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Evaluation of hepatocyte growth factor as a local acute phase response marker in the bowel: The clinical impact of a rapid diagnostic test for immediate identification of acute bowel inflammation

Ashraf E. Sorour^a, Johanna Lönn^b, Sravya Sowdamini Nakka^b, Tayeb Nayeri^d, Fariba Nayeri^{c,d,*}^a Department of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University, Cairo, Egypt^b Division of Clinical Medicine, School of Health and Medical Sciences, Örebro University, Örebro, Sweden^c Division of Infectious Diseases, Department of Medical and Health Sciences, University Hospital, Linköping, Sweden^d The Institute of Protein Environment Affinity Surveys (PEAS Institut), Linköping, Sweden

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ABSTRACT

Background: There are no rapid tests that can distinguish contagious gastroenteritis, which requires isolation at its onset, from exacerbation of chronic inflammatory bowel disease (IBD) or bowel engagement in the course of systemic inflammatory response syndrome (SIRS). Hepatocyte growth factor (HGF) is an acute phase cytokine that is produced at the site of injury. It has high affinity to sulfated glycan, and this binding affinity is lost during chronic inflammation. The fecal pH strongly impacts the prognosis for severe bowel disease. We developed a strip test to evaluate HGF as a local acute phase response marker in the bowel. This test assessed the binding affinity of HGF to sulfated glycans in fecal samples and determined fecal pH as an indicator of illness severity.

Methods: Fresh feces from patients with diarrhea ($n = 513$) were collected and tested blindly, and information about patient illness course and outcome was collected. Patients were classified based on the focus of inflammation and the cause of the symptoms. Objectively verified diagnoses of infectious gastroenteritis ($n = 131$) and IBD onset/exacerbation and bowel cancer ($n = 44$) were used to estimate the performance of the test strip. ELISA was performed on 101 freeze-thawed feces samples to determine the fecal HGF levels.

Results: The test rapidly distinguished infectious gastroenteritis from non-infectious inflammatory causes of diarrhea (sensitivity, 87.96%; specificity, 90.9%; positive predictive value, 96.6%; negative predictive value, 71.4%; accuracy, 89.1%). Fecal pH ($p < 0.0001$) and mortality within 28 days of sampling ($p < 0.04$) was higher in patients with sepsis/SIRS and diarrhea. The concentration of HGF was higher in strip test-positive stool samples ($p < 0.01$).

Conclusions: HGF is a good local acute phase response marker of acute bowel inflammation. Test-strip determination of the binding affinity of fecal HGF to sulfated glycan was a rapid, equipment-free way to assess patients with diarrhea and to guide the diagnostic and therapeutic approaches on admission.

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1. Introduction

In order to inhibit disease transmission, patients with diarrhea are isolated at medical centers upon admission. Based on the patient's medical and epidemiological history, a wide range of tests and examinations may be performed before a definite diagnosis is made [1]. Subsequent treatment may include fluid and electrolyte replacement plus antibiotic treatment for patients with fever and stomach pain. However, appropriate treatment can be delayed

for a few serious diseases that include diarrhea as an initial symptom. Such conditions include the onset of inflammatory bowel disease (IBD) in young patients, colon cancer (which has varying periods of culture-negative diarrhea), and abdominal processes or abscess that cause reactive diarrhea [2]. Despite the growing problem of multidrug-resistant gram-negative bacteria, it is inappropriate to treat self-limiting infectious gastroenteritis with broad-spectrum antibiotics, but this is quite common in medical centers [3,4]. Various microbiological and immunological tests are performed on stool samples when patients with diarrhea are admitted to the hospital. However, these tests have limited sensitivity with respect to antibiotic consumption and/or low antigen burdens [5,6].

* Corresponding author at: Division of Infectious Diseases, Department of Medical and Health Sciences, University Hospital, Linköping, Sweden.

E-mail address: Fariba.Nayeri@lio.se (F. Nayeri).

Hepatocyte growth factor (HGF) is produced by mesenchymal cells during organ injury. It stimulates cell division [7] and cell motility [8] and promotes normal morphogenic structure [9] in epithelial cells adjacent to injured areas. It also induces the regeneration and repair of damaged tissue [10]. HGF is translated as a single-chain precursor and is activated at the site of injury by proteolytic cleavage, resulting in a double-chained active form of HGF [11]. High levels of systemic HGF have been detected during injuries caused by infection [12]. In bacterial meningitis and pneumonia, there is local production of HGF at the site of infection [13,14].

To identify the bowel as the focus of inflammation, proteins and cytokines that are produced locally at the site of injury can be detected in feces. HGF is produced both systemically and locally in infectious diseases [15–18], and determination of the HGF concentration in feces can be used to identify infectious gastroenteritis. However, there may also be high levels of HGF in feces due to chronic bowel diseases such as colon cancer and inflammatory bowel disease (IBD) [19,20], limiting the specificity of such a test. Furthermore, HGF produced during acute inflammation binds to heparan sulfate proteoglycan (HSPG) with high affinity but exhibits decreased affinity to HSPG when produced during chronic inflammation [21–26]. Based on these observations, we developed a metachromatic semi-quantitative test to detect the presence of growth factors such as HGF that show affinity to sulfated glycans in feces during infectious gastroenteritis [27].

Determination of fecal pH is a classic method for evaluating signs of malnutrition and infection in feces. Recently, the pH levels in the feces of severely ill patients were found to indicate the severity of a disease or increased mortality [28].

In order to confirm the results from previous studies [24], we developed a platform that could be used to evaluate whether the determination of substances with binding affinity to sulfated glycans, such as HSPG, could be used to distinguish between the various causes of diarrhea when patients with diarrhea were admitted to the hospital. Dextran sulfate (DS) has properties similar to those of HSPG in terms of binding to HGF [21]. We developed a new strip test that has two assay surfaces, one for measuring fecal pH and one for detecting the binding affinity of fecal HGF to DS.

In the present work, we performed a cohort study in which we assessed patients with symptoms of diarrhea and noted the outcomes during follow-up of up to one year. We evaluated local production of HGF as a local acute phase response marker in the bowel using the newly developed strip test and determined whether use of the test strip could distinguish infectious gastroenteritis versus onset/exacerbation of IBD and bowel cancer in these patients.

2. Patients and methods

2.1. Study population

A total of 513 fecal samples were collected in a blinded fashion from patients with bowel disturbances who contacted health care centers or hospital-connected home health care agencies, or who were admitted to the University Hospital in Linköping or to county hospitals in Norrköping and Motala, Sweden, from March 2012–December 2013. Each patient was followed for up to one year after inclusion in this study. Patients in hospital wards were isolated until they recovered from diarrhea.

2.2. Sample collection and processing

- The stool samples included in this study were collected in feces collecting tubes without additives (feces tube #80.734, Sarstedt, Nümbrecht, Germany).

- The samples were coded at collection and all identifying information was removed.
- The samples were then sent to project staff members for testing using the new strip test while the physician in charge performed diagnostic procedures and oversaw treatment.
- The strip tests results for each sample were documented in the project database.

2.3. The new strip test

2.3.1. Description

- The new strip test for feces samples has two sensing surfaces (pads). The upper sensing surface detects the presence of HGF protein in the feces based on its binding affinity to DS, while the lower sensing surface determines fecal pH (Fig. 1).
- A positive signal for the presence of HGF indicates an infection or acute inflammation, while a negative response could indicate chronic inflammation. The pH measurement provides additional information that can help make a diagnosis. For example, high fecal pH, i.e. pH 9–10, indicates a generalized inflammatory response, bacterial translocation, and septicemia.

2.3.2. Strip test pH assay results

- Stool samples were stored at room temperature for 15 min prior to analysis.
- For analysis, a micro brush (Amax Medical Dental Supply Ltd., Calgary, Canada) was soaked in deionized sterile Milli-Q (MQ) water and immersed in the feces for 10 s.
- The micro brush was wiped twice on each of the two strip pads.
- Fecal pH was determined by the pH sensor included in the strip. The pH was indicated by the color of the strip, which has a range from pH 4 to pH 10 (Fig. 1).
- The color change was observed within 60 s of application of the sample to the strip and was compared to the CMYK color chart [29] (Fig. 1).

2.3.3. Quality control and reproducibility of the new strip test

- Ten samples were analyzed 10 times with different batches of strips in order to determine method variability due to possible variations in strip preparation.
- Twenty strips from each batch ($n = 5$) were also tested with negative controls (MQ water) and positive controls (Recombinant HGF Standard, R&D Systems, Minneapolis, MN, USA). The control tests were run daily prior to the analysis of samples.
- All fecal samples were analyzed in duplicate with the same results.

2.3.4. Concentration of HGF in feces

- Due to differences in fecal shape and consistency, it was not possible to perform ELISA analysis on fresh samples. The method used to standardize the volume of fecal samples was described previously [30].
- Fecal samples were collected and stored at -20°C within 24 h of collection.
- Prior to handling, the samples were thawed at room temperature and mixed by vortexing. The narrow heads were cut off of plastic syringes (2-ml, latex-free Omnifix

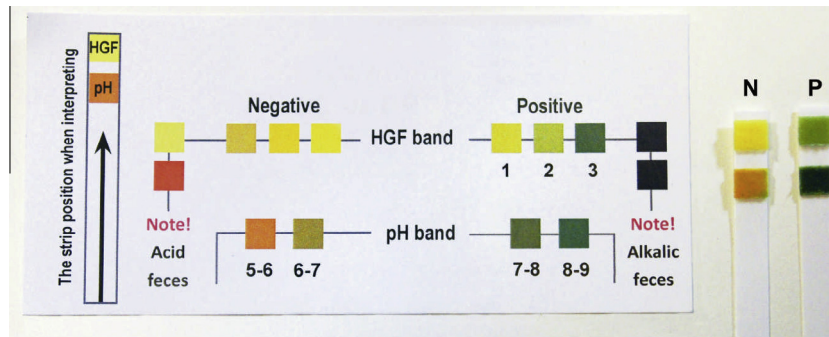


Fig. 1. The CMYK color chart for semi-quantitative evaluation of the presence of hepatocyte growth factor (HGF) in the bowel and for determining fecal pH. The strip on the left (N) is negative for HGF and shows a fecal pH of 5–6. The strip on the right (P) is positive for HGF and shows a fecal pH of 8–9.

syringes; B. Braun Melsungen AG, Melsungen, Germany). The plunger of each syringe was pulled out to create a small cylinder with an exact volume. The cylinder was filled with the fecal sample and incubated at -70°C for 15 min, then kept at room temperature for 1 min. The plunger of the syringe was then pushed down to empty the cylinder into a 20-ml scintillation vial (Sarstedt AB, Landskrona, Sweden) and diluted 1:6 with distilled water. The sample was then vortexed, centrifuged at 3000g for 15 min, and the supernatant transferred to new tubes (Nunc CryoTubes; Nunc Brand Products, Soeborg, Denmark). The supernatant was stored at -70°C until analysis.

- For analysis, the supernatant samples were thawed and centrifuged at 1000g for 15 min. Immunoreactive HGF levels were determined by ELISA using a commercially available kit (Quantikine HGF Immunoassay, R&D Systems) according to the manufacturer's instructions. The minimum detection level for the assay was $0.02\ \mu\text{g/L}$ for feces.

2.4. Reference tests

Stool samples were analyzed using the following routine tests at the Department of Microbiology, University Hospital in Linköping, Sweden:

1. *Detection of Clostridium difficile (C. difficile) toxin A and B:* these toxins were detected using two-step sandwich enzymatic immunoanalysis with fluorescent detection (automated ELFA-based VIDAS system; Biomerieux, Marcy l'Etoile, France).
2. *Isolation of C. difficile from fecal specimens:* stool samples were collected using sterile copan eswabs (Copan Italia S.P.A. via Perotti, Brescia, Italy) and inoculated on CCFE agar (Lab. 090 agar for fastidious anaerobes; LAB-M, Lancashire, UK) supplemented with cycloserine, cefoxitin, and fructose.
3. *Isolation of Salmonella, Shigella, and Campylobacter:* stool samples were collected using sterile copan eswabs and cultured on xylose lysine deoxycholate agar (XLD) (Lab. 032 agar, LAB-M, Heywood, UK) and blood agar (Colombia blood agar, Acumedia, Michigan, USA) and incubated in a 5% CO_2 incubator.
4. *Detection of Enterohemorrhagic Escherichia coli (EHEC):* stool samples were collected using sterile copan eswabs and analyzed by PCR (Bio-Robot EZ1 advanced XL system, Qiagen, Hilden, Germany) to detect EHEC.
5. *Detection of viral agents*

- a. *Detection of Calicivirus RNA:* stool samples were collected in feces collecting tubes without additives and analyzed by PCR (Bio-Robot M 48 QIA-symphony system, Qiagen) to detect *Calicivirus* RNA.
 - b. *Detection of Rotavirus antigen:* stool samples were collected in feces collecting tubes without additives and analyzed by Enzyme Immunoassay (EIA) (ProSpecT rotavirus, Thermo Scientific, Waltham, MA, USA) to detect the rotavirus antigen.
6. *Detection of stool parasites:* stool samples were collected in ParasiTrap BIOSEPAR tubes (Biosepar GmbH, Germany) containing formaldehyde-free fixation and transport medium and examined by light microscopy to detect stool parasites.
 7. *Detection of fecal hemoglobin:* qualitative analysis was performed to detect fecal hemoglobin using antibodies developed against human hemoglobin (Fecal Immunochemical test (FIT); OC FIT-CHEK, Polymedco. Inc. NY, USA).
 8. *Other tests:* Other X-ray and endoscopic techniques were used as indicated.

2.5. Database entry

- The project leader examined the medical records and the medical history of each patient, and patient research files were updated daily to include the results of routine laboratory tests, X-rays, endoscopic procedures, and the final diagnosis before patient discharge.
- Patients were tracked for one year after inclusion in this study to determine the ultimate diagnosis and outcome.
- In addition to the documentation in the research database, the results of 74 cases were documented immediately in the digital patient journal, *COSMIC*.

2.6. Statistical analysis

- GraphPad Prism Version 5 was used for statistical analysis.
- The specificity, sensitivity, and positive and negative predictive values of the new strip test were calculated manually.
- Logistic regression was used to analyze correlations between the results of the new strip test and the fecal hemoglobin results.
- The Kruskal–Wallis and Mann–Whitney *U* tests were used to analyze differences in fecal pH between the groups as the data was not normally distributed, and the chi-square test was used for analyzing mortality in the groups. The pH

Table 1

Ultimate diagnoses in the cohort of Swedish patients with diarrhea studied between March 2012 and December 2013.

Survey of patients with diarrhea (n = 513)	Age range years (median age) – females; in-patient	Etiological characteristics	Microbiological test in feces (result), further diagnostic procedures	Positive fecal strip test n, (%)
Verified infection, non-IBD n = 131	21–100 (73)–65; 101	Bacteria n = 101 <i>E. coli</i> n = 1 <i>C. difficile</i> n = 81 <i>Salmonella</i> n = 9 <i>Campylobacter</i> n = 10 Virus n = 27 <i>Rota virus</i> n = 8 <i>Calici virus</i> n = 19 Parasite n = 3	(Positive)	117 (89.31%)
Verified infection, developed IBD (n = 2)	23–44 (nd)–1; 2	<i>Salmonella</i> n = 2	(Positive), endoscopies, bowel biopsies	0 (0%)
<i>C. difficile</i> (clinically verified) n = 40	30–90 (76)–16; 27	Previously positive toxin, diarrhea treated efficiently with metronidazole/oral vancomycin	(Negative)	36 (90%)
Short non-recurrent episode < 2 weeks n = 97	3–100 (75)–54; 54 (median 9 days)	Positive epidemiology, unclear etiology	(Negative)	74 (76.3%)
Sepsis/SIRS n = 66	29–102 (67)–35; 66	Severe colitis n = 13 Septicemia n = 18 Severe pneumonia n = 8 Abdominal emergencies n = 16 Neutropen fever n = 11	(Negative)	59 (86.4%)
Chronic diarrhea > 2 weeks n = 36	21–93 (66)–21; 12	Depression, IBS, non-verified IBD, constipation	(Negative)	6 (16.7%)
IBD n = 38	18–77 (42)–24; 8	Exacerbation n = 32 Onset n = 6	(Negative), endoscopies, bowel biopsies	4 (10.5%)
Onset of malignancy n = 6	64–88 (74)–4; 3	Colon cancer n = 4 Ventricle cancer n = 1 Lymphoma terminal ileum n = 1	(Negative), endoscopies, bowel biopsies	0 (0%)
Side-effect medicine n = 20	28–98 (71)–9; 17	Carbapenem, PT, rifampycin, trimetoprim n = 7 Warfarin, adalimumab n = 2 Metformin, simvastatin, morfin n = 3 Photopheresis, anti epileptic n = 3 Nutrition n = 5	(Negative)	0 (0%)
Other infectious foci n = 35	25–92 (78)–19; 27	Urinary tract infection n = 11 Lung inflammation n = 6 Focal infections/abscess n = 16 Fungemia n = 2	(Negative), other cultures yielded growth accordingly	4 (11.4%)
Other disturbances n = 18	38–95 (79)–12; 15	Abdominal bleeding Uremia and electrolyte disorders Terminal phase cancer patients Liver diseases	(Negative)	2 (10.5%)
Resolved symptom, no recurrence n = 24	41–92 (69)–9; 12	Formed feces	<i>C. difficile</i> = 6 <i>Campylobacter</i> = 1 <i>Calici virus</i> = 1 <i>Blastocystis</i> = 1	0 (0%)

IBS inflammatory bowel syndrome; nd non-defined; PT piperacillin–tazobactam.

is reported as a mean value with 95% CI, and mortality is reported as a %. In this study, $p \leq 0.05$ was considered statistically significant.

- The Mann–Whitney *U* test was used to assess the significance of differences between fecal HGF concentrations and the strip test results.

2.7. Ethics committee approval

The ethics committee in Linköping, Sweden, approved the study protocol (M151-09, 2010/284-32).

2.8. External review

The SP Technical Research Institute of Sweden studied the test results and the ultimate patient outcomes to evaluate the sensitivity of the test (report designation 3P03321).

3. Results

3.1. Diarrhea and differential diagnoses in randomly selected cases over a one-year period

Acute infectious gastroenteritis was verified in 131 of the 513 fecal samples that were included in this study. In patients who had negative microbiological test results, the differential diagnosis was assessed by evaluating the course of treatment and the outcome during the 1-year follow-up. Several sub-groups were identified that are described in Table 1 and Fig. 2.

3.2. Estimates of strip test performance

Patients with microbiologically verified infectious gastroenteritis ($n = 131$) were compared to verified cases of IBD exacerbation/onset ($n = 38$) and gastrointestinal malignancy ($n = 6$). The strip test distinguished infectious gastroenteritis with a sensitivity of

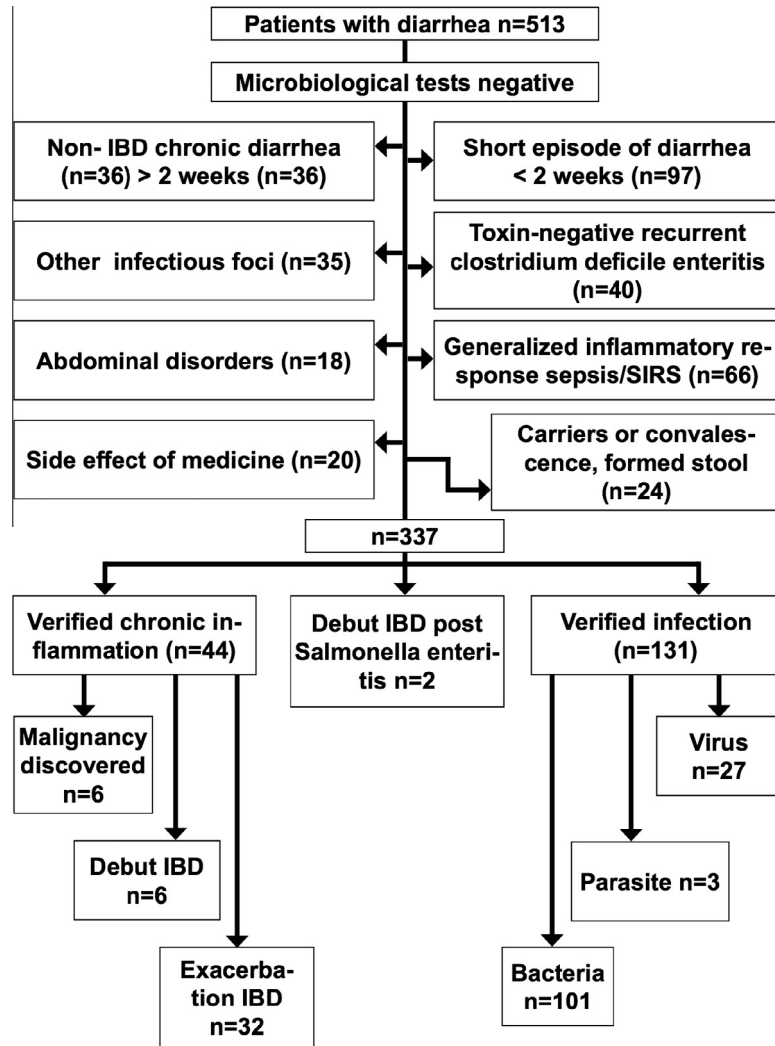


Fig. 2. The study population of patients presenting with diarrhea.

87.9%, a specificity of 90.9%, a positive predictive value (PPV) of 96.6%, and a negative predictive value (NPV) of 71.4% (Table 2). The reference test for *C. difficile* toxins A and B identified cases with recurrent enteritis with a sensitivity of 66.6% versus 90.9% using the strip test (Table 1).

There was no significant correlation between the strip test results and the presence of blood in feces in the same sample ($n = 36$, $R^2 = 0.07$). Additionally, no significant differences were detected in the strip test results obtained from the same versus different batches of the strips.

3.3. Fecal pH and mortality within the first month of sampling in groups

Fecal pH was measured and documented in patients with loose feces at the time of sampling. The patients were followed-up and divided into groups based on laboratory and clinical outcome. The sub-group with short non-recurrent episodes of diarrhea (<2 weeks; $n = 97$; Table 1) was omitted because no further investigation was needed during the follow-up period to define the cause of the short-term episode of diarrhea. The other cases consisted of three major groups: (1) microbiologically- and clinically-verified infectious gastroenteritis ($n = 171$; 10 patients died 2–26 days after admission and a median of 19 days after sampling, mean fecal pH 7.0–8.0); (2) non-infectious cases ($n = 178$; 12

patients died 1–30 days after admission and a median of 11 days after sampling, mean fecal pH 5.0–6.0); and (3) cases with a generalized inflammatory response (sepsis/SIRS; $n = 66$; 11 patients died 1–27 days after admission and a median of 6 days after sampling, mean fecal pH 9.0–10.0). The groups were then compared ($p < 0.0001$ for differences in pH and $p < 0.04$ for differences in mortality between the groups; Fig. 3, Table 1).

3.4. Fecal HGF concentration

The HGF concentration was measured by ELISA in 101 feces samples. Of these 101, 49 were positive using the strip test and 52 were negative using the strip test. The HGF levels were significantly higher in the positive test group ($p = 0.003$; Table 3).

4. Discussion

High levels of HGF are produced both locally and systemically in injuries caused by infection [3]. Low levels of serum HGF in patients with pneumonia correlates significantly with poor prognosis [31], and application of HGF to the site of an injury, such as to the site of a chronic ulcer, accelerates the healing process [32]. The gastrointestinal mucosa has a remarkable ability to repair damage, and growth factors play an important role in the regeneration

Table 2

The sensitivity and specificity of the strip test for distinguishing between infectious gastroenteritis and chronic inflammatory bowel disease (IBD).

Strip test result	Infectious diarrhea Microbiologically verified as infectious				All subgroups	Non-infectious diarrhea Verified chronic inflammation			All subgroups	Total
	Bacteria	Virus	Parasite	Suspected onset of IBD <i>Salmonella</i>		Onset of IBD	Exacerbation of IBD	Malignancy		
Positive	91	23	3	0	117	0	4	0	4	121
Negative	10	4	0	2	16	6	28	6	40	56
Total	101	27	3	2	133	6	32	6	44	177

The test strip sensitivity was 87.96% and the specificity was 90.9%. The positive predictive value was 96.6%, and the negative predictive value was 71.4%. The test strip accuracy was 89.1%.

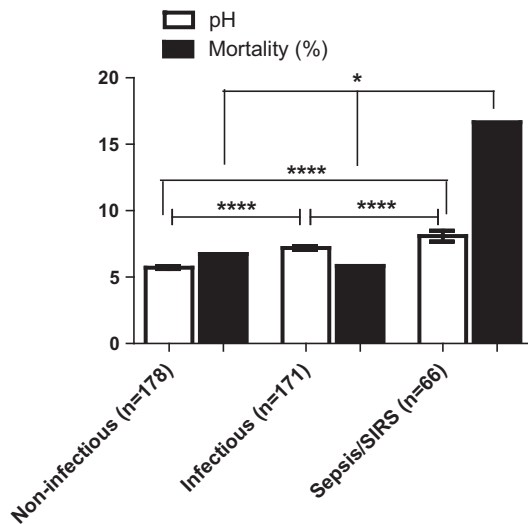


Fig. 3. The fecal pH (presented as the mean with 95% CI) as determined with the test strip and the 1-month mortality (%) in each group of patients with diarrhea. The Kruskal–Wallis and Mann–Whitney *U*-tests were performed to statistically analyze the pH data, and the chi-square test was used to analyze mortality data. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

of injured cells in gastrointestinal organs [33]. Nishimura et al. [34] has shown that of the cytokines (HGF, transforming growth factor- α , transforming growth factor- β , and keratinocyte growth factor), HGF is the most potent in terms of accelerating the repair of the damaged monolayer of epithelial cells derived from normal rat small intestine.

This study evaluated the ability of a new strip test to determine the binding affinity of acute phase proteins such as HGF to DS as a tool for assessing patients with diarrhea. This strip test showed high sensitivity and specificity for identifying infectious gastroenteritis in patients with diarrhea upon hospital admission.

The binding of HGF to HSPG on the cell surface and in the extracellular matrix plays an important role in activating an HGF precursor and in facilitating its interaction with the high-affinity c-met receptor [35]. We observed previously that binding of HGF to both high affinity (c-Met) and low affinity (HSPG) receptors can be studied using a surface plasmon resonance-based system to differentiate HGF that is biologically active during acute inflammation from HGF that is present during chronic inflammatory diseases [36]. We showed that unlike the biologically inactive form of HGF, the biologically active form of HGF has binding affinity to HSPG [22], and this affinity decreases significantly when DS is added to the samples. Thus, DS has properties similar to those of HSPG in terms of binding to active HGF [20]. In a previous study, we prepared a DS-containing gel and immobilized it on plastic loops. Notably, DS changes the color of methylene from blue to red, so we developed a method that took advantage of this

property. In this method, the binding of HGF to DS competes with the interaction of DS and methylene blue and inhibits the color change to red. Using this method, we tested fecal samples from patients and healthy volunteers to investigate the ability of the method to distinguish infectious gastroenteritis with high sensitivity and specificity [27]. We then developed this strip test based on the binding affinity of active HGF to DS.

Determination of fecal pH yields important information about ion exchange in the bowel [37] and has been used as a non-specific way to diagnose some bowel infections. This method was recently demonstrated to predict outcome in severely ill patients [28]. Since patients with generalized inflammatory response, or SIRS in the course of severe trauma and/or sepsis, have HGF with high affinity to DS [27] and have high fecal pH [20], we determined both the binding affinity of HGF to DS and fecal pH. We observed that patients with infectious gastroenteritis had higher fecal pH (pH 7.0–8.0) than those with non-infectious diarrhea (pH 5.0–6.0; Fig. 3) and healthy controls (data not shown). However, fecal pH ≥ 9.0 was seen in cases with severe colitis and SIRS, and this was associated with significantly increased mortality (Fig. 3). Thus, including a pH sensor in the strip test may differentiate cases with self-limiting infectious gastroenteritis from cases at risk of bacterial translocation, septicemia, and SIRS. This would mean that antibiotics could be used in a more directed way. Fecal pH ≤ 4.0 predicted an unfavorable outcome (data not shown).

Routine diagnostic tests for identifying infectious gastroenteritis have low sensitivity (45.1%) [38]. We observed that the reference test for detecting the *C. difficile* toxin in feces identified cases with *C. difficile* enteritis with a sensitivity of 66.6%. However, technical improvements and the development of new diagnostic methods have made diagnosis more rapid and accurate.

During the development of the strip test, we assessed the concentration of HGF in feces by ELISA. We observed previously that the HGF concentration is significantly higher in feces during infectious gastroenteritis than in feces from diarrhea due to non-infectious causes [18]. Additional studies showed that the concentration of HGF in feces was significantly higher in chronic IBD compared to infectious gastroenteritis [20]. It is complicated to perform ELISAs on stool samples [30], and it was not possible to perform ELISAs on fresh samples. The strip test was developed based on the affinity of HGF to the receptor (HSPG) and not the concentration of HGF [26]. Thus, the concentration of HGF was determined in some samples, and the stool samples that were negative for HGF on the strip test included samples from cases with IBD as well as with other causes of diarrhea (Table 1). Therefore the data was not normally distributed (Table 3).

Limitations: the strip test could not differentiate between the various etiologies of infectious gastroenteritis. However, patient isolation and avoiding antibiotic treatment are common management strategies for infectious gastroenteritis. Difficulty in detecting changes in the color is another limitation of the strip test, and one that could be overcome by instrumentation. The performance of the strip test did not change significantly when

Table 3Results from ELISA determination of the hepatocyte growth factor (HGF) concentration in stool samples from patients with diarrhea ($n = 101$).

Strip test result	Elisa ng/ml range (median) $p < 0.01$	Verified infection bacterial or viral	Verified non-infectious diarrhea
Positive ($n = 49$)	0.02–77.2 (0.93)	37	2
Negative ($n = 52$)	0.02–64.3 (0.47)	5	41

freeze-thawed feces were tested (data not shown) [30]. The binding affinity to sulfated glycans/HSPG may not be limited to HGF in feces i.e. there may be competitive binding by other proteins as well. Although the strip test showed high sensitivity and specificity in the current investigation, the lack of a gold standard for comparison is a major limitation of this study.

Further studies are planned to further assess the performance of the strip test and its possible impact on antibiotic use in different patient groups and diseases.

5. Conclusions

In summary, HGF is a good local acute phase response marker for acute bowel inflammation. We developed a new rapid test for stool specimens that evaluates local production of HGF as a local acute phase response marker in the bowel. We suggest that this method can be used for the simultaneous determination of fecal pH and the binding affinity of fecal HGF to DS in order to assess patients with symptoms of acute diarrhea. The strip test provides useful information for making decisions about patient isolation and for planning appropriate diagnostic procedures and therapy. More data is needed before a test built on this platform can complement or replace currently available tests.

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Appendix A. Supplementary material

An Excel data file containing all of the information collected and used in this study is available on a local disc (at lio.se). The test results of 72 cases are also documented in the medical records of patients. These are available for viewing. Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.cyto.2014.07.255>.

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