DIAGNOSTIC APPROACH TO ACUTE INFECTIOUS DIARRHEA: THE STATE OF THE ART

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Abstract

A continuously increasing number of enteric microorganisms is being recognized as responsible for acute infectious diarrhea. Accordingly, the need of a low cost and effective approach to diagnosis and management of diarrhea is at present a pressing problem for both policy makers and physicians. This paper reviews the available information on the issue, attempting to answer whether fecal leukocytes, occult blood, fecal lactoferrin or any combination of these screening tests with clinical data allow the identification of a majority of cases of inflammatory, invasive diarrhea. After a preliminary section dealing with pathophysiological considerations on the inflammatory response of intestinal mucosa to infection by pathogenic agents and a summarized revision of the pioneering studies on the value of fecal leukocytes in the discrimination between bacillary and amebic dysentery, a critical analysis is made of later studies which addresses to the reliability of different approaches in the discrimination between invasive and noninvasive diarrhea. Several methodological flaws found in the revised studies are discussed, particularly those related to the role of fecal leukocytes as a diagnostic clue for inflammatory diarrheas. The most relevant problems identified are related to the study designs, estimates of diagnostic accuracy, the effect of variation in study validity on estimates of diagnostic accuracy, and the generalizability of results. Likewise, potential confounding and bias sources (such as different clinical and epidemiologic features of acute diarrhea, and frequent finding of mixed-agent diarrheas) are emphasized, particularly for studies performed in developing countries. Fecal lactoferrin appears to be a promising indicator of inflammatory diarrheas, although its ultimate utility in different clinical and field settings require further studies. Finally, the need to conduct a scientific overview (meta-analytic study) of primary studies performed on the issue is emphasized. Such a quantitative method is a potentially useful strategy to settle the yet unsolved questions mentioned above.

Key words: Acute diarrhea, diagnosis, clinical features, fecal screening tests, fecal leukocytes, fecal occult blood, fecal lactoferrin.

ENFOQUE DIAGNÓSTICO DE LAS DIARREAS AGUDAS INFECCIOSAS: EL ESTADO ACTUAL DEL PROBLEMA

Resumen

El número de microorganismos reconocidos como enteropatógenos capaces de producir diarrea aguda infecciosa está en incremento continuo. Por ello, tanto los clínicos como las autoridades de salud

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requieren con apremio de un enfoque efectivo y de bajo costo para el diagnóstico y el manejo de la diarrea. Este artículo revisa la información disponibles sobre el tema, intentando responder si la presencia de leucocitos fecales, sangre oculta, lactoferrina fecal, o una combinación de estas pruebas de tamizaje con datos clínicos, puede permitir la identificación de la mayoría de casos de diarrea inflamatoria. Luego de una sección preliminar relativa a la fisiopatología de la respuesta inflamatoria de la mucosa intestinal frente a la infección por organismos patógenos y una breve revisión de los estudios pioneros sobre el valor de los leucocitos fecales en la discriminación entre disentería bacilar y amebiana, se realiza un análisis crítico de los estudios posteriores dirigidos a evaluar la confiabilidad de diferentes enfoques en la distinción entre diarrea invasiva y no invasiva. Se discute varias limitaciones metodológicas halladas en los estudios revisados, particularmente aquellos relacionados al rol de los leucocitos fecales como un indicador de diarreas inflamatorias. Los problemas más relevantes identificados tienen que ver con los diseños de estudio, el efecto de la variación en la validez de los estudios sobre la exactitud diagnóstica, y la factibilidad de generalizar los resultados. Igualmente, se enfatiza sobre las fuentes potenciales de confusión y sesgo (tales como manifestaciones clínicas y epidemiológicas diferentes, y el hallazgo frecuente de diarreas mixtas), particularmente para estudios realizados en países en desarrollo. La lactoferrina fecal parece ser una prueba prometedora para distinguir las diarreas inflamatorias, si bien su utilidad final en diferentes contextos clínicos y de campo requiere de estudios adicionales. Finalmente, se puntualiza sobre la necesidad de llevar a cabo un estudio cuantitativo (meta-analítico) de los estudios primarios realizados sobre el tema, como una estrategia potencialmente útil para resolver las preguntas que todavía quedan sin respuesta definitiva.

**Palabras claves:** Diarrea aguda, diagnóstico, manifestaciones clínicas, pruebas fecales de tamizaje, leucocitos fecales, sangre oculta, lactoferrina fecal.

**DIAGNOSTIC DES DIARRHÉES INFECTIEUSES AIGUÈS: LE POINT SUR LA QUESTION**

**Résumé**

On reconnaît actuellement un nombre croissant de micro-organismes responsables de la production de diarrhées infectieuses aiguës. Il est donc nécessaire pour les médecins et les autorités administratives médicales de compter sur un système de diagnostic efficace et peu coûteux. C'est ce que nous étudions dans cet article. Nous essayons de déterminer si la présence de leucocytes fécaux, de sang fécal occulte, de lactoferrine fécale, ou une combinaison de ces tests de triage avec les données cliniques permettent l'identification d'une majorité de cas de diarrhée inflammatoire. Après une section préliminaire destinée à la révision de la physiopathologie de la réponse inflammatoire de la muqueuse intestinale à l'infection par organismes pathogènes et une brève revue des premières études sur la valeur des leucocytes fécaux pour la discrimination entre les dysenteries bacillaires et amibiennes, nous faisons un analyse critique des plus récentes approches diagnostiques consacrées à la discrimination entre les diarrhées invasives et non-invasives. Plusieurs limitations méthodologiques trouvées sont discutées, notamment celles concernant le rôle des leucocytes fécaux en tant qu'indicateurs de diarrhées inflammatoires. Les problèmes les plus importants sont ceux qui concernent la préparation des études, l'effet de la variation dans la validité des études sur l'exactitude diagnostique du test d'intérêt, et la possibilité de généralisation des résultats. Nous soulignons aussi des problèmes potentiels de confusion et de biais, surtout dans les études réalisées dans des pays en voie de développement, tel que la variation des tableaux cliniques et épidémiologiques par rapport aux pays industrialisés, et l’identification très fréquente des diarrhées aiguës avec plus d’un germe (diarrhées mixtes). La lactoferrine fécale semble être un test de triage potentiellement utile pour identifier les diarrhées inflammatoires, bien que la démonstration définitive de son utilité dépende d’études futures dans différents contextes cliniques et épidémiologiques. Finalement, nous discutons sur la nécessité de réaliser une révision scientifique d’ampleur (approche métanalytique) des études primaires réalisées. Une telle approche métanalytique est soulagée comme une stratégie quantitative potentiellement utile pour répondre à des questions qui restent encore insuffisamment résolues.

**Mots-clés:** Diarrhée aiguë, diagnostic, données cliniques, tests de triage fécaux, leucocytes fécaux, sang occulte, lactoferrine fécale.
INTRODUCTION

The development of rapid tests directed to the etiologic diagnosis of acute diarrheas has created high expectations among microbiologists and physicians (Eisenstein & Engleberg, 1986; Thorne, 1988; Tenover, 1988; Relman, 1989). Most of them are based on application of pathogenic mechanisms involved and their corresponding genetic background, and represent an exciting promise of molecular biology (Hinojosa-Ahumada et al., 1991; Litwin et al., 1991; Yan et al., 1991; Dennehy et al., 1994; Dinulos & Matson, 1994; Weiss, 1995). However, they present considerable limitations of diagnostic accuracy and we think that they will remain substantially unavailable and unaffordable tools in developing countries. Maybe these new tools provided by molecular genetics are more rewarding when applied to the unravelling of cellular pathogenic mechanisms of diarrhea. In this regard, enteric toxins provide powerful tools to dissect mediators involved in inflammatory diarrhea and to improve our understanding of novel ways of signal transduction in host cells (Guerrant, 1994).

Diarrhea may be caused by means of enterotoxin production, cytotoxin production, through interference with absorption, or through invasion of the intestinal mucosa (Table 1).

Table 1 - Pathophysiology and Types of Acute Diarrhea.

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Noninflammatory</th>
<th>Inflammatory</th>
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<tr>
<td></td>
<td>Enterotoxin or reduction of small bowel</td>
<td>Mucosal invasion or</td>
</tr>
<tr>
<td></td>
<td>absorptive capacity</td>
<td>cytotoxin-mediated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>inflammatory response</td>
</tr>
<tr>
<td>Site</td>
<td>Upper small bowel</td>
<td>Colon, distal small bowel</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>No fecal leukocytes, low levels of fecal</td>
<td>Fecal leukocytes, high levels of fecal</td>
</tr>
<tr>
<td></td>
<td>lactoferrin</td>
<td>lactoferrin</td>
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<tr>
<td>Example</td>
<td><em>V. cholerae</em>, ETEC(1),</td>
<td><em>Shigella, Salmonella,</em></td>
</tr>
<tr>
<td></td>
<td>Norwalk-like agents, rotaviruses,</td>
<td><em>C. jejuni, invasive</em></td>
</tr>
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<td></td>
<td><em>Giardia</em>, <em>Cryptosporidium</em>,</td>
<td><em>E. coli, C. difficile (2),</em></td>
</tr>
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<td></td>
<td><em>Cyclospora</em></td>
<td><em>E. histolytica (2)</em></td>
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</table>

*Vibrio cholerae* and enterotoxigenic *Escherichia coli* (ETEC) strains are the best known agents leading to diarrhea by means of their particular enterotoxins (Carpenter, 1982; Bishop & Ullsen, 1988). Following adhesion of microorganisms to the enterocytes, the produced enterotoxin leads finally to a so-called secretory, watery diarrhea, which is often profuse. It

(1) Moderately elevated titers of fecal lactoferrin may be detectable (Miller et al., 1994).
(2) High titers of fecal lactoferrin usually detectable (Miller et al., 1994).
does not usually contain fecal leukocytes on microscopic examination. However, the mechanism by which enterotoxins induce an increased transport of water and electrolytes to the intestinal lumen appears to be more complex than previously thought. The reader may find a detailed discussion of this issue elsewhere (Spangler, 1992; Leclerc, 1993; Kaper et al., 1995).

Other agents that produce watery diarrhea not involving production of enterotoxins or similar molecules include rotaviruses, Norwalk-like viruses, and parasites such as *Giardia* and *Cryptosporidium*. All of them seem to have the upper small bowel as the target organ. Viral agents infect mature villous enterocytes which are replaced with functionally immature enterocytes, there is loss of brush border disaccharidases and monosaccharide carriers, blunting of villous fronds, and loss of absorptive capacity and stimulation of motility (Schreiber et al., 1973; Hodes, 1980), but the response of the host does not seem to involve a significant activation of neutrophil chemotaxing cytokines. Another microorganism that has recently been recognized and classified as the coccidian parasite *Cyclospora Ceylanensis* also seems to produce consistently watery noninflammatory diarrheas that frequently last several weeks. The pathogenic mechanisms of diarrhea associated with this novel enteropathogen are not clear (Ortega et al., 1993; Ortega et al., 1994; Soave & Johnson Jr., 1995; Zerpa et al., 1995). Similarly, several fundamental questions regarding the exact pathogenic events leading to diarrhea in patients infected with *Cryptosporidium* and *Giardia* still remain to be answered (Fayer & Ungar, 1986; Adam, 1991; Current & Garcia, 1991; Wolfe, 1992). Chemotaxation of neutrophils does not appear to be a relevant aspect of the intestinal mucosal response to these parasitic diarrheas.

On the other hand, there are agents that have a preferential tropism for the distal small bowel or the colonic mucosa, leading to an inflammatory diarrhea. They may produce their effects by means of a cytotoxin, such as verotoxin (Shiga-like toxin)-producing *Escherichia coli* strains (Pickering et al., 1994); and Shiga toxin-producing *Shigella* strains (O'Brien & Holmes, 1987; Sansonetti, 1991). *Entamoeba histolytica* is a protozoan that typically produces a dysenteric colitis. Infection with cytotoxicigenic *E. coli* and cytotoxicigenic *Shigella* strains and with *E. histolytica* lead most characteristically (but not necessarily most frequently) to dysentery with grossly bloody diarrhea. However, the stools do not contain fecal leukocytes. There are studies demonstrating that *E. histolytica* and cytotoxicigenic bacteria such as *Clostridium difficile* are able to elicit an inflammatory response with presence of fecal leukocytes, but these leukocytes are destroyed and thus are not detectable on microscopic examination (Guerrant et al., 1981; Ravdin & Guerrant, 1981; Petri et al., 1989; Guerrant et al., 1991).

Another way of production of inflammatory diarrhea is by means of an invasive capacity. *Campylobacter, Shigella* and *Salmonella* are the best characterized invasive pathogens (Takeuchi, 1967; Takeuchi et al., 1965; Rout et al., 1974; Mandal & Mani, 1976; Cantey, 1985; Skirrow, 1984; Walker et al., 1986; Mathan & Mathan, 1991). This inflammatory diarrhea usually arises by mucosal invasion by the agent, typically in the colon or distal small bowel (Formal et al., 1983). These agents induce an inflammatory response with edema, mucosal bleeding of variable intensity, formation of microabscesses, ulceration and leukocytic chemotraction and infiltration. Compromise to the lamina propria is proportional to the severity of the inflammatory response. The inflammatory cellular and vascular response represents a defensive reaction of the body to eliminate invading pathogens (Sprinz, 1969).
In brief, diarrheas may be classified as inflammatory (those produced by invasive agents such as *Shigella, Salmonella, Campylobacter*) and noninflammatory (those produced by viruses, parasites such as *Giardia*, and enterotoxigenic bacteria). Inflammatory diarrheas involve presence of polymorphonuclear leukocytes in stool specimens, although cytotoxigenic agents (verotoxigenic *E. coli*, Shiga toxin-producing *Shigella, C. difficile, E. histolytica*), even if they are able to induce chemoattraction of neutrophils, very often produce diarrhea without fecal leukocytes.

The decision of whether to request or not microbiologic investigations and whether to begin antimicrobial treatment in patients with acute infectious diarrhea is a daily challenge for practitioners irrespective of their particular work setting. One can be willing to be highly confident on the accuracy of clinical and epidemiological data in order to solve these problems. However, a straightforward medical decision making on the basis of such data is not an easy task. Ideally, such aids should be simple, not expansive, and highly reliable.

Thus a critical reappraisal of the currently recommended approaches to acute infectious diarrhea is needed. The main question to be answered is: can the examination for fecal leukocytes, occult blood, fecal lactoferrin, or a combination of two or more of these tests be used either as a presumptive evidence of invasive, potentially treatable bacterial diarrhea or as a screening tool to determine which patients deserve stool cultures? In addition, does the use of a combined clinical-epidemiologic and fecal screening tests-based approach result in a significant increase in the diagnostic and management effectiveness? Are such approaches generalizable to settings and patients with different characteristics?

The development of a simple and reliable approach should result in a substantial reduction of the number of stool cultures requested and a prompt beginning of antimicrobial therapy for those patients suffering from potentially severe inflammatory diarrheas. Appropriate early use of oral rehydration solutions for supplying the increased losses thus preventing severe dehydration and a continued feeding will be sufficient treatment of most acute infectious diarrheas, and rational use of the fecal examination and of microbiologic studies derived from a selective diagnostic approach will identify those in need of more specific or intensive therapy.

In the attempt to answer the above posed questions we perform here a critical review of the diagnostic approach to acute infectious diarrhea.

We conducted a computerized search from MEDLINE bibliographic database from 1966 to 1995 by using all key words and key phrases begginning with the words “diarrhea” with subheadings of “diagnosis”, “fecal leukocytes”, “occult blood”, or “fecal lactoferrin”. Additional articles were identified by examining reference lists of primary and review articles and contacting experts in the field and first authors of relevant articles to determine whether they might be aware of any additional research published.

1. ROLE OF Fecal Leukocytes

1.1. Preliminary Studies

The search for fecal leukocytes was evaluated at the beginning of this century (Willmore & Savage, 1913; Graham, 1918; Willmore & Sherman, 1918; Anderson, 1921; Haughwout, 1924). Fecal leukocytes were present in almost every case of bacillary dysentery
and conversely were absent in the majority of amebic dysentery cases. Thus, Willmore (1918) concluded that

"a diagnosis between the two great types of dysentery, amebic and bacillary, can be made not only more rapidly but even with greater accuracy by simple direct examination of the stools than it can by cultural methods."

However, only *Shigella* and *E. histolytica* were assessed in these earliest studies. In addition, these studies were hampered by problems inherent in field studies of tropical diarrhea, namely coexistent parasitic infections, limited knowledge of bacterial pathogens and poor bacteriologic isolation rate.

After these reports, it was most surprising that only a few studies were performed for more than 40 years. While the interest on research related to the etiology and pathogenesis of diarrhea increased steadily, the role of fecal leukocytes or other potentially useful screening tests in the diagnosis of diarrhea remained an issue largely ignored. A testimony of this trend is provided by reviews performed in the 1960s and the 1970s on the pathogenesis of diarrhea. They scarcely mention or do not comment at all the role of fecal screening tests, particularly fecal leukocytes, in the diagnostic approach to diarrhea (Sprinz, 1969; Grady & Keusch, 1971; Plotkin et al., 1979).

A noteworthy exception was that of Wolff (1969). This author published an extensive article related to the experience of his team with fecal samples obtained in many countries. He criticized that nearly all textbooks mentioned the microscopic examination of stools in the differentiation of diarrheas, but at the same time they failed to recommend an easy procedure for the preparation of such slides. Since 1961 Wolff and colleagues had been trying many different methods for obtaining a "cytodiagnosis" of fecal smear upon microscopic examination. He stated that it is possible to obtain good results with any of the procedures, while warning that Gram stain is complicated and it should only be used by experts. Wolff concluded that the diagnosis of bacillary dysentery (shigellosis) can be made when erythrocytes, polymorphonuclear cells and macrophages are present in the fecal smear. He found that pathogenic *E. coli* also produces an inflammatory exudate, and that in cases with only a few red cells and mainly damaged, old white cells, salmonellosis should be strongly suspected. This important work has some limitations with regard to the report of results. One of them is that the author did not mention explicit quantitative criteria for reporting fecal leukocytes as positive or negative. The other one is that he did not provide clinical and epidemiological data of patients. In addition, he did not mention the negative results of smears when the stool cultures were also negative, making impossible to infer the true positive and false positive rates of fecal leukocytes test. Nevertheless, this study has made a significant contribution to the practicability of the stained smears of fecal samples, even in difficult field conditions.

Maybe the principal contribution of these earlier studies has been the suggestion of the notion that it is possible to know with certainty whether a diarrhea case is due to an agent capable of disrupting the colonic mucous barrier. Further studies attempting to ascertain whether such pioneering results were consistently repeated have not been performed for a long time.
1.2. Later Studies

It was only in the early 1970s that several investigators turned again their attention toward the problem of developing simple diagnostic tests for identifying invasive agents. Results of preliminary studies and some later studies are shown in Table 2.

<table>
<thead>
<tr>
<th>Author</th>
<th>Diagnostic test</th>
<th>Diagnostic contribution</th>
<th>Relevant problems</th>
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<tr>
<td><strong>Pioneering studies</strong></td>
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<td>Willmore &amp; Savage, 1913; Graham, 1918; Anderson, 1921; Haughwout, 1924; Wolff, 1969;</td>
<td>Fecal leukocytes</td>
<td>Discrimination between amebic and bacillary dysentery, index of disruption of colonic mucous barrier</td>
<td>Coexistent parasitic infections, different techniques used, poor microbiologic isolation rate</td>
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<td><strong>Later studies</strong></td>
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<td>Harris et al., 1972; Peirce et al., 1974; Guerrant et al., 1975; Pickering et al., 1977; Blaser et al., 1979; Korseniowski et al., 1979;</td>
<td>Fecal leukocytes</td>
<td>Index of colitis and of colonic mucosa disruption, better results when no prior antimicrobials, discrimination of bacterial and parasitic cases Diagnosis of C. jejuni, higher sensitivity of fresh cup stool specimens</td>
<td>Positivity criterion ill defined, Shigella and Salmonella were agents predominantly assessed, problems posed by natural and experimental diarrhea not addressed Inadequate design for assessment of fecal leukocytes in cases associated to C. jejuni diarrhea</td>
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Nelson & Haltalin (1971) studied the reliability of assessing the likelihood of bacterial diarrhea on the basis of clinical data in outpatient pediatric patients. The correct prediction of the type of diarrhea was made in approximately 70% of cases solely upon the basis of history and clinical examination. The authors did not define well the clinical guidelines used and they did not provide information about whether they used some scoring system or other
quantitative way of assessing the diagnostic accuracy of the clinical data. In addition, the incidence of bacterial diarrhea, particularly Shigella, was exceptionally high. These problems make difficult the applicability of these results to general practice.

Harris et al. (1972) evaluated examination of the acute diarrhea stool for leukocytes as a possible aid toward early diagnosis of the cause, using a methylene blue, wet-mount preparation. Sixty-six of 68 subjects experimentally infected with Shigella, Salmonella or invasive E. coli showed presence of fecal leukocytes, while the isolation rate of these causative agents was only 60%. The authors did not find fecal leukocytes in healthy controls, nor in viral, parasitic and enterotoxin-associated diarrhea. Furthermore, nine of 11 patients with naturally acquired salmonellosis and all seven patients with shigellosis had fecal leukocytes. Fecal leukocytes were observed in cases of shigellosis regardless of the gross appearance of the stool. This study did not include a systematic assessment of parasitic diarrhea. It did not address the question of whether an experimental infection due to the ingestion of a pure bacterial inoculum and a naturally acquired infection due to the ingestion of contaminated water or food lead ultimately to the same pathogenic events. In addition, the criterion of positivity for fecal leukocytes was not clearly defined. These factors make difficult the generalizability of the test as an index of invasive bacterial diarrhea. We think that the principal contribution of this study is the demonstration of fecal leukocytes in a diarrheal stool as a reliable index of colitis with disruption of the distal intestinal mucosa. We can not ascertain from the available data whether other enteric pathogens such as invasive E. coli and Campylobacter jejuni are associated with the same frequency to diarrheal stools with positive fecal leukocytes (inflammatory diarrheas).

In other study, Peirce et al. (1974) found that fecal leukocytes were present in two of 22 children (9%) with parasitic infection and three of 38 children (8%) with nonspecific diarrhea. All five patients with shigellosis who had not received prior antimicrobial therapy had fecal leukocytes, whereas only five of 12 children (42%) with culture-proven shigellosis had the test positive. Only two of 22 (9%) children with diarrhea of parasitic origin had fecal leukocytes. The definition of positivity for the fecal leukocytes test is quite ambiguous, as

“fecal smears were considered to contain leukocytes only if multiple cells could be seen on many high-power fields. If only occasional white blood cells (WBCs) were seen, results of the study were considered negative”.

The number of subjects with an invasive agent isolated in the stool was small, especially those without prior antimicrobial therapy, making difficult the interpretation of sensitivity, specificity and predictive values. The contribution of this study is that it clearly shows the importance of stool examination prior to the institution of any antimicrobial therapy. Thus, attention should be paid to the inclusion of patients without previous antimicrobial therapy in primary studies.

Guarrant et al. (1975) examined the stools for fecal leukocytes as evidence of colonic involvement and investigated enterotoxigenic and invasive properties in the isolated agents. Fecal leukocytes were present in nine of the 11 patients (82 per cent) with invasive pathogens. Six of nine patients without a demonstrable pathogen in their stools had fecal leukocytes. In this pediatric population E. coli appears to produce most acute diarrheas due to enterotoxigenic and invasive pathogenic mechanisms. Although the number of stool specimens assessed for both fecal leukocytes and toxigenic or invasive properties was quite small, the authors were
able to demonstrate a clear relationship between presence of fecal leukocytes and invasive capability of the isolated agents. Conversely, the absence of fecal leukocytes correlated well with the absence of an invasive agent and with the presence of an enterotoxin producing pathogen. No viral agents were sought in this study. It would be extremely useful to extend these observations regarding the relationship between the presence of fecal leukocytes and the enterotoxigenic or invasive properties of the isolated agent to other enteric pathogens such as Shigella, Campylobacter and Aeromonas.

In another study (Pickering et al., 1977), 90 adults and 200 pediatric patients had their stool specimens examined for fecal leukocytes and enteric pathogens during episodes of acute diarrhea lasting less than three days. Fecal leukocytes were found in 24 of 35 subjects (69%) with diarrheal stools from whom Shigella was isolated, and in 4 of 11 (36%) specimens from which Salmonella was isolated. In addition, fecal leukocytes were present in a significantly greater number of patients with Shigella than in subjects without enteric pathogens isolated or than in subjects with other bacterial and protozoal pathogens. Eleven of 13 invasive Shigella isolates (85%) were associated with numerous fecal leukocytes, while none of five other invasive bacteria were related to positive fecal leukocytes. This study also found that diarrhea cases associated to viruses, Giardia, E. histolytica, or enterotoxigenic E. coli rarely showed positive fecal leukocytes. The most common causes of colitis were Shigella strains. This report extended the two previous studies of Harris et al. (1972) and Peirce et al. (1974). One problem with this study is that the analysis did not differentiate whether subjects were from developed or developing settings nor whether they were adults or children. Prevalence of etiologic agents is known to be different from setting to setting (Guerrant et al., 1990). Similarly, positivity criterion for fecal leukocytes test was also the same used by the previous report of Peirce et al. (1974), making quite difficult generalizability and comparison with other studies. In spite of these problems, this study substantiated further that the absence of fecal leukocytes may be suggesting diarrhea due to virus, Giardia, E. histolytica, or enterotoxigenic E. coli. It also supports the view that presence of fecal leukocytes is a reliable indicator of colitis. Furthermore, it revealed that correlation with rate of isolation of specific agents able to produce a colonic mucosal inflammation is a complex problem that merits additional studies.

Blaser et al. (1979), studying Campylobacter associated diarrhea, found that stool examination revealed blood in 60% and polymorphonuclear leukocytes in 78% of the patients. Unfortunately, we do not know whether microscopic examination for fecal leukocytes were performed in Salmonella and Shigella cases. In addition, the study design has not been specifically directed to the assessment of value of fecal leukocytes in the diagnosis of Campylobacter, and the number of fecal samples studied for this test was small (14 samples studied). Similarly, as in previous studies, a precise criterion for considering fecal leukocytes test as positive was not ascertained. In spite of these problems, this study suggested that, in addition to Shigella and Salmonella, Campylobacter should be borne into mind as another possible etiologic agent in dysenteric, inflammatory diarrheas.

A prospective study (Korseniowski et al., 1979) found that the sensitivity of fecal leukocytes test in shigellosis was 95% when cup specimens were obtained, and 44% when swab or diaper specimens were examined. Of 20 cup specimens that were culture-positive for shigellosis, occult blood assessed by guaiac method was positive in 85%. Only 45% of the
patients with shigellosis who provided cup specimens had grossly bloody dysentery. In 12 of the 101 patients (12%) fecal leukocytes were found, suggesting an inflammatory intestinal process, but no invasive pathogens were identified. As none of these patients had either recurrent or chronic diarrhea, these patients appear to indicate a false-negative culture for invasive pathogens. Some limitations of this otherwise useful study include the lack of a systematic investigation of Campylobacter, and the small number of other invasive agents different from Shigella identified in the stools, which do not allow the evaluation of the role of fecal leukocytes in the differential etiologic diagnosis of invasive diarrheas. Contrasting with this study, Ronsmans et al. (1988) found in a prospective, community-based study, that a history of bloody diarrhea in children aged under 5 years was as predictive of the presence of shigella infection (positive predictive value 50%, negative predictive value 86%) as more complex diagnostic algorithms incorporating other clinical features, or microscopic examination of stools for presence of fecal leukocytes or red blood cells. Thus the authors recommend that primary health workers treat with the appropriate antimicrobial agent all patients with a history of bloody diarrhea. However, they acknowledge that as a history of bloody diarrhea may correctly identify 69% of all patients with Shigella infection, the number of patients unnecessarily treated will equal those who have shigellosis, because the positive predictive value of bloody diarrhea for shigellosis is 50%. This study used rectal swabs. Additionally, these swab specimens were previously placed in merthiolate-iodine-formaldehyde ("MIF") for later microscopic examination. We are not aware of studies validating the "MIF" stain-preservation technic for fecal leukocytes examination. This method was originally proposed for microscopic identification of intestinal protozoa (Sapero & Lawless, 1953).

Another group of investigators (Speelman et al., 1987) found that the mean number of fecal leukocytes per mm² was significantly higher in shigellosis than in amebiasis and correlated with estimations of fecal leukocytes per hpf in a wet mount preparation. Patients with shigellosis more often had over 50 fecal leukocytes per hpf. Fecal red blood cells were similar in number in shigellosis and amebiasis. The contribution of this work is related to quantification of intensity of the colonic mucosal inflammatory response reflected in the number of fecal polymorphonuclear leukocytes, an issue that had not been previously assessed.

In a retrospective study (Stoll et al., 1982), simple visual inspection of stool for blood correctly identified 44% of all patients infected with Shigella. Blood noticeable on simple visual inspection of the stool was closely associated with the presence of 10 or more red blood cells per hpf. More than 10 white blood cells per hpf were found in stool specimens of 80% of patients with shigellosis, in 40% of patients infected with C. jejuni, in 59% of patients infected with E. histolytica, and in 38% of patients not infected with Shigella. A limitation of this study may be that it was not specifically designed for assessing the role of blood cells or fecal leukocytes in the differential diagnosis of invasive diarrhea. Prospectively conducted studies may reveal whether the poor performance of fecal leukocytes in Campylobacter associated diarrheas found in this report is a consistent finding. We can not rule out the presence of a factor of distortion in results, due to the lack of a systematic examination of all studied samples for red blood cells and fecal leukocytes.

Hossain & Albert (1991) found that the best predictor of shigellosis (positive predictive value 85%, negative predictive value 83%) was the presence of more than 25 WBC/hpf
together with the presence of RBC regardless of number. The study was performed in Teknaf, Bangladesh, an area hyperendemic for shigellosis (Hossain et al., 1990). It remains to be seen whether the presence of >25 WBC/hpf considered by the authors as the significant cut-off level is equally applicable to other settings where the frequency of shigellosis is lower.

Blaser et al. studied patients with diarrhea associated to Campylobacter, Salmonella, or Shigella (Blaser et al., 1983). During a 15-month multicenter study, fecal specimens submitted to clinical microbiology laboratories at eight hospitals in different parts of the United States were examined. Abdominal pain, bloody diarrhea, fever, tenesmus, and abnormal sigmoidoscopic findings were present significantly more often in patients infected with C. jejuni than in a control group of patients with diarrhea. Of patients with fecal leukocytes and a history of fever, almost half of them were infected with one of these three enteric pathogens. Fecal cultures had the highest yields when obtained from patients within 7 days from the onset of symptoms. Fecal specimens that were watery or that contained stools with erythrocytes or leukocytes were more likely to yield one of the three pathogens than those specimens without these findings. However, the sensitivity and predictive values of these features were low. The presence of macroscopic blood was the most specific index of isolation of the pathogens. Combinations of these findings improved the specificity but decreased the sensitivity in the diagnosis. The authors acknowledge that the interpretation of the results of such a large survey can be affected by inherent methodological biases, including lack of randomization of the participating hospitals, a higher proportion of patients with severe illnesses for whom stool cultures were requested, and the lack of data concerning prior antimicrobial therapy.

In another report (Stoll et al., 1983), visible blood was more common in stools infected with Shigella (51%) and E. histolytica (39%) than in those from patients infected with enterotoxigenic E. coli, rotavirus, C. jejuni, Vibrio cholerae O:1, or Giardia. Shigellosis was most likely to have more than 50 fecal leukocytes/hpf than all other patients and that patients infected by E. histolytica. Diarrheas associated to noninvasive, noncitotoxysgenic agents had loose stools with fewer red blood or white cells. One third of patients had more than one pathogen isolated, but the analysis did not include these patients. This study found that some of patients with noninvasive single agent diarrhea had more than 20 fecal leukocytes/hpf. Again, this study shows the potential value of fecal leukocytes in distinguishing an invasive process, and to discriminate between bacillary and amebic dysentery. Sensitivity, specificity and predictive values for purposes of decision making in the clinical setting are relatively low, thus remaining a problem that needs further investigation.

In a prospective study of acute diarrhea performed in hospital inpatients in Birmingham (Alvarado, 1983), fecal leukocytes were present in 91% and 87% of cases due to Shigella and Campylobacter, respectively, whereas controls and diarrheal cases due to viruses, parasites or toxigenic bacteria did not have fecal leukocytes. Interestingly, mixed-agent diarrheas had fecal leukocytes in 80% of cases. The problem of mixed-agent diarrheas is much more frequent in developing settings, and it needs to be adequately challenged.

In a stepwise approach to acute diarrhea (DeWitt et al., 1985), fecal leukocytes sensitivity was 85%, specificity was 88%, positive predictive value was 59%, and negative predictive value was 97%. A cluster of three historical variables (abrupt onset of diarrhea, greater than four stools per day, and no vomiting before the onset of diarrhea) was the best
of any clinical combination to define a subgroup of patients with high risk of having a positive stool culture (27% vs 4% if any of the variables was absent). An internal validation of the study was made in a random sample of 80% of the cases. Ascher & Edusada-Corpus (1991) confirmed that the historical cluster of variables combined with positive fecal leukocytes smear (the same criteria used by DeWitt et al., 1985) has an 83% positive predictive value for bacterial diarrhea. In the absence of these factors there is a negative predictive value of 97%. They tested this system on a second series of children and obtained nearly the same diagnostic accuracy. These findings support a plausible approach to the clinical workup of children with acute diarrhea. However, as Ascher and Edusada-Corpus acknowledged, the high diagnostic index values obtained may reflect the patients' higher socioeconomic setting and accessibility to better sanitary facilities. Accordingly, there remains the question of whether the performance of this approach may drop significantly in children from developing countries because of the higher prevalence of enterotoxigenic E. coli and Vibrio cholerae.

Using laboratory findings, another stepwise approach to diarrhea was prospectively performed by Thorson et al. (1985). The value of fecal leukocytes in the detection of an inflammatory, invasive bacterial diarrhea was confirmed, as with fresh cup specimens the sensitivity of the methylene blue examination was 96% (23 of 24 specimens), and the predictive value was 77% (23 of 30 specimens). When stools positive for fecal leukocytes were examined by dark-field microscopy or carbol-fuchsin Gram stain for making a diagnosis of Campylobacter diarrhea, the sensitivity was 75% and the predictive value of a positive test by either dark field study or Gram stain was 75%. When examined by dark field or Gram stain, these specimens allow diagnosis of Campylobacter in a substantial proportion of cases. The potential applications of this study are exciting. However, in general practice it is not always possible to obtain immediately fresh cup specimens that yield high positive fecal leukocytes. Rectal swabs or diaper specimens are more easy to obtain but they yield low positive results. It would be useful to combine this laboratory-based stepwise approach with the combined approach suggested by DeWitt et al. (1985) and Ascher & Edusada-Corpus (1991) to see whether predictive values increase. Generalizability of the present findings to developing settings is again a question needing to be addressed.

Fontana et al. (1987) proposed a two-step predictive method to assess the probability of bacterial origin in childhood acute diarrhea. The patients were divided in high, intermediate and low probability groups according to a clinical score. The patients in the intermediate group were further assigned to the high or low probability group according to the presence or absence of fecal leukocytes. The method was assessed in a second series of pediatric patients to validate the reproducibility of findings. This stepwise method allowed a correct classification of patients in 86% of cases in the first series and 81% in the second series. One problem with this study is that for the distribution of the total scores among the patients of the first series, cut-offs were empirically chosen. Because they did not find a single cut-off value which assured good sensitivity and specificity, the authors decided to divide the patients into high, intermediate and low probability groups, thereafter assigning the intermediate group of patients to high or low probability groups if fecal leukocytes were present or absent, respectively. It remains to be seen whether this approach is as simple as it is suggested by the authors. The task may be quite cumbersome for the busy clinician. Maybe the approach suggested by DeWitt et al. would be more easily applied in the daily
clinical practice, because it does not need use of a scoring system for its application to each individual case. Future studies extending the proposal of DeWitt et al. (1985) and Ascher & Edusada-Corpus (1991) to children from developing countries taking into account validation of the results with another external series of patients would be illuminating.

What about watery diarrheas and the probability of evolution toward a dysentery? There are no systematically recognized indicators for predicting such cases before the apparition of gross blood in stools. Patwari et al. (1993), attempted to evaluate various clinical and laboratory predictors of dysentery in outpatient children. Their results suggest that the severity of invasive disease, clinically evidenced as mucoid stools and/or presence of >10 fecal leukocytes/hpf or red blood cells may be a better indicator of the invasive nature of the infection and subsequent development of dysentery rather than mere isolation of invasive enteropathogens. The authors recognize that the low rate of isolation of invasive agents may be due to the inclusion criteria which only allowed enrolment of patients with acute diarrhea without visible blood and to the fact that C. jejuni was not included in the microbiologic investigation. In spite of these limitations and others related to leukocytes searching procedure (we are neither told whether fresh stool specimens were stained with methylene blue, nor whether swab, diaper or cup specimens were used), this study is particularly important because it paves the way for future similar studies with more careful designs.

1.3. Areas Needing Further Investigation

The principal criticisms directed to the studies performed in the early 1900s are related to coexistence of parasitic diseases, limited knowledge of bacterial pathogens, and poor microbiologic isolation possibilities. These problems have been partially overcome with the advancement of new isolation techniques for Campylobacter and other enteric pathogens. However, there remains the problem of availability of an experienced technician for an accurate identification of fecal leukocytes. Another potential problem is that other cells, cysts or fungi may be mistaken for leukocytes (Vogtlin et al., 1983). Moreover, if the fecal sample is not immediately processed, the leukocytes are destroyed and are not detected. The optimal type of specimen collection seems also relevant, as fresh cup specimens may reveal more frequently fecal leukocytes than swab or diaper specimens (Korseniowski et al., 1979). In addition, primary studies should use standardized techniques for searching fecal leukocytes and for reporting results. Prior antimicrobial therapy reduces significantly the possibility of a positive fecal leukocytes test result.

2. ROLE OF OCCULT BLOOD AND FECAL LACTOFERRIN

2.1. Preliminary Studies

Taking into account the problems related to the use of fecal leukocytes, one study has suggested that the presence of fecal occult blood through the modified guaiac test may be an adequate substitute for fecal leukocytes (Vogtlin et al., 1983). The authors found that occult blood and fecal leukocytes were positive in over 80% of the patients infected with invasive pathogens (Salmonella, Shigella, Campylobacter), but no data are provided for performance of these tests in separate cases of Salmonella, Shigella, and Campylobacter. Moreover, in the group in which no pathogens was isolated, fecal leukocytes were found in 25% of patients and
occult blood in 31%, which means that in settings with similar etiologic patterns of acute diarrhea, if occult blood would be used as a screening test for further investigation, i.e., stool culture, up to one-third of patients would have an unnecessary and expansive stool microbiologic examination. In addition, this study does not provide clinical information, we do not know whether fresh cup or swab specimens were used, nor we are told when was a fecal smear stained with Giemsa considered to be positive.

Conversely, Paccagnini et al. (1987) found positive occult blood in 83% of children with diarrhea due to Salmonella and in 74% of Campylobacter diarrhea, and in about one-third of cases with rotavirus infection or with no pathogens. In contrast with the study of Vøgtlin et al., in this study rotavirus was the agent most frequently isolated. Fecal leukocytes were present in 54% of Salmonella cases and in 32% of Campylobacter cases. In rotavirus or in no pathogens groups fecal leukocytes were positive in only 10% of cases. We are not told whether patients with prior antimicrobial therapy were included. This is an important point, because stool specimens without antimicrobial therapy yield more frequently positive fecal leukocytes (Peirce et al., 1974). If the authors used swab or diaper specimens for fecal smears, this would explain additionally the poor performance of fecal leukocytes in cases of invasive diarrheas found in this study.

2.2. Later Studies

In young children of a developing country (Huicho et al., 1993), fecal leukocytes were found in 36, 16 and 18% of patients with Salmonella-Shigella-Campylobacter, rotavirus or enterotoxigenic E. coli, respectively. In addition, 43, 39 and 38% of these groups, respectively, as well as 16 controls had occult blood. 70% of Shigella cases had fecal leukocytes. Fecal leukocytes were seen in 27 and 8% of children with any combination of Salmonella-Shigella-Campylobacter or noninvasive pathogens combination, respectively. Likewise, 44 and 18% of these groups had occult blood. Agreement between both tests being positive was poor. Dysentery combined with both tests positive was associated with 15 (88%) cases where invasive agents were isolated, whereas combination of dysentery and fecal leukocytes was associated with 21 (72%) cases of invasive agents recovered. Several possible explanations for the lack of correlation between the two screening tests and the results of microbiologic examination found are discussed. It is suggested that a combined clinical-epidemiologic and screening tests-based approach to infectious diarrhea may be more rewarding.

Guerrant et al. (1992) have recently developed a marker for fecal leukocytes, fecal lactoferrin. This test proved in vitro to be a useful marker, even when fecal leukocytes are morphologically lost or are destroyed on stool specimens. Preliminary clinical experience showed that in patients with inflammatory diarrheas caused by Salmonella and C. difficile (a cytotoxigenic agent), lactoferrin was readily detected by lactoferrin latex agglutination. The test remained sensitive in fecal specimens, even after transportation, storage (overnight), swab, or toxin destroyed the leukocyte morphology. A recent clinical work undertaken by the same group of investigators (Miller et al., 1994) has shown that stool specimens of patients with shigellosis or C. difficile inflammatory colitis have high levels of fecal lactoferrin as compared with healthy controls or volunteers with experimentally induced cholera. Interestingly, subjects with experimentally induced enterotoxigenic E. coli showed moderately elevated titers of fecal lactoferrin. Such an evidence of a mild intestinal inflammatory
response induced by enterotoxigenic agents may account for the low-grade fever and possible increased complexity of infection with enterotoxigenic agents that may include cell invasion (Elsinghorst & Kopecko, 1992). These findings are exciting because they challenge the notion of a pure secretory diarrhea without mucosal inflammatory response in the intestinal infections due to enterotoxigenic pathogens.

2.3. Areas Needing Further Investigation

Potential cross-reactions between ‘hemoccult’ reagents and stool components which might lead to false-positive results need to be assessed. Presently, we do not know whether fecal lactoferrin levels are substantially reduced in patients with prior antimicrobial therapy. The role of this new marker of inflammatory diarrhea in developing settings warrants more study, particularly in pediatric patients. Likewise, in children from developing countries, the frequency and significance of moderately high levels of fecal lactoferrin in diarrhea associated to enterotoxigenic agents is a subject not yet addressed. Tables 2 and 3 show a summary of preliminary and later studies.

Table 3 - Summary of Later Representative Diagnostic Studies on Acute Diarrhea (Continued).

<table>
<thead>
<tr>
<th>Author test</th>
<th>Diagnostic contribution</th>
<th>Diagnostic</th>
<th>Relevant problems</th>
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<tbody>
<tr>
<td>Ronsmans et al., 1988; Stoll et al., 1982; 1983; Blaser et al., 1983; Patwari et al., 1993;</td>
<td>Macroscopic appearance of stools, fecal leukocytes</td>
<td>Usefulness of bloody diarrhea for Shigella and Campylobacter, different results for fecal leukocytes</td>
<td>Low positive predictive value of dysentery in shigellosis, different cut-off values for fecal leukocytes</td>
</tr>
<tr>
<td>DeWitt et al., 1985; Fontana et al., 1987; Ascher &amp; Edusada-Corpus, 1991;</td>
<td>Stepwise clinical and laboratory-based approach</td>
<td>Increased correct identification of patients with invasive diarrhea</td>
<td>Different methods of validation, different clinical criteria, need of further studies in poor countries, with higher prevalence of ETEC</td>
</tr>
<tr>
<td>Vogtlin et al., 1985; Paccagnini et al., 1987; Huicho et al., 1993;</td>
<td>Fecal leukocytes, occult blood,</td>
<td>Lack of correlation between screening tests and agents isolated</td>
<td>Scarce of clinical data on patients, different settings and age groups involved, need to consider false-positive results for ‘hemoccult’</td>
</tr>
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</table>
3. POTENTIAL PROBLEMS NEEDING CONSIDERATION WHEN DESIGNING, ANALYSING, AND REPORTING PRIMARY STUDIES RELATED TO VALUE OF FECAL SCREENING TESTS OR COMBINED CLINICAL AND SCREENING TESTS

Besides the criticisms addressed to the earlier and to the more recent studies, there are other potential problems needing consideration when designing, analysing, and reporting primary studies related to the value of fecal leukocytes or other tests in the approach to acute infectious diarrhea.

3.1. Problems Prevalent in Developing Settings

Watery diarrhea due to enterotoxigenic agents and viruses accounts for a substantial proportion of cases in developing countries. The majority of the proposed diagnostic approaches and algorithms for acute diarrhea are related to the discrimination between invasive and noninvasive diarrhea (Satterwhite & DuPont, 1976; Guerrant et al., 1985; Williams et al., 1986; Radetski, 1986; DeWitt, 1989; Guerrero, 1989; Guerrant & Bobak, 1991). Conversely, there is scarce information about studies attempting differential diagnosis of agents leading to watery diarrheas (Miranda-Langschwager et al., 1993).

The agents of acute diarrhea in developed and developing countries are known to be different (Guerrant et al., 1990). In addition, the clinical and epidemiologic features may vary from setting to setting and among different age groups. In this regard, several review articles on diarrhea consider that C. jejuni is an agent leading to inflammatory diarrhea, together with Shigella and Salmonella (Guerrant et al., 1985, 1986, 1987, 1991; Radetski, 1986; Ashkenazi & Pickering, 1989; DeWitt, 1989; Leung et al., 1989; Guerrero, 1989; Cerf, 1990; Becq-Giraudon, 1991). By contrast, we found in two prospective studies that Campylobacter in Peruvian children behaves as an endemic pathogen, leading more commonly to watery, noninflammatory diarrheas (Murga et al., 1993; 1995). It has been shown that most Campylobacter strains produce a cholera-like enterotoxin (Ruiz-Palacios et al., 1983; Walker et al., 1986; Pérez-Pérez et al., 1989), which may account for noninflammatory diarrheas associated to this agent, although the ultimate pathogenic and clinical roles of this enterotoxin remain unclear. Accordingly, the diagnostic and treatment algorithms widely used in cases of acute diarrhea might vary from setting to setting and might be different in adults and children.

Two or more potentially pathogens are frequently isolated in an individual fecal sample (DuPont et al., 1976; Evans et al., 1977; Korzeniowski et al., 1984; Stoll et al., 1983; Chunget al., 1989; Sethi et al., 1989; Pazzaglia et al., 1991; Arthur et al., 1992; Huicho et al., 1993; Murga et al., 1993; 1995; Zerpa & Huicho, 1995; Zerpa et al., 1995). This fact makes more complex the interpretation of results and the problem of identifying the real pathogenic agent. Some speculative hypotheses needing assessment include the possible synergistic interaction in these coinfections, and the converse possibility that the coexistence of the agents may be merely a consequence of an intestinal overinfection in settings with
overcrowding and poor sanitary facilities. The actual significance of such coinfections is still a problem without solution and it is a frequent source of confusion in the interpretation of diagnostic tests of acute diarrhea.

3.2. Estimation of diagnostic accuracy of the tests

Interdependence of sensitivity and specificity must be taken into account. The measures rely on a single threshold (cut-point or positivity criterion) for classifying a test as positive. Changing the threshold to increase sensitivity decreases specificity and vice versa. This point needs further consideration when designing and reporting value of fecal leukocytes, fecal lactoferrin and other screening or clinical tests in the approach to acute diarrhea. An alternative to reporting a single pair of sensitivity and specificity estimates is to report a range of pairs, which is obtained as the threshold is varied. Such a range of pairs is often reported as a receiver operating characteristic (ROC) curve (Centor & Schwartz, 1985), and it provides a quantitative measure of a system's diagnostic or predictive performance (Hanley, 1989). Another measure of test performance is the likelihood ratio, which is the ratio of probability of a particular test result in people with the disease to the probability of the same test result in people without disease (Klassen & Rowe, 1992). This measure avoids the loss of information caused by dichotomizing a test as positive or negative.

3.3. Assessment of the effect of variation in study validity on estimates of diagnostic accuracy

When analysing results of studies related to role of clinical or laboratory data in the diagnostic approach to acute diarrhea, it is important to consider whether they are prospective or retrospective. Frequently, retrospective studies have not been performed with the specific purpose of assessing the value of such data, being part of more wide clinical and/or epidemiologic studies. In addition, consideration should be paid to whether an appropriate reference standard was used, namely, whether the stool microbiologic tests reliably reflect the pattern of etiologic agents of acute diarrhea in the particular setting considered. Independent evaluation of the test or tests and reference standard is important, as test results may be biased if the test requires judgement and this judgement is made by someone who has knowledge of the reference standard. Fortunately, this issue does not seem to be a problem in the studies performed for the assessment of the value of fecal leukocytes and other laboratory or clinical tests in the diagnostic approach to acute diarrhea. Furthermore, verification bias may occur when the reference standard (stool culture) has been assessed on patients sampled differentially in the categories of test results. This bias may be avoided if diagnostic accuracy is assessed in consecutive patients who present the clinical problem of interest, i.e. acute infectious diarrhea.

3.4. Generalizability

To estimate the generalizability of a study one must know if valid estimates of diagnostic accuracy are applicable to the setting in which the reader works. We may decide that the estimate of diagnostic accuracy of the test or tests are applicable to our decision making if characteristics of the patients and the test are similar in the primary study and in
our population, if characteristics are not associated with diagnostic accuracy, or if a particular characteristic (for example sex or age, prior antimicrobial therapy, duration of diarrhea at the time of enrolment) affects diagnostic accuracy and estimates are provided separately for groups defined by this characteristic so that we can then apply them separately to each group.

4. CONCLUSION

A qualitative review such as the one that we have performed in this paper allows to critically comment the literature on the diagnostic approach to acute infectious diarrhea and to identify methodological flaws and problems that probably need further investigation. We believe that the fundamental questions posed at the beginning of this article have been partially answered. In this regard, it appears that the primary studies show that it may be feasible to develop a reliable diagnostic approach to acute diarrhea by distinguishing inflammatory from noninflammatory diarrheas by means of rapid screening tests (fecal leukocytes or the recently developed fecal lactoferrin) or alternatively using a combined clinical and laboratory-based stepwise analysis. However, the revision of the primary studies has revealed methodological flaws. These limitations do need to be weighted to ascertain with further accuracy whether the discriminatory performance of the diagnostic tests has been substantially affected. Accordingly, we think that a review including specified quantitative methods of identifying, selecting, and validating the primary studies can 1) provide an overall summary of diagnostic accuracy of the test(s) of interest, 2) determine study validity of primary studies, 3) generalizability of the test(s) of interest, and 4) identify areas for further research. In addition, new hypotheses may be generated or this approach may reveal deficits that need to be addressed in future primary studies before such a quantitative overview can be done. Thus a meta-analytic review of the primary studies applying recently developed methods (Irwig, 1994) would be a very useful complement to our qualitative review. Such an assessment may allow the development of more substantiated algorithms in the approach to acute diarrhea, so that clinicians may decide whether to use a diagnostic test (for instance fecal leukocytes, fecal lactoferrin, or any other single test or combination of tests) and how to interpret the result, and policy makers may assess the overall value of the test, compare it to alternatives, and decide whether the test should be available.

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