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ORIGINAL ARTICLE

Comparison of jejunal aspirate culture and methane and hydrogen breath test in the diagnosis of small intestinal bacterial overgrowth

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Abstract

Background Small intestinal bacterial overgrowth (SIBO) is still difficult to diagnose. Quantitative culture of small intestine aspirate is recommended to be the gold standard. The methane and hydrogen breath tests are easily repeatable, sufficiently sensitive and highly specific for SIBO diagnosis. Our goal is to contrast the diagnostic value of the breath tests with jejunal aspiration cultures.

Methods 40 adult outpatients (age < 60) were enrolled in our study. Randomly, within 2 days, both the methane and the hydrogen breath test and jejunal aspiration culture were performed on each patient and the results of both tests were evaluated and contrasted.

Results The jejunal culture was positive (10^5CFU / mL) in 14/40(35%) subjects, the lactulose breath test (LBT) was positive in 18/40 (45%) subjects, and the glucose breath test (GBT) was positive in 12/40 (30%). The GBT showed good agreement ($\kappa = 0.659$) and LBT showed poor agreement ($\kappa = 0.588$) with the jejunal aspirate culture. The sensitivity, specificity, positive and negative predictive values of LBT/GBT were 85.7/71.4%,76.9/92.3%, 66.6/83.3% and 90.9/85.7%, respectively.

Conclusions 35% of patients with suspected SIBO are identified using jejunal aspirate cultures. For the identification of SIBO, GBT is more specific than LBT, but has a lower sensitivity. In individuals with suspected SIBO, the breath test should be initially due to its good agreement with the jejunal aspirate culture.

Keywords Jejunal aspirate culture · Methane and hydrogen breath test · Sensitivity and specificity · Small intestinal bacterial overgrowth

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Introduction

Small intestinal bacterial overgrowth (SIBO) describes an increase in the number or variety of bacteria in the small intestine as a result of organic or functional stasis. Although some patients with SIBO exhibit abdominal symptoms such as abdominal pain, bloating or diarrhea, others are asymptomatic [1]. Early diagnosis of SIBO is difficult due to limited diagnostic techniques or lack of specific symptoms. Abnormal structure or motility of the small intestine, decreased immune function, drugs such as proton pump inhibitors(PPIs) or aspirin may lead to SIBO [2]. Quantitative culture of small intestine aspiration, although invasive, is considered the gold standard for the diagnosis of SIBO [3]. The test is technically difficult and can only detect bacteria in the proximal small intestine. There is no strong evidence to support the current guidelines for SIBO cut-off values for bacterial colony counts [4].

Methane and hydrogen breath tests are non-invasive techniques based on the detection of metabolic products from bacteria in expired air [5], which presents several advantages: easily repeatable, sufficiently sensitive and highly specific for the diagnosis of SIBO [6, 7]. The glucose breath test (GBT) and the lactulose breath test (LBT) have been promoted for the past few years due to their convenience and safety. However, their performance in the diagnosis of SIBO is still controversial. As a result of the heterogeneity of the substrates used, the hydrogen and methane levels cut-offs, and the time interval used for measurement, the interpretation of their results is somewhat unclear [6].

The aim of this study was to assess the incidence of SIBO in patients using jejunal aspirate culture, GBT, and LBT to evaluate the diagnostic accuracy of these methods.

Methods

Study subjects

Between October 2016 and September 2019, 40 adult outpatients (age < 60) with gastrointestinal symptoms that included diarrhoea, bloating, gas, or unexplained abdominal discomfort were enroled at outpatient department of Gastroenterology, the Second Medical Center, Chinese PLA General Hospital, Beijing, China, after obtaining informed consent from them. No patient had metabolic problems or had taken PPIs, probiotics, or antibiotics within the previous four weeks. In the four weeks prior to the trial, no patient had taken any medication that could affect gastrointestinal motility or increase the risk of developing bacterial overgrowth, such as prokinetic medications or narcotics. Patients with active inflammation, cardiac, respiratory, hepatic, renal, or thyroid disease, Crohn's disease or ulcerative colitis, hepatobiliary and pancreatic disease, or immunological disease were excluded from the study. The study protocol was approved by the IRB of Chinese PLA General Hospital (S2018-081-02).

Jejunal aspirate culture

Doctors and nurses wore sterile gloves before aspiration. The catheter (Freka-Trelumina CH 16/9,150cm) was slowly inserted from the nasal cavity under aseptic techniques, passed through the esophagus, the stomach and the duodenum, finally entered the jejunum. The guide wire was removed, and the catheter was fixed under the earlobe with the tape outside the nasal cavity, so that the pipe could be naturally bent and relaxed. X-ray photography was performed 24 h after intubation to confirm the arrival of the tube to the upper jejunum. The small intestine content was aspirated with a sterile vacuum pressure syringe and transferred to a sterile tube.

The jejunal aspirate was then diluted and injected into different culture mediums using the serial dilution method. The aerobic culture media used were Nutrient agar, MacConkey agar, 5% sheep blood agar, and Salmonella Shigella agar. The samples were placed on agar plates and kept there for 24 h at 37 °C. The anaerobic culture media employed were lactobacilli MRS agar, gut microbiota medium (GMM) and bifidobacterium medium. The jejunal aspirate was plated in anaerobic medium at 37 °C for 24–48 h. In our study, SIBO was considered to exist when there were 10^5 CFU/ml of bacteria in the small intestine.

Methane and hydrogen breath test

The instrument used in this study was the BreathTracker SC expiratory gas analyser made in Quintron Company (USA) with its hydrogen and methane concentration expressed as parts per million (ppm), the detection range defined as 0-500 ppm, with a sensitivity of 1 ppm and an accuracy of ± 5%.

Milk products, soybean products, roughage and fermented foods were prohibited from the day before methane and hydrogen breath test and patients were required to have a cooked rice diet plus a small volume of protein in dinner. The time from the day before the breath test to the day of the breath test was 14 h, during which the patients could drink boiled water, but not beverages in which hydrogen was produced due to bacterial decomposition. The patients were asked to defecate and brush their teeth but not to do strenuous exercise. Smoking was prohibited at 2 h before and during the breath test. The patients were asked to remain

conscious and quiet and not do any strenuous exercise during methane and hydrogen LBT. Standard curves were plotted to maintain the stability of the BreathTracker SC expiratory gas analyser after adjustment. The expiratory gas in resting state was collected into a bag for the measurement of methane and hydrogen concentrations. The patients were given 75g of glucose or 10g of lactulose dissolved in 200 ml of water and asked to exhale the gas into the bag every 20 min from the time the substrates were given and repeated 9 times to measure methane and hydrogen concentrations in the expiratory gas. GBT and LBT were performed on two separate days.

Diagnosis of SIBO

After the expiratory gas was tested, curves for the time-hydrogen concentration and the time-methane concentration in the expiratory gas were plotted with the time as abscissa, the methane and hydrogen concentration as the ordinate. Following glucose ingestion, a sustained increase in breath hydrogen of 20 ppm over the basal level was regarded as SIBO evidence. SIBO was defined as an increase in hydrogen in breath of 20 ppm over basal values within 90 min after lactulose treatment. A positive methane breath test was defined as a methane level ≥ 10 ppm at any time point in the study [8].

Culture results analysis

The results were considered SIBO-positive if one or more organisms (aerobic or anaerobic) were cultured with a colony count of 10^5 CFU/ml. Additionally, we measured the incidence of SIBO at a cut-off value of 10^3 CFU/ml.

Statistics

Data were analyzed with SPSS software, version 25.0 for Windows. Categorical variables were analyzed using the χ^2 test and Fisher's exact test as applicable. Nonparametric continuous data were analysed using the Mann–Whitney test. P-values below 0.05 were considered significant. Sensitivity, specificity, positive and negative predictive values, and diagnostic precision were calculated using standard formulas. The agreement between various methods for the diagnosis of SIBO was assessed by κstatistics. Values for κ of at least 0.81 were considered to show excellent agreement, 0.61–0.80 as good, and below 0.60 as poor.

Results

Demographics

In total, 40 adult outpatients were evaluated in the study. Subjects aged 28–57 (40.3 ± 8.4) years old, with positive methane and hydrogen LBT/GBT rate of 45%/30% respectively. We contrasted patients with small bowel colony counts of ≥ 10^5 CFU/ml and < 10^5 CFU/ml in terms of symptoms and GSRS scores. There was no difference between both groups. Furthermore, there was no change in the variables between patients with bacterial colony counts greater than 10^3 CFU/ml and less.

Breath test

LBT and GBT were performed in all patients, with 18/40(45%) positive in LBT and 12/40(30%) positive in GBT. We contrasted the predetermined cutoff value of 10^5 CFU/ml by jejunal aspirate culture with the results of LBT or GBT and found that the sensitivity, specificity, positive and negative predictive values of LBT/GBT were 85.7/71.4%,76.9/92.3%, 66.6/83.3% and 90.9/85.7%, respectively. A total of 10/14 (71.4%) with and 6/26 (23.1%) without SIBO in culture had high hydrogen on LBT (sensitivity 71.4%, specificity 76.9%).A total of 8/14 (57.1%) with and 4/26 (15.4%) without SIBO on culture had high methane on LBT (sensitivity 57.1%, specificity 84.6%) (Fig. 1). GBT showed good agreement (κ = 0.659) and LBT showed poor agreement (κ = 0.588) with jejunal aspirate culture. The agreement between LBT and GBT was also good (κ = 0.687).

Aspirate culture

In the study, 22(55.0%), 4(10.0%), and 14(35.0%) patients, respectively, exhibited bacterial colony counts of ≤ 10^3, > 10^3 to 10^5 and ≥ 10^5 CFU/ml. Among patients with colony counts ≥ 10^5 CFU/ml,8 (57.1%) had positive aerobic cultures and 6(42.9%) had both positive aerobic and anaerobic cultures (Fig. 2). Anaerobic cultures that were positive alone

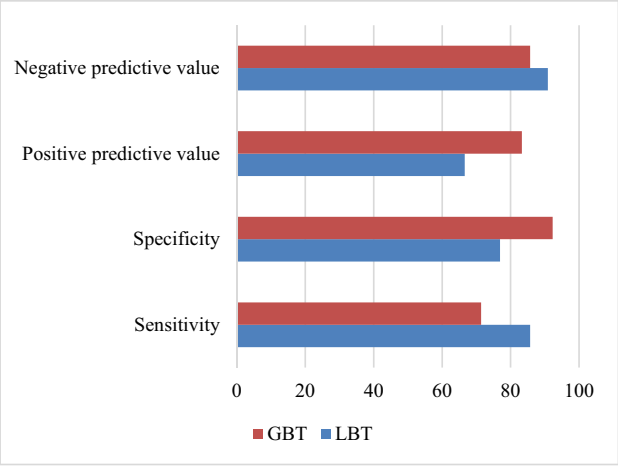

Fig. 1 Comparison of evaluation indicators for diagnostic breath tests

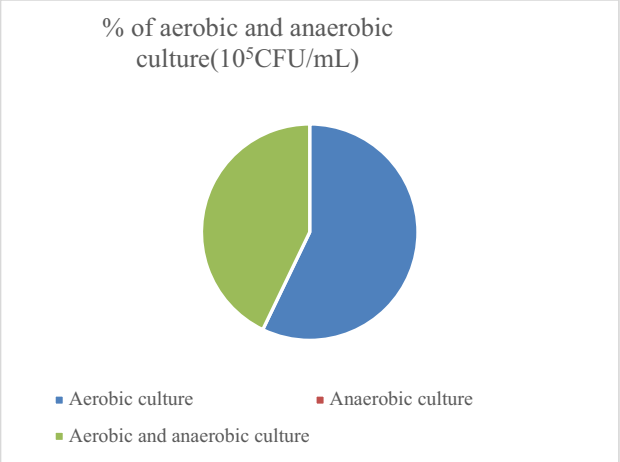

Fig. 2 Aerobic and anaerobic distribution of positive culture results ($> 10^5$ CFU/mL)

did not emerge in any of the patients. The most frequently isolated microorganisms included Alpha-haemolytic streptococcus, Klebsiella species, and Neisseria spp (Table 1).

Discussion

The diagnosis of SIBO remains controversial. Since the gold standard is not available for the diagnosis of SIBO, the number of bacteria in proximal duodenal and jejunal aspiration and culture 10^5CFU / ml is therefore the widely accepted diagnostic technique for SIBO [9]. However, this technique is invasive, difficult to operate, time-consuming, expensive, false positive because of bacterial contamination, and is thus not widely used in clinical practice. The methane and hydrogen breath test is recommended as the best technique for the diagnosis of SIBO because it is a feasible, simple, noninvasive and radiation-free technique [10, 11].

In this study, we evaluated and contrasted the jejunal aspirate culture with the methane and hydrogen breath test in patients with gastrointestinal symptoms. While 33.3% of the patients had positive GBT and 45.0% of the patients had positive LBT, 35.0% of the patients had positive jejunal cultures. The most typical organisms identified from cultures included Alpha-haemolytic streptococcus, Klebsiella species, and Neisseria spp. 42.9% had both positive aerobic and anaerobic culture, while 57.1% only showed a positive aerobic culture. Therefore, the sensitivity and specificity of LBT/GBT were 85.7/71.4%,76.9/92.3%, respectively. What cutoff is better suitable for determining an abnormal level of bacterial growth is up for debate. We compared the results of different cutoff values in our study and found that the positive culture rate was 45% at $\geq 10^3$ CFU/ml and 35% at $\geq 10^5$ CFU / ml. Depending on the criteria used to identify a positive culture and the characteristics of the patients, the positive rate may change [12]. In healthy subjects, the proximal jejunum can contain up to 10^4 CFU/mL of bacteria [4, 13]. 0.12% of healthy individuals had bacteria counts in the proximal jejunum ranging from 0 to 10^3 CFU/ml [14]. There is no standardisation of aspiration and culture. Compared to our method of inserting a sterile catheter into the jejunum, some used endoscopic suction to collect duodenal juice, which can cause a high risk of contamination.

Table 1 Culture results of jejunal aspirates

Culture results ($\geq 10^5$ CFU/ml)	Total, N = 40 (%)
Alpha-hemolytic streptococcus	14(35.0)
Klebsiella species	8(20.0)
Neisseria spp	6(15.0)
Escherichia coli	6(15.0)
Rothia spp. (Stomatococcus)	4(10.0)
Staphylococcus aureus	2(5.0)

Because of variations in the dose and species of drugs employed, the cut-off for determining a positive breath test, and the features of the patients, the outcomes of the former studies have been inconsistent [15]. 0.30%/45% of the participants in our study who had no gastrointestinal symptoms tested positive for GBT/LBT. GBT was positive in 31% [16] and LBT was positive in 34.3–84% [17, 18] of patients with IBS. GBT was positive in 26.7%, LBT in 18.3% and culture ($\geq 10^5$CFU/ml) in 39.5% of patients with malabsorption in another study [7].

With a cut-off value of 10^6 CFU/ml, a study that cultured proximal jejunum fluid at two different locations reported that GBT had a sensitivity and specificity of 62% and 83%, respectively [19]. The specificity for a positive GBT was usually 76–85%, although there had been considerable variation in sensitivity based on different cutoff values, aspiration site, technique, and bacterial concentration. While the low sensitivity of GBT may attribute to quick absorption or lack of availability as a substrate in the distal small intestine, it was made reliable by the high specificity of the test [20]. The shortcomings of our research include a poor sample size of only 40 subjects. Our aspiration was carried out from the proximal jejunum, and it was not known whether the aspiration performed in the distal jejunum or some other site of the small intestine could have resulted in a higher positive cultures was unsure. Despite the aseptic methods we focus on, we could not completely rule out the possibility of oral bacteria.

In conclusion, compared to LBT, GBT has a lower sensitivity but a higher specificity to detect SIBO. Due to the noninvasive nature and wide availability of this tests, breath test should be considered first in patients who have symptoms of SIBO. The agreement between jejunal culture and GBT

was good. Both GBT and LBT might be necessary in some patients with high suspicion of SIBO.

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Data availability The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Declarations

Ethics approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee of Chinese PLA General Hospital (S2018-081-02) and with the 1964 Helsinki declaration and its subsequent amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflicts of interest The authors declare that they have no conflict of interest.

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