Spinal Cord (2014) 52, 61–64

www.nature.com/sc

ORIGINAL ARTICLE

The prevalence of small intestinal bacterial overgrowth and methane production in patients with myelomeningocele and constipation

V Ojetti[1], G Bruno[1], V Paolucci[2], S Triarico[2], F D'aversa[1], E Ausili[2], A Gasbarrini[1] and C Rendeli[2]

Study design: Prospective study.
Objectives: The objective of this study was to assess the prevalence of small intestinal bacterial overgrowth (SIBO), methane (CH_4) production and orocecal transit time (OCTT) in children affected by myelomeningocele.
Setting: This study was conducted at the Catholic University in Rome, Italy.
Methods: Eighteen (6M/12F; 16.4 ± 7.6 years) children affected by myelomeningocele were enrolled. All subjects underwent H_2/CH_4 lactulose breath tests to assess SIBO and OCTT. All patients performed a visual analog scale to investigate abdominal pain, bloating and flatulence, and maintained a diary of the frequency and consistency of the stool during the previous 7 days. A nephro-urological clinical evaluation of the number of urinary tract infections (UTIs) and neurogenic bowel disease score were also performed.
Results: Thirty-nine percent (7/18) of the children showed SIBO and 61% (11/18) presented a delayed OCTT. Moreover 44.4% (8/18) produced high levels of CH_4. Interestingly, all myelomeningocele children who produced CH_4 showed a delayed OCTT and a higher incidence of UTI, with a lower frequency of evacuation, compared with those with a normal or accelerated OCTT.
Conclusion: The association between CH_4 and constipation suggests that CH_4 has an active role in the development of constipation. One of the most interesting features of our study is to identify a correlation between myelomeningocele, CH_4, delayed OCTT and UTI. The intestinal decontamination with locally acting drugs in these children may reduce the number of UTIs and improve intestinal motility.
Spinal Cord (2014) **52,** 61–64; doi:10.1038/sc.2013.131; published online 19 November 2013

Keywords: myelomeningocele; small intestinal bacterial overgrowth; methane; breath test

INTRODUCTION

The term spina bifida (SB) refers to a group of congenital neural tube defects with a variety of clinical manifestations, resulting from the lack of vertebral arches in the median line during the third and fourth week of gestational age.[1]

The incidence of SB is around 0.1–0.3% and the risk for parents with SB of having an affected baby is estimated to be about 4%.[2] The multifactorial etiology of SB involves both genetic and environmental factors, for example, socioeconomic status, geographic area, maternal obesity, epilepsy or diabetes, maternal exposure to drugs, alcohol or radiations.[3]

The open SB, called myelomeningocele (MMC), is characterized by a visible protrusion of spinal cord and/or meninges through the defect in the vertebral arch. MMC is a frequent form of SB with lifelong disabilities, characterized by the extrusion of spinal cord and by the development of Arnold—Chiari type II malformations and hydrocephalus in the central nervous system.[2] The level of neurological impairment influences muscle innervations, motor development, sensory and sphincter dysfunction.[3]

The injury in the lumbosacral spine compromises the sensory and motor functions of the perianal region, leading to a delayed colonic motility and anorectal dysfunction, which result in functional obstruction and severe constipation.[4]

Neuropathic bowel has a great impact on social integration of patients with MMC, which benefit from a well-organized treatment plan of constipation with stool softeners, oral laxatives, digital evacuation, enemas or transanal irrigation.[5] Moreover, the treatment of neuropathic constipation is crucial for reducing the risk of urinary tract infections (UTIs), which frequently occur in these patients. In fact, a majority of UTIs is caused by gastrointestinal organisms (such as *Escherichia coli*), thus a successful treatment of constipation may avoid the reinfection of the urinary tract from the rectal reservoir.[4–6]

It is well documented in literature that delayed orocecal transit time (OCTT) predisposes to small bowel bacterial overgrowth, because of the stasis, which, in turn, promotes excess bacterial proliferation and inflammation.[3]

Small intestinal bacterial overgrowth (SIBO) is defined by the presence of 1×10^6 CFU ml^{-1} of intestinal aspirate and/or the presence of colonic-type species.[3] In general, SIBO is prevented by the presence of the ileocecal valve, by the action of gastric acid and pancreatic enzymes, as well as by small intestinal motility. When one or more of these mechanisms fail, SIBO can occur.

The consequences of SIBO are different. They include the alteration of the absorption of many substances such as carbohydrates, proteins, lipids and vitamins that lead to a malabsorption syndrome with

[1]Department of Internal Medicine and Gastroenterology, Catholic University, Rome, Italy and [2]Spina Bifida Center Pediatric Department, Catholic University, Rome, Italy
Correspondence: Dr V Ojetti, Department of Internal Medicine and Gastroenterology, Catholic University, Rome 00168, Italy.
E-mail: veronica.ojetti@tin.it
Received 3 June 2013; revised 5 September 2013; accepted 9 October 2013; published online 19 November 2013

different clinical effects (bloating, flatulence, abdominal pain).[7] This variability is caused by many factors including the entity of contamination and species involved, the extension of the intestinal tract and the predisposing factors.

The gold standard for the diagnosis of SIBO was the jejunal aspiration and the culture of intestinal fluid, but it is invasive and expensive. Thus nowadays, the lactulose or the glucose breath tests are preferred, because they are not invasive, are less expensive and more tolerated. Lactulose breath test (LBT) had a higher sensitivity (68% vs 16.7%) but lower specificity (44% vs 70%) than the jejunal aspiration technique.[8] Furthermore, this test provides information about the OCTT.

The breath test is based on the concept that hydrogen generated in the intestine passes into the blood, then enters the lungs and is excreted in the breath. There is a strong association between the gas produced by bacterial fermentation and the hydrogen produced that is measured by a specific machine in parts per million (p.p.m.). The criteria for a positive SIBO were an increase in H_2 >20 p.p.m. by 90 min or 180 min. Moreover, the revised criteria applied by Pimentel for a normal test were 'no rise of breath hydrogen or methane concentration before 90 min of lactulose with a definitive rise never >20 p.p.m. during 180 min of measurement'.[8]

Around 15% of the general population has predominant methanogenic bacteria that metabolize hydrogen themselves that cannot be absorbed in enough quantities to be excreted via the lungs.

In this subset of patients, the simple execution of H_2 breath test cannot be enough to determine a possible sugar malabsorption or SIBO. The execution of a CH_4/H_2 breath test may avoid achieving false-negative patients. Gut bacteria that produce CH_4 are able to slow down small intestinal transit by nearly 70% in an *in vivo* model of transit.[9]

The association between CH_4 and constipation suggests that CH_4 has an active role in the development of constipation.[10,11]

Only a few papers investigate in children the role of the gut microbiota in the modulation of OCTT.

The aim of our study was to assess the prevalence of SIBO, CH_4 production and OCTT in children affected by MMC through LBT.

MATERIALS AND METHODS

Eighteen (6M/12F; 16.4 ± 7.6 years) children affected by MMC were enrolled at the Spina Bifida Pediatric Center of Gemelli Hospital, Catholic University, Rome. All children were of Italian origin and white.

Exclusion criteria were a history of severe cardiovascular disease, chronic renal failure, central neurological disease, diabetes, thyroid disease, inflammatory bowel disease, celiac disease, cirrhosis, connective tissue disease, and intestinal surgery.

At birth, all children were submitted within the first 24 h of life to neurosurgical intervention for neural tube defect repair.

We also excluded patients who take antibiotics, oral laxatives, probiotics or who underwent a colonoscopy within 1 month.

All patients were submitted to a H_2/CH_4 LBT to evaluate the OCTT and the presence of SIBO.

All parents gave written informed consent.

H_2/CH_4 LBT

Subjects were asked to have a carbohydrate-restricted dinner on the day before the test and fast for at least 12 h to minimize basal H_2 excretion.

Before the test, children performed a mouth wash with 20 ml chlorhexidine 0.05%. Physical exercise and food were not allowed for 30 min before and during the test. End-alveolar breath samples were collected immediately before lactulose ingestion (lactulose 10 g in 200 ml solution) and were taken every 15 min for 4 h with two-bag system, consisting of a mouthpiece, a T-valve and two collapsible bags; the first one collects alveolar air. Samples were analyzed immediately for H_2 and CH_4 with a Breath Tracker Quintron Gas Chromatograph (Quintron Instrument Company, Milwaukee, WI, USA). Results were expressed as p.p.m.

OCTT was defined as the time elapse between lactulose ingestion and a sustained increase in H_2 excretion of $\geqslant 10$ p.p.m. above the baseline value, which is about 90 ± 15 min in normal subjects. Therefore, OCTT was calculated on the basis of the colic peak of H_2 excretion.

Assessment and study outcomes

At enrollment all patients were invited to fulfill these criteria:

- A diary where they recorded the presence of abdominal symptoms (bloating, abdominal pain, flatulence), the severity of which was assessed by a visual analog scale, the score ranging from 0 (absent) to 10 (severe).[12]
- A diary of the frequency and consistency of the stool (according to the Bristol stool scale)[13] during the previous 7 days (Table 1).
- A nephro-urological clinical evaluation of the number of UTIs and urine examination with urine culture, indicating the type of germ involved and the type of treatment used.
- Questionnaire on the impact of intestinal problems on quality of life scores to determine the neurogenic bowel disease.

Statistical analysis

Statistical analysis was carried out with STATA 11 (College Station, TX, USA). Variables concerning H_2 excretion and clinical score were expressed as mean values $\pm$ s.d. Correlations were assessed using the Spearman's ρ test and Fisher's exact test. The calculated *P*-values that were <0.05 were considered statistically significant.

RESULTS

In the present study, we enrolled 18 children and their demographic characteristics are shown in Table 2. At baseline, the mean of bowels per week in all patients in the study was 2.8 (s.d.: $\pm$ 1.4). Eight children had encopresis (44%).

None of our children used laxatives, three patients used enemas and 11 underwent anorectal digital extraction. The number of patients affected with UTI was six with a mean UTI per year of 4.2 ± 1.6. The most common bacteria isolated from urine culture was *E. coli*.

For the prevention of UTI: five patients were given intravescical antibiotic prophylaxis, six patients were given a regular cycle of oral antibiotic prophylaxis with quinolones (not given in the last month) and seven patients were not given any antibiotic prophylaxis.

According to the Bristol stool scale (Table 1), four patients belonged to type 1 (separate hard lumps, like nuts), five patients to type 2 (sausage-shaped, but lumpy); four patients to type 3 (sausage but with cracks on its surface) and five patients to type 4 (smooth sausage or snake).

Table 1 The Bristol stool scale

Seven types
Type 1: separate hard lumps, like nuts (hard to pass)
Type 2: sausage-shaped, but lumpy
Type 3: like a sausage but with cracks on its surface
Type 4: like a sausage or snake, smooth and soft
Type 5: soft blobs with clear cut edges (passed easily)
Type 6: fluffy pieces with ragged edges, a mushy stool
Type 7: watery, no solid pieces. Entirely liquid

Table 2 The demographic characteristics of enrolled patients

Number of patients	18
Sex (male/female)	6/12
Age (years), mean (s.d.)	16.4 (7.6)
Mean weekly stool frequency (s.d.)	2.8 (1.4)
Encopresis, *n* (%)	8 (44)
Hard stool consistency (BSS1–2), *n* (%)	9 (50)
Manual extraction, *n* (%)	11 (61)
UTI, *n* (%)	6 (30)
Mean UTI/year (s.d.)	4.2 (1.6)

Abbreviations: BSS, Bristol stool scale; UTI, urinary tract infection.

The mean of visual analog scale for gastrointestinal symptoms at enrollment are abdominal pain: 2.5 ± 2.4; bloating: 3.8 ± 2.5 and flatulence: 2.9 ± 2.8.

With regard to neurogenic bowel disease score, 5 children had a very low score; 2 a low score, 10 a moderate score and just 1 had high score.

LBT

Seven out of eighteen (39%) children were SIBO positive. Sixty-one percent (11/18) children presented a delayed, 33% (6/18) a normal and just one patient had an accelerated OCTT.

Eight out of eighteen (44.4%) children produced high levels of CH_4 with a mean peak of 25 ± 13 p.p.m. Interestingly, all SB children who produced high levels of CH_4 showed a delayed OCTT.

All the results are reported in Figure 1.

Clinical symptoms

Patients with SIBO showed a higher visual analog scale score of abdominal pain (3.6 ± 0.9 vs 1.8 ± 1.1; $P = 0.07$), bloating (4.3 ± 1.3 vs 3.4 ± 1.1; $P =$ ns) and flatulence (3.4 ± 1.2 vs 2.5 ± 0.8; $P =$ ns) compared with those without SIBO (Figure 2).

A higher prevalence of UTI was observed in methane producers (4/8 patients) compared with non-methane producers (2/10 patients; 50% vs 20%, respectively; Figure 3).

Methane producers showed a higher consistency of stool (according to Bristol stool scale) compared with non-methane producers (2.1 ± 1.2 vs 2.9 ± 1.1; $P =$ ns).

Conversely, SIBO-positive children showed a lower consistency of stool compared with negative ones without reaching a statistical difference (2.7 ± 0.9 vs 2.4 ± 1.2; $P =$ ns).

DISCUSSION

Our study showed for the first time that in the 18 patients affected by MMC, 40% had a SIBO and 60% had a delayed OCTT.

The more interesting data are that around 50% of our children had methanogenic flora, which produced large amounts of CH_4 associated with a delayed OCTT and higher prevalence of UTI.

Patients affected by MMC have an altered intestinal motility secondary to neurological damage resulting in severe constipation. This study through LBT confirms that a large percentage of our constipated children showed a delayed OCTT.

It is well known that pathologies associated with an alteration of intestinal motility, such as diabetes and progressive systemic sclerosis, are associated with a higher prevalence of SIBO.[14] SIBO is a clinical condition characterized by a rising of colonic bacteria along the small intestine. Acid barrier, mucosal and systemic immunity, and intestinal clearance are some of the main mechanisms restricting bacterial colonization in the upper gut. If one of these mechanisms fail, SIBO occurs.

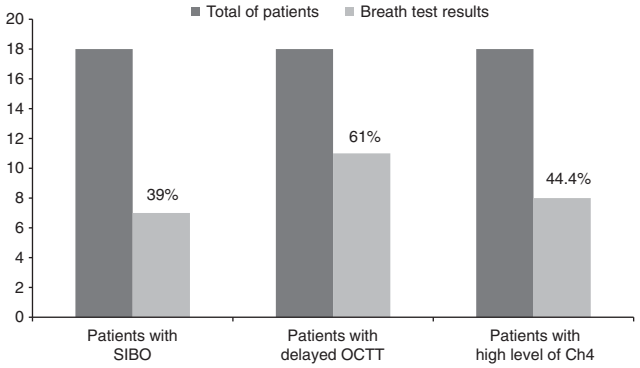

Figure 1 Results of breath test.

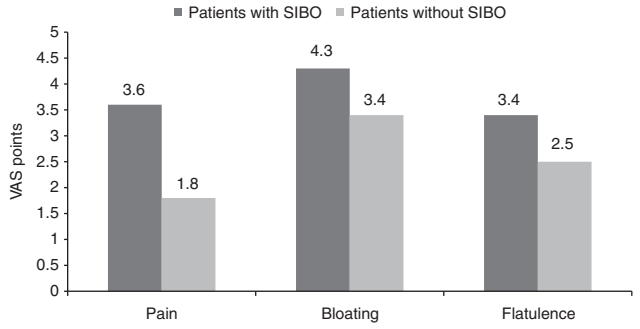

Figure 2 Prevalence of pain, bloating and flatulence in patients with SIBO vs without SIBO.

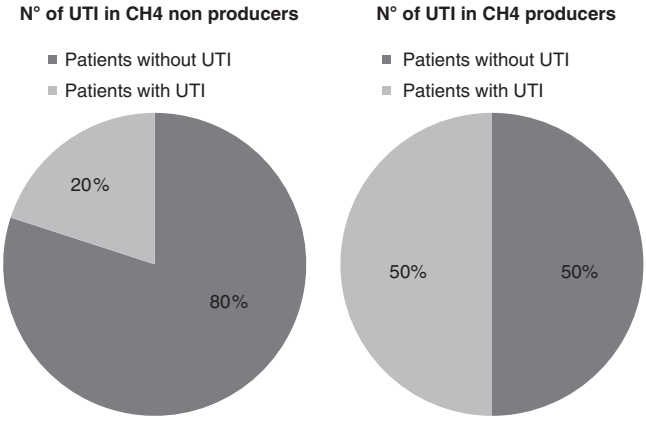

Figure 3 Number of UTIs in CH_4 non-producers vs CH_4 producers.

A recent paper showed that children with intestinal failure are at a risk of developing SIBO because of anatomical and other factors, but larger studies are needed to better determine the relationship between these conditions.[15]

In our study, we confirm that children with MMC have a higher prevalence of SIBO compared with those observed in the control group of the literature.[16] Moreover, these children, as expected, showed a higher visual analog scale score of abdominal pain, bloating and flatulence compared with those without SIBO.

We can hypothesize that in children with MMC, the alteration of intestinal innervation is responsible for the slowing down of the

orocecal transit and that this can lead, as shown by other studies, to SIBO.

Recent studies have demonstrated that CH_4, produced by methanogenic flora, was able to slow down small intestinal transit.[17]

The more interesting data are that half of the children analyzed excrete CH_4 through a breath test. CH_4 production has been linked to some diseases such as chronic constipation, predominant constipated irritable bowel syndrome, diverticulosis and colon cancer.[18]

Among intestinal microflora, *Methanobrevibacter smithii*, which uses H_2 to reduce CO_2 to CH_4, is responsible for almost all CH_4 produced in the intestine.[9] There is evidence now to suggest that methanogenic organisms may actively participate in the control of intestinal motor function. Previous studies have shown that patients affected by constipation-type irritable bowel syndrome produce CH_4, whereas irritable bowel syndrome patients with diarrhea have a higher excretion of hydrogen in breath samples.[19]

One of the possible mechanism hypotheses of slow colonic transit time because of CH_4 production might be related to abnormality in intestinal motility or a reduction in serotonin production.

Even in young patients, the colonic transit time is significantly more prolonged in constipated children who produce CH_4.[20]

On the other hand, the presence of methanogenic flora associated with a slowing down of transit time can be a cause or consequence of deterioration in the constipation of these children.

Our study, in fact, demonstrated that methane producers have a delayed OCTT and high predisposition to UTI.

Previous studies have shown that recurrent UTI were found in a significant number of children who had functional constipation with encopresis. With successful treatment of the constipation, most of them prevented UTI. It has been suggested that the distended rectum in a constipated child presses on the bladder wall and produces a bladder outflow obstruction that may cause detrusor instability.[1–3]

Another pathogenetic hypothesis is that constipation can determine bacterial stasis with huge proliferation and translocation of the same bacteria in the genitourinary system. To validate this concept, some studies have also shown that treatment with probiotics, restoring a normal balance of bacterial flora, prevents vaginal infections.

One of the most interesting features of our study is to identify a correlation between CH_4 production, delayed OCTT and UTI. The CH_4 intestinal decontamination with locally acting drugs without systemic effects in these children may, in fact, reduce the number of UTIs and improve intestinal motility.

Considering the limitations of our study (a modest sample size, the poor sensitivity/specificity of LBT), further analyses with a larger cohort of patients are necessary to confirm and give clinical relevance to our data.

DATA ARCHIVING

There were no data to deposit.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

1 Venkataramana NK. Spinal dysraphism. *J Pediatr Neurosci* 2011; **6**(Suppl 1): S31–S40.
2 Frey L, Hauser WA. Epidemiology of neural tube defects. *Epilepsia* 2003; **44**(Suppl 3): 4–13.
3 Northrup H, Volcik KA. Spina bifida and other neural tube defects. *Curr Probl Pediatr* 2000; **30**: 313–332.
4 Rendeli C, Ausili E, Tabacco F, Focarelli B, Pantanella A, Di Rocco C *et al.* Polyethylene glycol 4000 vs lactulose for the treatment of neurogenic constipation in myelomeningocele children: a randomized-controlled clinical trial. *Aliment Pharmacol Ther* 2006; **23**: 1259–1265.
5 Ausili E, Focarelli B, Tabacco F, Murolo D, Sigismondi M, Gasbarrini A *et al.* Transanal irrigation in myelomeningocele children: an alternative, safe and valid approach for neurogenic constipation. *Spinal Cord* 2010; **48**: 560–565.
6 Romańczuk W, Korczawski R. Chronic constipation: a cause of recurrent urinary tract infections. *Turk J Pediatr* 1993; **35**: 181–188.
7 Bures J, Cyrany J, Kohoutova D, Förstl M, Rejchrt S, Kvetina J *et al.* Small intestinal bacterial overgrowth syndrome. *World J Gastroenterol* 2010; **16**: 2978–2990.
8 Pimentel M, Chow EJ, Lin HC. Normalization of lactulose breath testing correlates with symptom improvement in irritable bowel syndrome. a double-blind, randomized, placebo-controlled study. *Am J Gastroenterol* 2003; **98**: 412–419.
9 Kim G, Deepinder F, Morales W, Hwang L, Weitsman S, Chang C *et al. Methanobrevibacter smithii* is the predominant methanogen in patients with constipation-predominant IBS and methane on breath. *Dig Dis Sci* 2012; **57**: 3213–3218.
10 Pimentel M, Lin HC, Enayati P, van den Burg B, Lee HR, Chen JH *et al.* Methane, a gas produced by enteric bacteria, slows intestinal transit and augments small intestinal contractile activity. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G1089–G1095.
11 Kunkel D, Basseri RJ, Makhani MD, Chong K, Chang C, Pimentel M. Methane on breath testing is associated with constipation: a systematic review and meta-analysis. *Dig Dis Sci* 2011; **56**: 1612–1618.
12 Ojetti V, Pitocco D, Scarpellini E, Zaccardi F, Scaldaferri F, Gigante G *et al.* Small bowel bacterial overgrowth and type 1 diabetes. *Eur Rev Med Pharmacol Sci* 2009; **13**: 419–423.
13 Koh H, Lee MJ, Kim MJ, Shin JI, Chung KS. Simple diagnostic approach to childhood fecal retention using the Leech score and Bristol stool form scale in medical practice. *J Gastroenterol Hepatol* 2010; **25**: 334–338.
14 Leiby A, Mehta D, Gopalareddy V, Jackson-Walker S, Horvath K. Bacterial overgrowth and methane production in children with encopresis. *J Pediatr* 2010; **156**: 766–770.
15 Awad RA. Neurogenic bowel dysfunction in patients with spinal cord injury, myelomeningocele, multiple sclerosis and Parkinson's disease. *World J Gastroenterol* 2011; **17**: 5035–5048.
16 Scarpellini E, Giorgio V, Gabrielli M, Lauritano EC, Pantanella A, Fundarò C *et al.* Prevalence of small intestinal bacterial overgrowth in children with irritable bowel syndrome: a case-control study. *J Pediatr* 2009; **155**: 416–420.
17 Lee KM, Paik CN, Chung WC, Yang JM, Choi MG. Breath methane positivity is more common and higher in patients with objectively proven delayed transit constipation. *Eur J Gastroenterol Hepatol* 2013; **25**: 726–732.
18 Roccarina D, Lauritano EC, Gabrielli M, Franceschi F, Ojetti V, Gasbarrini A. The role of methane in intestinal diseases. *Am J Gastroenterol* 2010; **105**: 1250–1256.
19 Ghoshal UC, Kumar S, Mehrotra M, Lakshmi C, Misra A. Frequency of small intestinal bacterial overgrowth in patients with irritable bowel syndrome and chronic non-specific diarrhea. *J Neurogastroenterol Motil* 2010; **16**: 40–46.
20 Soares AC, Lederman HM, Fagundes-Neto U, de Morais MB. Breath methane associated with slow colonic transit time in children with chronic constipation. *J Clin Gastroenterol* 2005; **39**: 512–515.