EDUCATIONAL COMMENTARY- FECAL LACTOFERRIN

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Learning Outcomes

Upon completion of this exercise, the participant will be able to:

- discuss the conditions in which fecal leukocytes may be present in stool.
- review the disadvantages of the fecal leukocyte smear.
- explain why lactoferrin can be used as a surrogate marker for fecal leukocytes.
- list the tests available to check for the presence of lactoferrin in stool samples.

The presence of polymorphonuclear neutrophils (PMNs) or an increased level of their markers in feces indicates an inflammatory process in the mucosa of the intestinal lining. This inflammation can be caused by microorganisms or autoimmune conditions such as Crohn’s disease. Differentiating the pathogenic conditions from more benign processes can be costly, time-consuming, and require the patient to go through invasive procedures. Fecal lactoferrin testing can be an effective screening tool in these cases.

Diarrheal Diseases

Acute diarrheal diseases caused by viruses usually do not cause an inflammatory response and are generally self-limiting. Simple oral hydration is often the only treatment needed. Diarrhea caused by organisms such as Salmonella, Shigella, Campylobacter, and Clostridium difficile can produce an inflammatory response with an increase of white blood cells (WBCs) in stool. These diseases are more serious, and in the case of C. difficile, antimicrobial therapy should be initiated. The presence of WBCs in stool samples indicates that further workup, such as a stool culture or C. difficile testing, is appropriate.

A chronic disease can be fairly benign, such as idiopathic enteritis or irritable bowel syndrome (IBS). Other chronic diseases can be more serious, such as inflammatory bowel disease (IBD) or ulcerative colitis. The inflammatory response of IBD results in an increase in PMNs in the intestinal mucosa. IBS does not produce an inflammatory response and there is no significant increase of WBCs in the feces. While patients who have IBS may experience diarrhea, cramps, gas, bloating, and constipation, this condition usually does not cause permanent damage to the colon. However, IBD can result in permanent damage to the gastrointestinal tissue and can lead to an increased chance of developing colon cancer. Invasive diagnostic tests such as colonoscopy are usually used to differentiate between IBS and IBD. Using a test for fecal leukocytes as a screening step can help the physician determine if a more extensive workup is needed.
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Fecal Testing

The standard test for the detection of WBCs in the stool is a fecal leukocyte stain. This test can be very subjective, depending on the technologist who performs the smear. The Gram stain, methylene blue stain, and the trichrome stain are among the available methods. Fecal leukocyte staining requires a fresh or properly preserved stool sample, personnel with microscopic training and skills, and extended staining processes, especially when utilizing the trichrome technique. In settings such as the emergency department or a clinic, collecting a proper specimen for fecal leukocytes is often difficult, and trained personnel are not always present to perform the stain. The WBCs are also vulnerable to lysis unless they are properly preserved and processed. In addition, organisms such as *C. difficile* can produce toxins that destroy WBCs. While the fecal leukocyte smear can be done quickly by properly trained and competent personnel, the sensitivity of the test ranges from 45% to 95%.

Because of the difficulty of collecting and performing a stain for fecal leukocytes, another way of determining the presence of PMNs in stool samples was needed. Leukoesterase was considered, but all stool samples gave a positive reaction for this enzyme. Researchers determined that an increase in lactoferrin in the stool might be used as a surrogate marker for PMNs.

Lactoferrin is an iron-binding glycoprotein with antimicrobial properties. It is present in the secondary granules of PMNs, milk (both human and bovine), and mucosal secretions such as tears. It is not present in monocytes or lymphocytes. It is stable in stool samples and is not degraded by the toxins of *C. difficile*. The test had to be specific in detecting increased levels of lactoferrin and sensitive enough to be of diagnostic value. Lactoferrin is present in low levels in normal stool samples, and testing methods were modified to account for its presence.

Testing for Lactoferrin

Several tests detect fecal lactoferrin. In the latex agglutination test, latex beads are coated with rabbit antihuman lactoferrin. When lactoferrin is present it will cross-link the latex beads and a positive (agglutination) reaction will result. This test has been standardized using a 1:50 dilution. The dilution eliminates a positive result due to the level of lactoferrin normally present in feces.

In the enzyme-linked immunosorbent assay, antibodies to lactoferrin are immobilized onto wells of a microtiter plate. If present, the lactoferrin will bind to these antibodies. The unpreserved stool sample is first diluted then added to the sample wells of the plate. The dilution step is performed to eliminate the detection of the normal level of lactoferrin found in the stool sample. After incubation and a wash step, the conjugate is added and the plate is incubated then washed again to remove any unbound material. A substrate is then added, and the plate is read on a photometer. The development of color, as indicated by an increased optical density, detects the presence of increased lactoferrin in the stool sample.
The third test is the immunochromatographic test. Like the two other procedures, this test uses antibodies to lactoferrin. In this test the sample is diluted, then added to a sample well of a membrane cassette. Lactoferrin, if present in the sample, will be bound to antibodies conjugated to gold particles. As the sample migrates across the membrane it will form a complex with anti-lactoferrin antibodies and will appear as a red line on the cassette. If no lactoferrin is present in the sample, the gold particles will not bind to the membrane and no line will be visible. A control strip contains anti-IgG antibodies. If the test is done correctly, a red line will appear as a control on the procedure.

The three tests described above are more costly to perform than the leukocyte stains, but the ease of use and the stability of the sample make them desirable. The latex agglutination test may be performed on stool samples that have been stored at 2° to 8°C for up to 48 hours. For the enzyme-linked immunosorbent and immunochromatographic assays, samples may be stored at room temperature for up to 2 weeks.

Fecal lactoferrin tests are not recommended for breast-fed children. Milk, especially human milk, contains high levels of lactoferrin which can lead to false-positive results.

Summary
Leukocytes are present in stool samples due to inflammatory conditions such as *C. difficile* infection, autoimmune diseases such as Crohn’s disease, or ulcerative colitis. When using a fecal smear to detect leukocytes, a fresh or properly preserved sample is essential for a valid test result because fecal leukocytes are fragile and can easily degrade. The availability of staff with proper training in microscopy is also essential.

Lactoferrin is an enzyme present in the secondary granules of PMNs. It is stable in unpreserved stool samples and does not require the presence of intact PMNs for detection. The presence of an elevated amount of lactoferrin in the feces can help the physician determine whether a more extensive workup is needed to determine the cause of the diarrheal illness. The lack of an elevated amount of lactoferrin indicates that the condition is fairly benign, and supportive care may be all the treatment that is needed.

Several test methods have been developed to check for the presence of lactoferrin: latex agglutination, enzyme-linked immunosorbent assay, and immunochromatographic assay. These tests are easy to perform and require no specialized skills.
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