Lactoferrin as a Predictor of Disease Severity for Clostridium difficile Disease

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INTRODUCTION

Clostridium difficile infection (CDI) involves a range of clinical presentations from self-limiting diarrhea to life-threatening pseudomembranous colitis and mega colon. Patients usually experience at least 3 watery stools without blood per day and may have abdominal pain or cramping. Currently, a combination of clinical presentations and various laboratory parameters have been proposed for stratifying patients from mild to severe disease. While blood cell (WBC) count, serum albumin and creatinine levels are the most commonly used lab indicators for disease activity of CDI, while most episodes resolve with treatment, up to 25% of these cases relapse and require a second round or more of antibiotics. An initial episode of mild CDI is often treated with metronidazole, while vancomycin is saved for moderate to severe or relapsing cases. Human lactoferrin is a glycoprotein that is present in most mucosal secretions and a primary component of the granules of activated neutrophils. During the onset of intestinal inflammation, activated neutrophils infiltrate the intestinal lumen causing an increase in fecal lactoferrin.

AIM

To investigate the link between elevated levels of lactoferrin and disease severity.

METHODS

- 39 patients suspected of having C. difficile disease based on clinical history of prior antibiotic use, more than 3 unformed stools per day and the presence of fecal glutamate dehydrogenase (GDH) were enrolled over a 6 month period with informed consent. A total of 53 healthy subjects (no intestinal illness) were included as controls (normal lactoferrin range = 1.45 ±0.4 µg/mL).
- Disease severity: Mild = diarrhea no other symptoms, Moderate = diarrhea with some symptoms of pain, fever, vomiting, elevated WBC count, nausea, comorbidities. Severe = diarrhea and most symptoms listed.
- Tissue culture: Human foreskin cell monolayers and toxin B neutralizing sera were used for specific neutralization with feces and cultures.
- Bacterial and toxigenic culture: Ethanol spore enrichment with CCFA was used to isolate culture-positive specimens. Isolates were subcultured to BHI and grown for 72h then tested by tissue culture for the presence of toxin B.
- PCR ribotyping analysis: DNA was extracted from broth cultures using the QIAamp Mini Kit (Qiagen, Valencia, CA). Control CD strains (ARL ribotypes 001, 002, 003, 012, 014, 017, 027, 033, 036, 046, 053, 054, 078, 106, 110, 126 and 154) were used for ribotyping standards.
- C. difficile -Chek-60™ test: This is a microwell ELISA that provides results for the presence of antigen (GDH).
- BIO-UCAMP®: quantitative ELISA for measuring stool lactoferrin concentration (µg/mL).
- Statistical analysis: Student T-test for p-value, mean ±standard error by Microsoft Excel.

RESULTS

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>Patient Group</th>
<th>Percent of GDH Positive</th>
<th>Percent of Lactoferrin Elevated</th>
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<tbody>
<tr>
<td>Age</td>
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<tr>
<td>% Males</td>
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<td>WBC</td>
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<td>Comorbidities</td>
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<td>Diabetic</td>
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<td>Renal failure</td>
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| Table 2. C. difficile Detection Rates for Stratified Patient Populations

CONCLUSIONS

- Elevated levels of lactoferrin are useful as an aid for stratifying patients based on disease severity.
- Ribotype ARL 027 is associated with higher lactoferrin levels and more severe disease as determined by clinical assessment.
- Toxinogenic culture and the presence of fecal GDH performed similarly in detecting ARL 027 and non027 C. difficile.

References cited

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