FECAL LACTOFERRIN TESTING

A CLINICAL AID FOR DETERMINING SEVERITY IN CLOSTRIDIUM DIFFICILE INFECTION
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FECAL LACTOFERRIN TESTING IN CDI

INTRODUCTION

Infectious diarrhea caused by Clostridium difficile often involves intestinal inflammation. Toxigenic strains of C. difficile produce toxins A and B, which are harmful to the gut and cause colitis. Lactoferrin is a glycoprotein secreted by activated neutrophils as part of the human immune response. Lactoferrin levels in feces are used as a biomarker for fecal white blood cells that increase during infectious colitis (Figure 1). Recent studies have shown that fecal lactoferrin measurements can indicate severity of inflammatory infectious diseases such as those caused by toxigenic C. difficile. Fecal lactoferrin can help physicians assess severity of infection through quantitation of inflammation, to determine an appropriate course of treatment.

Figure 1: Mucosal Inflammation

CLINICAL UTILITY OF LACTOFERRIN TESTING FOR CDI

Clostridium difficile is a gram-positive anaerobe and the leading known cause of antibiotic-associated diarrhea. Infection with C. difficile is most common in patients with recent antibiotic use, which disturbs
normal gut flora and allows C. difficile to colonize the intestine. C. difficile infection (CDI) produces symptoms such as diarrhea, fever, nausea, and abdominal pain. Mild cases of C. difficile disease may resolve on their own, while moderate to severe cases require selective antibiotic treatment or fecal microbiota transplant. Although most infections are cleared with antibiotics, clinical relapse is seen in approximately 20 to 30% of patients. Infection with Clostridium difficile can be particularly problematic in patients over 65 years of age, accounting for the majority of cases and placing patients at a higher risk of severe disease and relapse. Measurement of both blood and fecal biomarkers can give a more accurate indication of C. difficile infection, and allow for proper treatment. Elevated fecal lactoferrin, presence of toxin in the stool, elevated white blood cell count, elevated creatinine levels, low serum albumin levels, and identification of ribotype 027 are predictors for disease severity.

CDI involves a range of clinical presentations including mild self-limiting diarrhea to life-threatening pseudomembranous colitis and megacolon. Most cases are diagnosed based on clinical evaluations, history of antibiotic use and the presence of toxin in the stool (toxins A and B). Microwell and membrane EIA tests for detecting glutamate dehydrogenase (GDH) and toxin in fecal specimens are often used in combination with molecular assays like PCR tests for the toxin B gene (tcdB). The current gold standard methods for CDI are tissue culture combined with specific neutralization for stool toxin and toxigenic culture for the organism. All of these methods are suitable for detecting the presence of toxigenic C. difficile and its toxins as an aid to diagnosis but do not provide information regarding severity of disease. In general, about 30% of patients with CDI present with fever, 50% have a slightly raised white blood cell count (WBC) and 20% experience mild abdominal pain. Mild cases respond well to antibiotics and even to simply stopping the inciting agent. Moderate to severe CDI cases need early detection for a better outcome and for decreased mortality.

**Challenges in diagnosing and managing CDI**

Stratifying CDI patients based on severity is not a new concept but it has gained attention because of the increase in incidence and frequent severity of CDI over the past decade. In a study published by L. Kyne et al., the authors performed a detailed characterization of disease status in an outbreak of CDI in Dublin, Ireland. This particular outbreak involved 14 patients who were stool cytotoxin positive but asymptomatic. Of the symptomatic patients, 25% had mild self-limiting disease with no antibiotic treatment, 35% had moderately severe CDI responding to antibiotic treatment and 40% developed severe disease with prolonged symptoms lasting between 11 to 36 days. A total of 8% of the severe CDI patients progressed to colitis with pseudomembranes and toxic megacolon. The authors concluded that early indicators of disease severity are needed in order to lower the morbidity and mortality.

Currently, a combination of the clinical presentation plus blood biomarkers of inflammation have been proposed for stratifying patients by disease activity (mild to severe; Table 1). White blood cell count (WBC), serum albumin level (indicator of leakage into the bowel), and creatinine level for monitoring kidney failure are the most commonly used lab indicators for disease activity for CDI. Mild to moderate cases of CDI usually present with a WBC ≤ 15,000/µL, normal serum creatinine (< 2.0 mg/dL) and albumin levels (≥ 2.5 g/dL). Symptoms range from less than 10 watery stools without blood per day to mild cramping lasting for up to an average of 4 days. The common treatment for patients with an initial episode of mild to moderate CDI is 500 mg metronidazole 3 times daily for 10 days. Most cases resolve with no further complications but up to 25% of these cases may relapse and require a second round of antibiotics. Relapses are not limited to a single episode; patients can relapse multiple times. Stratifying patients for severity to optimize treatment will improve patient outcome and assist with antibiotic stewardship protocols by identifying patients who do not need antibiotics.
Patients over the age of 65 are at a higher risk for CDI and often suffer from more severe disease leading to multiple relapses. Severe fulminant CDI is characterized by having 11 or more liquid stools per day for more than 10 days. Fecal specimens often contain mucus and may be bloody. Defined lab parameters for fulminant C. difficile colitis are WBC ≥ 15,000/µL, a rising serum creatinine (50% increase and levels ≥ 2.0 mg/dL) indicating poor kidney function and albumin levels dropping below 2.5 g/dL showing loss of protein because of exudation of serum into the bowel. Clinical presentations may involve pseudomembranes on endoscopy, severe abdominal pain and cramping, and colonic thickening observed by CT scan. Toxic megacolon stemming from ileus may occur causing nausea, vomiting, severe dehydration and extreme lethargy. Treatment for moderate to severe cases of CDI usually involves vancomycin, fidaxomicin and, in some cases, fecal transplantation.

Utility of lactoferrin testing in routine diagnostics

Lactoferrin testing helps confirm inflammation in CDI

Not all individuals who are colonized with C. difficile have disease which requires treatment. For example, C. difficile is present in up to 70% of infants who are colonized but are typically asymptomatic. In adults, some strains of C. difficile do not produce the toxins which cause diarrhea, and even toxigenic strains may not actively produce toxin in individuals with healthy gut flora. In a recent study by Boone et al. that evaluated PCR-positive CDI patients, lactoferrin levels as an indicator of intestinal inflammation were higher in patients who were stool toxin-positive compared to PCR-positive-stool toxin-negative (Figure 2). This demonstrates that a sample with a tcdB-positive PCR result does not necessarily indicate that toxins are actively being produced by C. difficile. In a similar study by LaSala et al., patients who were both PCR- and stool/toxin-positive had a median lactoferrin level of 80 µg/mL compared to 24 µg/mL for the PCR-positive stool/toxin-negative group (p=0.004). A patient could thus be needlessly treated for C. difficile when they are only colonized, or could have another infection causing their diarrhea. Lactoferrin testing is beneficial in the differentiation of C. difficile colonization and disease. However, it is important to realize that patients who are symptomatically colonized with C. difficile are at greater risk to develop symptoms if they go on antibiotics. They also could spread disease to others in a setting such as a hospital or extended-care facility.
Severe CDI

CDI is a complicated disease and determining treatment is challenging. Ribotype 027 is an outbreak strain of *C. difficile* more highly associated with development of severe disease than other toxigenic strains. This strain is resistant to fluoroquinolones, and *in vivo* produces a greater quantity of spores, which are the primary mode of infection when ingested. Ribotype 027 is also more likely to produce toxin than other *C. difficile* strains, which results in significantly higher fecal lactoferrin levels than other ribotypes (Figure 3). The evidence is increasing for the importance of determining if a patient is infected with ribotype 027 and for identifying the extent of damaging inflammation to ensure that a proper course of antibiotic treatment is selected. Milder cases of *C. difficile* are usually cleared with metronidazole, while vancomycin and fidaxomicin are recommended in severe cases and for patients whose disease was not resolved with metronidazole. Though guidelines do not currently recommend initial treatment of ribotype 027 infections with vancomycin or fidaxomicin, an increasing amount of research is linking this ribotype with severe disease. Fecal lactoferrin testing offers an aid for determining severity of disease when dealing with this potentially harmful strain.

Fecal lactoferrin is a sensitive biomarker which can be very beneficial in determining the severity of *C. difficile* disease. Determining if a patient has high lactoferrin levels alerts a physician to more severe intestinal damage caused by the toxins. Utilizing fecal lactoferrin as a biomarker alongside stool toxin testing, blood biomarkers, and identification of ribotype 027 infection will result in a more comprehensive understanding of *C. difficile* disease and appropriate selection of treatment for infected patients.
Advantages of lactoferrin testing

**Lactoferrin testing is reliable.**

Elevated levels of fecal lactoferrin correlate with endoscopic and histologic patterns of intestinal inflammation. Lactoferrin is stable in feces for up to 2 weeks at room temperature and refrigerated, and for much longer periods at ≤-20°C. It is more reliable than microscopy for detecting fecal leukocytes since it does not rely on intact leukocytes which degrade in stool within hours.

**Lactoferrin testing is non-invasive.**

Lactoferrin testing relies only on a fecal sample and thus may be coupled easily with *C. difficile* diagnostic testing. It provides a patient-friendly first read on inflammation and helps to rapidly identify those patients with more severe CDI and who warrant further investigation.

**Lactoferrin testing is sensitive and specific for intestinal inflammation**

Lactoferrin testing is more sensitive than microscopy for detecting fecal leukocytes since cells degrade within hours.

Limitations of lactoferrin testing

Lactoferrin testing is specific for intestinal inflammation and not the particular disease. Results should be used in combination with other laboratory results and patient history. In addition, patients with low amounts of inflammation having lactoferrin levels near baseline may require repeat testing depending on changes in clinical presentations.
SUMMARY

_Clostridium difficile_ infection is a complicated disease and determining severity for optimal medical treatment in older sicker patients can be challenging. Determining the presence of intestinal inflammation using lactoferrin offers a noninvasive diagnostic aid for assessing patients with CDI. Identifying patients with moderate to severe disease will aid the direction of antibiotic treatment for improved patient outcomes.

REFERENCES CITED


TYPES OF LACTOFERRIN TESTING

Lactoferrin testing formats

Qualitative test formats

Qualitative lactoferrin testing provides detection of elevated levels of fecal lactoferrin, a marker for fecal leukocytes and an indicator of intestinal inflammation. It provides convenient results for smaller clinics with appropriately licensed in-house laboratories.

The LACTOFERRIN EZ VUE™ test is an immunochromatographic test for the qualitative detection of elevated levels of lactoferrin, a marker for fecal leukocytes and an indicator of intestinal inflammation. The LACTOFERRIN EZ VUE™ test is a simple-to-use lateral flow format that provides results in 10 minutes.

The LACTOFERRIN CHEK™ test is an ELISA for the qualitative detection of elevated levels of lactoferrin, a marker for fecal leukocytes and an indicator of intestinal inflammation. The LACTOFERRIN CHEK™ test is a simple-to-use microwell format that has a turnaround time of an hour and fifteen minutes.

Quantitative test format

Quantitative lactoferrin testing provides a measurement of fecal lactoferrin. It helps assess C. difficile disease severity and guide treatment.

The LACTOFERRIN SCAN™ test is a quantitative ELISA for measuring concentrations of fecal lactoferrin, a marker of fecal leukocytes. An elevated level is an indicator of intestinal inflammation. Measuring lactoferrin levels can aid in determining the severity of CDI and assist with treatment decisions.

How to order testing

Both qualitative and quantitative lactoferrin testing can be easily ordered through most laboratories. A stool specimen collected in a cup can be tested. Specimens can be stored at 2-8°C or at room temperature for up to 2 weeks before being tested.