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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

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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.

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].

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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