Quantitative Fecal Lactoferrin in Toxin-Positive and Toxin-Negative Clostridium difficile Specimens

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Quantitative fecal lactoferrin was measured in 112 patients tested for toxigenic Clostridium difficile using glutamate dehydrogenase (GDH) and toxin immunoassays combined with tcdB PCR. Lactoferrin levels were higher in the GDH-positive/toxin-positive group (79 µg/ml) than in the GDH-positive/toxin-negative/PCR-positive (21 µg/ml) and the GDH-negative groups (13 µg/ml). Differences in fecal lactoferrin levels suggest variable presence or severity of C. difficile infection among toxin-positive and toxin-negative patients.

Diagnostic testing for Clostridium difficile infection (CDI) may be accomplished through (i) organism detection by anaerobic culture or glutamate dehydrogenase (GDH) immunoassay with subsequent confirmation of toxigenicity, (ii) toxin detection by cell cytotoxicity neutralization assay (CCNA) or enzyme immunoassay (EIA), and (iii) nucleic acid amplification tests (NAAT) for tcdA, tcdB, and/or other C. difficile genes. Given that the rate of positivity of NAAT assays and GDH immunoassays approaches that of toxigenic culture (1, 2), while those of CCNA and EIA are significantly lower (3), universal testing by NAAT or by multistep GDH screening algorithms is endorsed by IDSA/SHEA consensus (4). Because toxin production is necessary for organism pathogenesis, however, debate persists as to whether direct toxin measurements may offer a theoretical advantage with respect to clinical specificity (5, 6). If toxin positivity were a better indicator of CDI than NAAT positivity, it would follow that levels of fecal lactoferrin (a measure of colonic inflammation) should be higher in toxin-positive patients than in NAAT-positive/toxin-negative patients. The aim of this study, therefore, was to assess fecal lactoferrin values among toxin-positive, NAAT-positive/toxin-negative, and NAAT-negative/toxin-negative patients.

Based upon results from a single academic medical center of a routine, two-step C. difficile diagnostic algorithm (7), which incorporates GDH and toxin A/B lateral flow EIA (C. Diff Quick Check Complete; TechLab, Blacksburg, VA) with tcdB PCR (Gene Xpert; Cepheid, Sunnyvale, CA), 112 previously frozen, unique patient stool samples were selected for study inclusion. Fecal lactoferrin levels were determined for all samples using quantitative enzyme-linked immunosorbent assay (ELISA) (IBD Scan; TechLab, Blacksburg, VA) as per the manufacturer’s instruction with a modification (to improve assay precision) that initial specimen aliquots were weighed to the nearest 0.001 g and diluted 1:10 (wt/vol) in kit diluent. A final dilution of 1:500 was used for all samples. Patient electronic medical records were queried to determine history of immunosuppression, inflammatory bowel disease (IBD) and/or prior bowel surgery, results of other stool studies, and peripheral white blood cell (WBC) count within 48 h of C. difficile testing. Data were analyzed using a statistical software program (Minitab 16), and Mann-Whitney U test and Fisher’s exact test were used to study group differences in lactoferrin levels.

Coefficient of variation for lactoferrin ELISA, determined by testing specimen triplicates over 3 days, ranged from 28% for levels of <10 µg/ml to 8% for levels of >100 µg/ml. Fecal lactoferrin values for all 112 specimens were compared between the following cohorts: group 1, negative for C. difficile (GDH−); group 2, negative for toxigenic C. difficile (GDH+/toxin−/PCR−); group 3, positive for toxin-producing C. difficile (GDH+/toxin+/PCR+); and group 4, positive for toxin-competent C. difficile (GDH+/toxin+/PCR+) (Table 1). No differences in fecal lactoferrin levels were observed between groups 1, 2, and 4, whereas group 3 demonstrated significantly higher lactoferrin values than group 1 (P = 0.006), group 2 (P = 0.002), and group 4 (P = 0.015) (Mann-Whitney U test). A significantly higher proportion of samples from group 3 also yielded lactoferrin values above lactoferrin thresholds of 80 or 100 µg/ml compared with all other groups (Table).

Peripheral WBC counts were available for 94% (105/112) of patients. Analysis revealed higher WBC values for group 3 than group 4 (P = 0.045, Mann-Whitney U test), a finding that corroborates results reported elsewhere (7). Rates of immunosuppression (defined as any history of organ transplantation, hematologic malignancy, or chemoradiation for solid tumor therapy) were similar between group 3 (12/25, 48%) and group 4 (9/30, 30%) (not significant), though they were slightly overrepresented among the lower fecal lactoferrin values within groups (i.e., 8/12 group 3 patients and 9/26 group 4 patients with fecal lactoferrin values of <80 µg/ml) were immunosuppressed, compared with 4/13 group 3 and 0/4 group 4 patients with fecal lactoferrin values of ≥80 µg/ml). The proportion of patients with IBD was greater in group 4 (5/30) than in group 3 (0/25) (nonsignificant). Among eight patients from groups 1 and 2 (i.e., negative for toxigenic C. difficile) having lactoferrin levels of ≥80 µg/ml, five had acute-onset, self-limited diarrheal illnesses for which no etiology was established. Two others had enteric bacterial pathogens recovered in culture (Campylobacter and Salmonella), and one patient had...
ischemic bowel necrosis requiring colectomy. Additional factors that potentially interfere with fecal lactoferrin assay performance characteristics (e.g., dietary intake of dairy products, recent colonic endoscopy, etc.) were not evaluated.

The observation of significantly higher fecal lactoferrin levels among patients with detectable *C. difficile* toxin (group 3) than among all toxin-negative patient cohorts suggests that colitis is either more prevalent or more pronounced in the former relative to the others.

These differences are particularly noteworthy given the small study sample size in addition to the greater number of IBD patients and fewer immunosuppressed patients observed in group 3 than in group 4 (both findings that might be expected to diminish differences in fecal lactoferrin levels). Because toxin is central if not essential to *C. difficile* pathogenesis (8), a potential drawback to exclusive reliance on NAAT is that detection of toxin genes may be less specific for the disease state than is the detection of toxin itself (5, 6). Interestingly, Ryder et al. used a novel, real-time cell culture impedance method to demonstrate a “dosage effect” for *C. difficile* toxin on CDI severity, yet the authors were unable to correlate toxin concentration with *tcdB* gene copy number (9). Furthermore, two studies incorporating clinical data suggest that the prevalence and/or severity of CDI are greater among toxin-positive patients than among toxin-negative patients (10, 11). Taken together with the present observations, these data support the idea that *C. difficile* toxin is more closely linked to colitis/disease than is the toxin gene.

Further investigation of CDI dynamics should aim to establish whether NAAT-positive/toxin-negative patients are more prevalent or more pronounced in the former relative to the others.

Finally, a number of other studies have explored the potential utility of fecal lactoferrin measurement in the setting of CDI. Early work using a latex bead format as a potential diagnostic marker of CDI showed a high statistical association between the two by multivariate analysis (19) but fairly poor sensitivity (44 to 75%) and specificity (46 to 61%) in detecting CDI outright (20, 21). Applying a semiquantitative titer methodology, Steiner et al. observed significantly higher fecal lactoferrin levels in CDI patients with severe disease than in those having mild manifestations (22). Wren et al. subsequently discussed the prospect of assessing lactoferrin by lateral flow EIA as an adjunct to CDI laboratory diagnostics, pointing out that elevated levels may be indicative of more severe disease (23). Given the labor-intensive nature of quantitative fecal lactoferrin ELISA in addition to its marginal utility in delineating patients with *C. difficile* toxin-positive and/or NAAT-positive specimens from those without, routine use of the methods described here in a diagnostic setting would seem formidable.

In summary, given high colonization rates of toxigenic *C. difficile* (14, 15) coupled with frequent orders for *C. difficile* testing on stool specimens from patients without objective diarrhea (17, 18), detection of some degree of asymptomatic carriage would appear inevitable. The present study suggests that detection of *C. difficile* toxin is associated with higher rates of intestinal inflammation than is detection of toxin genes. Additional prospective research that incorporates laboratory diagnostic data, fecal lactoferrin levels, therapeutic intervention, and patient outcomes is needed.

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**REFERENCES**


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