophils. Both toxins are chemotactic, bringing in more neutrophils, and both toxins activate inflammasomes. Activated neutrophils express increased levels of granule proteins such as lactoferrin, defensins, myeloperoxidase, and elastase. Activated neutrophils also release proinflammatory cytokines (IL-1β, IL-6, TNF-α), possibly exacerbating the disease, and they migrate into the intestinal lumen via diapedesis. In the lumen, the neutrophils lyse and release lactoferrin, calprotectin, defensins, myeloperoxidase, and elastase.

How is CDI severity assessed?

Do higher levels of biomarkers for intestinal inflammation correlate to more severe disease?

Can host biomarkers help determine treatment?



1. Infection with toxigenic *C. difficile* and release of toxins A and B.
2. Toxins damage mucosal cells, triggering influx of neutrophils. Toxins are chemotactic, bringing in more neutrophils, and they activate inflammasomes.
3. Activated neutrophils express increased levels of granule proteins such as lactoferrin, defensins, myeloperoxidase, and elastase.
4. Activated neutrophils release proinflammatory cytokines (IL-1β, IL-6, TNF-α).
5. Cells undergo diapedesis and enter intestinal lumen.
6. Neutrophils lyse in intestinal lumen and release lactoferrin, calprotectin, defensins, myeloperoxidase, and elastase.

Assessment of CDI Severity

Clinical Definition	Supportive Data
Mild to moderate	WBC ≤ 15,000 cells/μL and Serum Creatinine < 1.5 mg/dL
Severe, uncomplicated	WBC ≥ 15,000 cells/μL or Serum Creatinine > 1.5 mg/dL
Severe, complicated	Criteria for severe infection plus hypotension, shock, ileus, or megacolon

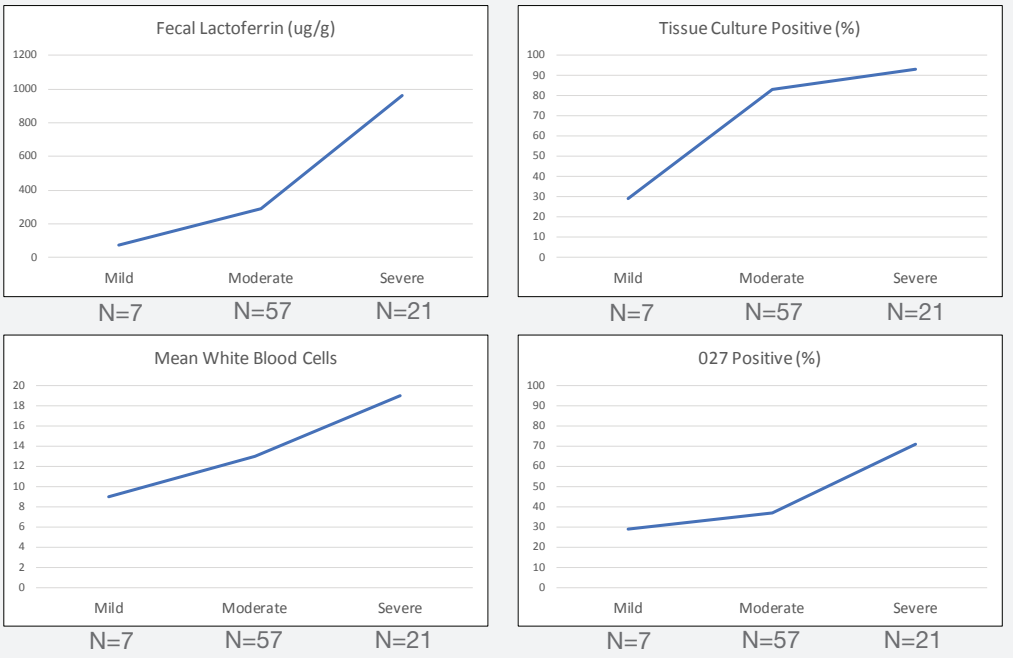
In general, more severe CDI is associated with:

- Higher WBC counts
- Low serum albumin
- Higher lactoferrin levels

What is lactoferrin?

Lactoferrin is an especially good biomarker for severity of CDI. It is an 80 kDa glycoprotein present in activated neutrophils. When the intestines are inflamed, activated neutrophils are shed into the stool, and lactoferrin is released and is present in high levels. It is a good marker because it is resistant to degradation and is highly stable in feces. Studies have demonstrated the correlation of fecal lactoferrin with severe CDI when elevated in conjunction with other laboratory markers and with clinical assessment of severity. The utility of lactoferrin has been demonstrated further in studies that followed the severity of CDI in patients infected with ribotype 027, an especially virulent strain of *C. difficile*. Additionally, an important role for host inflammatory biomarkers is supported by studies showing that the severity of CDI correlates with the level of intestinal inflammation, more so than with bacterial burden.

Lactoferrin levels in CDI patients



Fecal lactoferrin and calprotectin in patients with *Clostridium difficile* infection: A prospective case-control study

- Fecal lactoferrin and calprotectin were higher in CDI patients (N=135) than in patients who were negative for *C. difficile* and in patients who were colonized with nontoxigenic strains.
- Patients who had free toxin in their feces had significantly higher fecal lactoferrin and calprotectin than patients who were negative for *C. difficile* and in patients colonized with nontoxigenic strains.
- There was a correlation between intestinal inflammation and toxin.

Ribotype 027:

The most prevalent ribotype of *C. difficile* among inpatients admitted from long-term care facilities (LTCF)

- LTCF residents (N=28) had higher antibiotic use and more 027 infections than those admitted from home.
- LTCF residents who had 027 infections had higher mortality and more inflammation than those with non-027 infections (10 other ribotypes).
- Fecal lactoferrin levels were >95 µg/mL in 027-infected patients versus >36 µg/mL in non-027 patients.
- LTCF patients are typically sicker and more frail, and in this study, more likely to suffer from 027 infections.

Ribotype 027

Outbreak hypervirulent strain

Grows to higher numbers in the intestine

Produces more toxin in the intestine

Resistant to fluoroquinolones

Markers of intestinal inflammation, not bacterial burden, correlate with clinical outcomes in *Clostridium difficile* infection

- CDI was defined as a positive toxin test, ≥3 bowel movements a day, diarrhea persistence, intensive care unit admission, toxic megacolon, colectomy, and/or death.
- Fecal lactoferrin was higher in patients with a severe HINES VA score.
- Fecal bacterial burden, based on *tcd*B PCR, did not correlate with severity score.
- HINES VA score and leukocytosis correlated with severe disease.
- Concluded that intestinal inflammation determines clinical severity and prolonged CDI may be caused by sustained host response.

HINES VA Severity Score

- Fever (≥38˚ C)
- Ileus by clinical exam or X-ray
- Hypotension with SysBP <100 mmHg
- White blood cell count [x10^9/L]
- CAT scan findings of colonic wall thickening, dilated colon, or ascites

Does severity of CDI affect treatment?

Most patients with CDI will be treated with vancomycin or fidaxomicin, and most patients respond within several days. A positive response to antibiotic therapy can be followed by lactoferrin testing. CDI patients who do not respond to antibiotic therapy or who have a greater likelihood of relapse can be monitored by quantitative lactoferrin testing, which may help determine if a fecal transplant is needed. In addition, highly elevated lactoferrin levels may help to predict patients at risk of pseudomembranous colitis, patients who may need colectomies, and those possibly at an increased risk of death from CDI.

Conclusions

- When antibiotics are given for CDI, they kill vegetative cells and the toxins are no longer produced.
- In most cases, inflammation has been triggered by the action of the toxins.
- Once inflammation is initiated, the host response plays a major role in how severe the CDI becomes.
- Inflammation will help control the infection by killing the organism but the host response can become uncontrolled, resulting in more severe disease.

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