JOURNAL OF CLINICAL MICROBIOLOGY, May 1994, p. 1360–1361
0095-1137/94/$04.00+0

Vol. 32, No. 5

Comparison of Fecal Lactoferrin Latex Agglutination Assay and Methylene Blue Microscopy for Detection of Fecal Leukocytes in *Clostridium difficile*-Associated Disease

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Received 23 September 1993/Returned for modification 9 December 1993/Accepted 31 January 1994

The fecal lactoferrin assay was more sensitive (75%) than methylene blue microscopy (40%) for the detection of leukocytes in *Clostridium difficile* toxin-positive fecal samples. Although limited sensitivity and specificity precludes its use as a laboratory screening test, it may be a more useful initial test in an algorithm for clinically suspected *C. difficile*-associated disease.

The definitive diagnosis of colitis caused by *Clostridium difficile* remains problematic. In the presence of characteristic epidemiologic and clinical features, the most important diagnostic test is the detection of *C. difficile* toxins A, B, or both in stool samples by a cell culture cytotoxicity assay or enzyme immunoassay (1, 3, 4, 9). The presence of leukocytes in fecal samples is a nonspecific marker for inflammatory diarrhea, but their presence may be highly suggestive of *C. difficile*-associated disease (CAD) in the appropriate clinical setting. Fekety and Shah (4) recently proposed an algorithm for the management of antibiotic-associated diarrhea and colitis that emphasized the importance of fecal leukocyte testing in the initial patient evaluation. A potential drawback is that by methylene blue microscopy, fecal leukocytes are detected in only approximately 50% of stool specimens from patients with CAD (2, 5). Guerrant and colleagues (8) recently described a latex agglutination method for the detection of fecal lactoferrin as a marker for fecal leukocytes and found that in a small selected sample, 16 of 17 (95%) patients with high *C. difficile* cytotoxin titers (≥1,000) were also positive by the lactoferrin assay. In contrast, Garner et al. (6) found that fecal lactoferrin testing was only 69% sensitive for detecting CAD in a retrospective analysis of 361 stool samples. Therefore, given the limited experience and conflicting results, the purpose of our study was to compare the fecal lactoferrin assay with methylene blue microscopy for the detection of fecal leukocytes in a large number of stool samples also tested for *C. difficile* toxin in order to clarify further the potential utility of fecal lactoferrin testing in the initial evaluation of CAD.

A total of 325 stool samples submitted to the laboratory for *C. difficile* toxin testing were also examined for fecal leukocytes by both methylene blue microscopy and the fecal lactoferrin assay. Of the 325 specimens, 267 were unselected weekday specimens evaluated prospectively over a 2-month period. The remaining 58 specimens consisted of selected toxin-positive and random toxin-negative samples that were previously examined for *C. difficile* toxins by enzyme immunoassay (EIA) and cytotoxicity assay (CTA) and for fecal leukocytes by methylene blue microscopy on the original day of receipt; the samples were subsequently stored frozen at −20°C. In general, fresh specimens were stored at 4°C and were processed within 24 h of collection; frozen specimens were thawed and tested for fecal lactoferrin.

Fecal wet preps stained with methylene blue were examined microscopically for the presence of polymorphonuclear leukocytes (7), generally within 12 h of collection. Twenty oil immersion fields were searched, and the results were recorded as positive (≥1 polymorphonuclear leukocyte) or negative.

The fecal lactoferrin latex agglutination assay (Fast 'N' Flammatory; Tech Labs, Inc., Blacksburg, Va.) was performed according to the manufacturer's instructions by using 1:50 and 1:200 dilutions of specimen. A positive reaction was defined as, at the minimum, definite, fine agglutination with a milky background. A negative control was included with each specimen, and a positive control was performed on each day of testing.

The EIA for the detection of *C. difficile* toxins A and B (Cytoclone A + B EIA; Cambridge Biotech Corp., Worcester, Mass.) was also performed according to the manufacturer's instructions. The range for indeterminate results was expanded slightly, and those samples giving indeterminate results were then tested by a CTA. The spectrophotometrically determined results (dual wavelength) were recorded as positive (>0.225), indeterminate (0.125 to 0.225), or negative (<0.125). The CTA was performed on 9 samples that gave an indeterminate EIA result or was performed as the original sole toxin assay on 16 selected samples that were subsequently stored frozen. For the CTA we used a human diploid embryonic fibroblast cell line (MRC-5) and serial dilutions of specimen in a microtiter format as described previously (10). Plates were examined after 24 and 48 h for a characteristic cytopathic effect, which was neutralized by antitoxin in a control well. The titer was determined as the reciprocal of the highest dilution exhibiting a 50% cytopathic effect and was interpreted as positive when it was ≥90. Titers of ≥90 obtained by the CTA described above were generally clinically significant on the basis of previous observations in our institution (10).

Sixty-seven of the 325 specimens were cytotoxin positive: 43 by EIA only and 8 by CTA only (of 9 EIA-indeterminate samples) among the prospectively evaluated samples and 16 by both EIA and CTA among the selected samples. The corresponding sensitivity of the lactoferrin assay was 75%, whereas it was 40% for the methylene blue smear (Table 1). The specificity was 46% for the lactoferrin assay and 92% for the methylene blue smear. On the basis of an overall 10% prevalence of *C. difficile* toxin positivity in our laboratory (unpub-

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TABLE 1. Analysis of 325 stool specimens for *C. difficile* toxin and for fecal leukocytes by lactoferrin assay and methylene blue assay

Test results	No. (%) positive by the following assay:		
	C. difficile toxin(s)	Fecal lactoferrin	Methylene blue
Positive	67[a]	50 (75)	27 (40)
Negative	258	139 (54)	20 (8)

[a] Determined to be positive by either EIA only (n = 43), both EIA and CTA (n = 16), or CTA only (n = 8; titers ≥90).

lished data), the calculated positive and negative predictive values were 16 and 82%, respectively, for the lactoferrin assay and 5 and 87%, respectively, for the methylene blue smear.

In the present study, the fecal lactoferrin latex agglutination assay demonstrated greater sensitivity than methylene blue staining for the detection of fecal leukocytes in stool samples positive for *C. difficile* toxin. Lactoferrin appears to be relatively stable in refrigerated and frozen stool samples and is a useful marker for fecal leukocytes even when they are morphologically undetectable because of breakdown during transport or storage or destruction by cytotoxic fecal specimens (8). The optimal timing for examination of stool samples for leukocytes by the methylene blue assay is generally thought to be within several hours of sample collection, which is difficult to do in many busy laboratories. The methylene blue staining of most samples within 12 h in our study is probably representative of the practical limits of performance of the methylene blue assay. In contrast, the stability of fecal lactoferrin in stool samples would allow more feasible batch processing of specimens.

If clinicians use a management algorithm that includes examination for fecal leukocytes in the initial evaluation of patients suspected of having CAD, then the lactoferrin assay would be a more appropriate initial test for determining further workup, such as testing for cytotoxin. However, the overall limited sensitivity and specificity probably preclude its use as a screening test for CAD and emphasize that clinical suspicion best dictates the need for further *C. difficile* toxin testing by EIA or CTA.

We thank Tech Labs, Inc., for providing the Fast 'N' Flammatory kits used in the study.

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