What tests should you use to assess small intestinal bacterial overgrowth in systemic sclerosis?

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ABSTRACT

Objective. *Small intestinal bacterial overgrowth (SIBO) plays a major role in the pathogenesis of malabsorption in SSc patients and is a source of great morbidity and even mortality, in those patients. This manuscript reviews which tests are valid and should be used in SSc when evaluating SIBO.*

Methods. *We performed systematic literature searches in PubMed, Embase and the Cochrane library from 1966 up to November 2014 for English language, published articles examining bacterial overgrowth in SSc (e.g. malabsorption tests, breath tests, xylose test, etc). Articles obtained from these searches were reviewed for additional references. The validity of the tests was evaluated according to the OMERACT principles of truth, discrimination and feasibility.*

Results. *From a total of 65 titles, 22 articles were reviewed and 20 were ultimately extracted to examine the validity of tests for GI morphology, bacterial overgrowth and malabsorption in SSc. Only 1 test (hydrogen and methane breath tests) is fully validated. Four tests are partially validated, including jejunal cultures, xylose, lactulose tests, and 72 hours fecal fat test.*

Conclusion. *Only 1 of a total of 5 GI tests of bacterial overgrowth (see above) is fully validated in SSc. For clinical trials, fully validated tests are preferred, although some investigators use partially validated tests (4 tests). Further validation of GI tests in SSc is needed.*

Introduction

Involvement of the gastrointestinal tract (GIT) in SSc is extremely frequent; it is a leading cause of morbidity and the third most common cause of mortality in this disease. Oesophageal abnormalities occur in up to 90% of patients, stomach involvement can be documented in 50% or more of patients and small bowel, colonic and anorectal involvement occur in 50–70% of SSc patients (1-3).

The pathogenesis of GIT involvement is thought to include early vascular damage to the vasa nervorum of the nerves innervating the GIT. This leads to neurological dysfunction, particularly involving autonomic pathways (4-5). The activation of the immune system may contribute to neurological dysfunction by production of antibodies which specifically inhibit M3-muscarinic receptor-mediated enteric cholinergic neurotransmission (6). With damage to innervation, smooth muscle atrophies and is eventually replaced by fibrotic tissue which further leads to motility disorders of the GIT. At present, there is no single validated, objective, specific test to assess the global involvement of the GI tract although the GIT2.0, a patient reported outcome measure has been validated (7).

Hypomotility of the small bowel may cause intestinal pseudo-obstruction and small intestinal bacterial overgrowth (SIBO). SIBO is one of the main pathogenetic factors of malabsorption which is associated with 50% mortality over 8.5 years, in SSc patients (8). Diagnosis of SIBO is problematic. Quantitative culture of small bowel contents is considered the gold standard, but the lack of standardisation of the normal bowel flora in the small intestine limits its accuracy. A variety of indirect tests, including breath tests and biochemical tests based on bacterial metabolism of a variety of substrates, have been used over the years in an attempt to facilitate the diagnosis of SIBO. Our aim is to review the procedures used to evaluate SIBO in SSc patients and to assess their validity.

Methods

We performed systematic literature searches in PubMed, Embase and the Cochrane library from 1966 through November 2014 for English language, published articles examining bacterial overgrowth and malabsorption in SSc.

The keywords used were *systemic sclerosis* and *scleroderma* and they were combined with text words such as small bowel, bacterial overgrowth, malabsorption, breath tests, small bowel cultures, xylose test, lactulose tests, orocecal transit test, hydrogen, glucose, gut. Case reports, case series with less than 8 patients, reviews, articles with non-separable data for SSc patients, duplicates and articles in languages other than English were excluded. The titles and abstracts obtained were screened for inclusion by both a rheumatologist and a gastroenterologist. Titles and abstracts which included at least one validity criterion were selected and the data were extracted from the complete articles. Articles obtained from these searches were reviewed for additional references (Fig. 1). Reviews were not included but their reference lists were examined for additional articles.

The validities of the tests were evaluated as to whether they conformed to OMERACT (Outcome Measures in Rheumatologic Clinical Trials) principles (truth, discrimination and feasibility). The principles used to assess the truth, discrimination and feasibility of measures, which are accepted worldwide, are described in Table I.

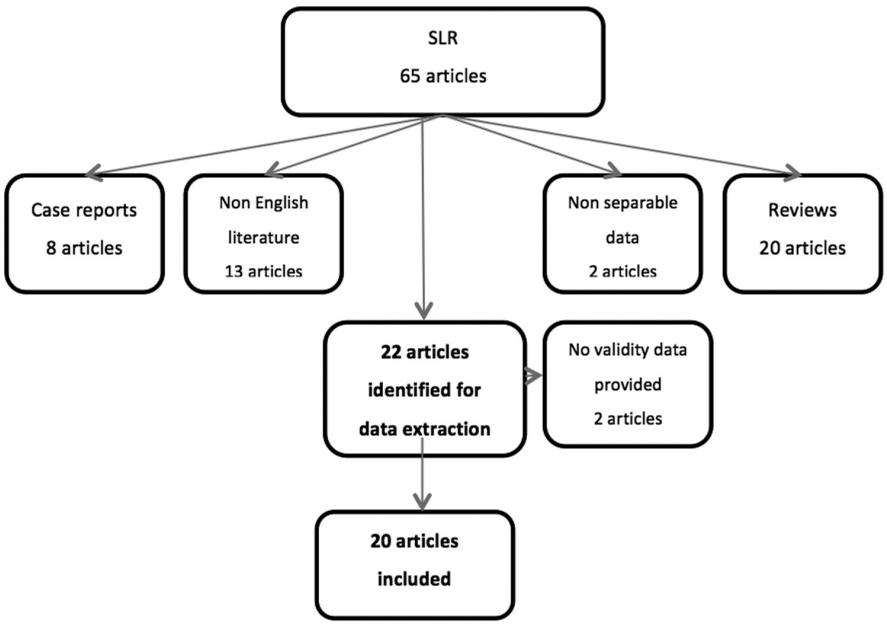

Fig. 1. Medline search of publications on the use of the diverse procedures for small intestinal bacterial overgrowth assessment in systemic sclerosis.

Table I. Omeract principles.

Face validity	apparent but untested statistical validity (whether a test measures what it was meant to measure, in the subjective opinion of the scientist);
Content validity	the items on the test represent the entire range of possible items the test should cover;
Construct validity	the extent to which operationalisations of a construct (*e.g.* practical tests developed from a theory) do actually measure what the theory says they do;
Criterion validity	the correlation between the test and a criterion variable (or variables) taken as representative of the construct. It compares the test with other measures or outcomes (the criteria) already held to be valid;
Convergent validity	the degree to which a measure is correlated with other measures that it is theoretically predicted to correlate with;
Reproducibility	requires stability when a measure is done repeatedly;
Sensitivity to change	requires that when an effective drug is used, the measurement changes by a statistically or clinically important amount;
Feasibility	requires that a measure should be easy to perform, requires little time and requires minimal amount of equipment.

Results

Validated or partially validated tests (Table II)

1. Jejunal culture

Jejunal cultures are only partially validated in SSc.

Three out 4 articles met inclusion/exclusion criteria and were extracted. The gold standard for diagnosis of bacterial overgrowth has been quantitative culture of an aspirate of luminal fluid from the jejunum. Growth of 10 million or more organisms/ml in either aerobic or anaerobic conditions is the criterion for a positive culture. Problems with use of jejunal cultures as a test for bacterial overgrowth include lack of standardisation of the collection method, the requirement for intubation of the upper GI tract and their relative high cost.

Truth

Face validity: the test meets the criteria for face validity in SSc. In a few studies, the prevalence of small intestinal bacterial overgrowth (SIBO) was 30–62.5% in SSc patients exhibiting gastrointestinal symptoms; in these series, SIBO was defined as microbial concentration (>10^5 CFU/ml) in the jejunal aspirate culture (9-11).

Content validity: the procedure was used in SSc pts with limited and diffuse disease and various disease duration (9, 10), thus meeting the criteria for content validity.

Construct validity: a study performed in 24 SSc patients with symptoms of malabsorption and in 9 normal controls, found significant bacterial counts: >10(5) colony forming units per ml (cfu/ml) of jejunal fluid in 8 patients (33%) compared to none in the controls. Seven out of these 8 patients had also a positive breath test (10). By implication, although no statistics were done, this study meets convergent construct validity.

Criterion validity: **c**riterion validity for bacterial overgrowth were met. A study performed in 20 unselected SSc patients and 18 controls found positive jejunal cultures (10^6 organisms/ml) in 7 SSc patients compared to none in

Table II. Validation of procedures used in systemic sclerosis (SSc) to assess small intestinal bacterial overgrowth.

	Small bowel absorption tests									
	Face	Content	Construct	Criterion	Reliability reproducability	Sens. to change	Feasibility	Assessment of.	Used in SSc	Validated in SSc
Jejunal cultures	Yes	Yes	Yes	Yes	Yes	Na	±	Bacterial overgrowth	Yes	partially
Hydrogen and methane breath tests	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Bacterial overgrowth	Yes	Yes
D-xylose test	Yes	Yes	No	NA	Yes	No	±	Bacterial overgrowth	Yes	partially
Lactulose test	Yes	Yes	No	NA	±	Yes	Yes	permeability	Yes	partially
72-hour fecal fat test	Yes	Yes	No	NA	±	Yes	No	absorption	Yes	partially

the controls. The difference in bacterial counts between the two groups was statistically significant (9).

Sensitivity to change/discrimination. There are no studies in SSc assessing sensitivity to change or discrimination .

Reliability and feasibility

The presence of greater than one million organisms/ml in either aerobic or anaerobic conditions is conventionally regarded as the criteria for a positive culture. However, bacterial overgrowth, particularly due to coliforms and enterococci, may occur in apparently healthy individuals with no evidence of malabsorption (12, 13) and so the clinical relevance of such a positive result may be difficult to determine. Although anaerobic organisms are primarily associated with malabsorptive syndromes, isolation and categorisation of bacterial anaerobes are not routinely performed in many laboratories. The lack of standardisation of bacterial counts, the possibility of sampling errors, and the need for intubation have a negative impact on the reliability and feasibility of the procedure.

Jejunal cultures are only partially validated in SSc with poor feasibility and no data documenting sensitivity to change or discrimination.

2. Breath tests

Various breath tests have been proposed as non-invasive tests for small intestinal bacterial overgrowth (SIBO). Bile acids breath tests have reasonable content validity *versus* bacterial counts from the jejunum (14). "Because this test has poor sensitivity and specifity, it is not used frequently and will not be reviewed (15, 16).

A. Hydrogen and methane breath tests

These are currently the most used diagnostic methods. Hydrogen breath tests reflect hydrogen which is produced by anaerobic bacteria in the small bowel, is absorbed and then exhaled. It requires 3 hours and is non-invasive. It is done after administering a carbohydrate substrate (glucose, lactulose or xylose) orally and a sequential end-expiratory breath is taken every 15 minutes for 3 hours. Sensitivity and specificity vary widely, in this case from 62% to 93% and from 78% to 100%, respectively (15, 17-19). The procedure as well as the optimal substrate to use are quite well standardised, although the criteria for considering a breath test as SIBO-positive or -negative are not fully established, hence the problems associated to the presence of false positive and false negative tests. (20, 28).

The glucose hydrogen and methane test is validated in SS.

Five out of five papers were extracted.

Truth

Face validity: glucose hydrogen and methane breath tests meet the criteria for face validity (see above). Hydrogen breath tests were found to be positive in SSc patients with symptoms of SIBO (9, 10).

Content validity: the procedure was used in SSc pts with limited and diffuse disease and various disease duration (9, 10, 21-24), thus meeting the criteria for content validity.

Construct validity: a study performed in 51 consecutive SSc patients assessed the prevalence of SIBO (21): all patients underwent glucose hydrogen and methane (H2/CH4) breath test, answered questionnaires about intestinal symptoms, underwent oesophageal manometry, gastroscopy and biochemical tests (ESR, haemoglobin level, total serum protein and albumin, ferritin, B12 and folic acid). Twenty-two patients (43.1%) had positive breath tests. Significant correlations were found between positive breath tests and diarrhoea (p=0.0034), abdominal pain (p=0.0001), constipation (p=0.00001), bloating (p=0.0246), GSS>5 (p=10^6), Scleroderma health assessment score (p=0.0086), ESR (p=0.003), haemoglobin (p=0.002), serum protein and albumin (p=0.066 and p=0.024 respectively), severe abnormal oesophageal motility disorder (p=0.046). Eleven out of 22 patients with SIBO underwent small bowel manometry. The manometry was abnormal in all of them (21). This test, therefore, meets construct validity criteria.

Criterion validity: a study performed in 24 SSc patients with symptoms of malabsorption and in 9 normal controls, found positive breath tests in 7 out of 8 patients with significant bacterial counts: >10(5) colony forming units per ml (cfu/ml) of jejunal fluid compared to none in the controls (10). This test meets requirements for criterion validity.

Sensitivity to change/discrimination: Sensitivity to change was assessed by administrating octreotide in SSc pts with bacterial overgrowth and in normal controls. Hydrogen breath test was

performed before and after 3 weeks treatment, Substantial symptomatic and hydrogen breath excretion rate improvement was observed (23).
Two other studies assessed efficacy of antibiotic treatment in patients with SIBO by performing glucose hydrogen and methane (H2/CH4) breath test before and 1-3 and 6 months after treatment: normalisation of the tests was achieved in 52.4% and in 42.8% of the patients, respectively (21, 24).
Both sensitivity to change and discrimination criteria were met.

Reliability
The main limitations are lack of standardisation, false negative results and inability to evaluate bacterial overgrowth-related antibiotic sensitivity/ resistance (18).

Feasibility
Feasibility is reasonable.
Glucose hydrogen and methane breath test is validated in SSc.

B. The D-xylose test
The D-xylose test is not validated and meets very few criteria in SSc.
The 14C D-xylose breath test depends on the capacity of the intestinal bacteria to release 14C CO_2 which is absorbed and ultimately eliminated in the breath where it can be quantified. Radioactive 14C or the stable isotope 13C can be used to label 1 g of xylose. The sensitivity of 14C d-xylose test ranges from 14.3% to 95%, and specificity from 40% to 94% (25, 26).
Four out of 5 papers answered the criteria.

Truth
Face validity: the D-xylose test meets the criteria for face validity. The test correlates with fecal fat and jejunal flora and was found to be abnormal in 13% of SSc patients (27, 28).
Content validity: the procedure was used in SSc pts with limited and diffuse disease and various disease duration (11, 27, 28), thus meeting the criteria for content validity.
Construct validity: in a study aimed to assess the passive permeability of the small bowel, 17 SSc patients underwent cellobiose/mannitol test, jejunal biopsies and xylose test. The passive permeability of the small bowel was normal in all patients, although, seven patients had a low xylose test result. No correlation was found between impaired xylose test and pathologic biopsies of jejunal mucosa in 17 consecutive SSc pts. The authors' conclusions were that passive intestinal permeability is unaltered in systemic sclerosis, and that malabsorption, when it occurs, is caused by other factors (11). Construct validity criteria have not been met for this test.
Criterion validity: there are no studies in SSc patients evaluating criterion validity.
Sensitivity to change/discrimination: There are no studies in SSc patients evaluating sensitivity to change or discrimination.

Reliability
The test it is not commonly performed in clinical practice because it is not widely available and has been largely replaced by hydrogen breath tests which are considered to be more accurate (29). In addition, disorders associated with impaired gastric emptying may lead to false negative results, while rapid intestinal emptying may lead to false-positive results (29, 30).

Feasibility
The feasibility is relatively low.
The D-xylose test is essentially unvalidated in SSC as it does not meet construct, criterion validity, is not reliable, is not discriminatory and has low feasibility.

C. Lactulose breath test
The lactulose breath test is only partially validated in SSc.
The lactulose hydrogen breath test (LHBT) is the most widely used hydrogen breath test. LHBT is a non-invasive, inexpensive, and well-tolerated technique, able to assess oro-cecal transit time (OCTT) with accuracy (31-33) and to detect SIBO (31, 32). The intestinal flora ferment the lactulose, resulting in the production of hydrogen and/or methane. The presence of bacterial overgrowth in the small bowel and cecal flora are detectable. Most investigators accept that the first rise in H2 be within 90 minutes of lactulose ingestion (32), although in some patients, an early rise in H2 greater than 20 ppm above the baseline can be considered as a measurement of OCTT rather than SIBO (34) There are few data with respect to this test for detection of SIBO in SSc (28, 32, 33, 35).

Truth
Face validity: Lactulose breath test meets the criteria for face validity. Lactulose test was positive for SIBO in 46% of a cohort of 99 SSc pts evaluated for GI involvement (36).
Content validity: the test was performed in limited and diffuse SSc patients with various disease duration (32, 33, 35, 36), thus meeting the criteria for content validity.
Construct validity: although differences in bacterial overgrowth were found between 55 SSc patients and 60 matched controls, construct validity was not tested (33). No other studies have examined construct validity in SSc. Construct validation has not been achieved for this test.
Criterion validity: there are no studies assessing criterion validity in SSc.
Sensitivity to change/discrimination: Sensitivity to change of lactulose test was assessed in a trial performed on 54 SSc pts and 60 matched healthy controls (33). Oral cecal transit time and the presence of SIBO were assessed by a lactulose breath test. Patients with SIBO were treated with rifaximin 1,200 mg/day for 10 days. A second lactulose test was performed 1 month after the end of therapy. SIBO was found in 30\54 SSc pts compared to 4\60 controls. Eradication of SIBO was achieved in 73.3% of patients, with a significant reduction of symptoms in 72.7% of them (33). Sensitivty to change and discrimination has been documented.

Reliability
The criteria for a positive breath test are problematic and not well validated. The reliability of the test was not validated in SSc. Data extrapolated from other conditions suggest a negative impact of

diabetes mellitus, hypochlorhydria on the reliability of the test (37, 38).

Feasibility
The feasibility is reasonable.
The lactulose breath test is partially validated in SSc as neither criterion validitiy nor construct validity have been found. Reliability is not well documented.

3. The 72-hour fecal fat test
This test is partially validated in SSc.
Quantitative fecal fat tests measure and report the amount of fecal fat which is collected over a period of three days. The fat content is extracted with solvents and measured by saponification. Normally up to 7 grams of fat are found when subjects ingest 100 grams of fat per day. In patients with malabsorption the amount of fat excreted is markedly increased. Two articles met inclusion/exclusion criteria and were extracted.

Truth
Face validity: the 72-hour fecal fat test meets the criteria for face validity. The test was performed in SSc patients on a 100 g fat diet and revealed a 100% abnormality among the patients with x-ray abnormalities (32).
Content validity: the test was performed in SSc patients with diffuse and limited disease and various disease duration (32, 39), thus meeting the criteria for content validity.
Construct validity: there are no studies assessing construct validity in SSc.
Criterion validity: the test is the gold standard to diagnose steatorrhoea, thus by definition meets criterion validity.
Sensitivity to change/discrimination. Sensitivity to change was assessed in SSc patients with malabsorption and bacterial overgrowth. Treatment with antibiotics significantly reduced the amount of fecal fat in these patients (39). Both sensitivity to change and discrimination criteria are met.

Reliability
Reliability has not been tested.

Feasibility
The test is time consuming and logistically difficult as it requires three day stool collection and a complete dietary intake record. Additionally, some patients with fat malabsorption have diarrhoea and therefore accurate and complete collection is difficult.
The 72-hour fecal fat test is partially validated in SSc for face, content, criterion validity and sensitivity to change, but construct validity and reliability have not been examined and feasibility is low.

Discussion

The prevalence of SIBO in SSC has been reported to be 30-62% (9, 10, 20, 22, 35). SIBO is associated with a greater prevalence of diarrhoea, abdominal pain and gas-related symptoms (bloating and abdominal tenderness) and has a significant impact upon the patients' quality of life and prognosis (21). SIBO plays a major role in the pathogenesis of malabsorption in SSc patients. However SIBO assessment in SSC is not standardised. A standardised approach should lay ground to assess and compare the beneficial effect of therapeutic interventions in SSC clinical studies.
The present review is the first study to carefully examine the validation of the procedures for the assessment of SIBO in SSc, according to the OMERACT criteria.
Only one out of the five tests examined, is fully validated in SSc-glucose hydrogen and methane breath tests. The test is non- invasive, feasible, but the main limitations are lack of standardisation, false negative results and inability to evaluate bacterial overgrowth-related antibiotic sensitivity/resistance. Jejunal cultures are only partially validated in SSc with poor feasibility and no data documenting sensitivity to change or discrimination. The lactulose breath test is partially validated in SSc as neither criterion validity nor construct validity have been found. The 72-hour fecal fat test is partially validated in SSc for face, content, criterion validity and sensitivity to change, but construct validity and reliability have not been examined and feasibility is low. The D-xylose test is essentially unvalidated in SSC as it does not meet construct, criterion validity, is not reliable, is not discriminatory and has low feasibility.
The paucity of high quality studies regarding GI involvement and use of new technologies in SSC patients imposes serious limitations upon the ability to do the high quality trials needed to develop new therapies.
There is need for more, well –planned studies and new, more accurate tests to assess SIBO in SSc.
In summary, we have outlined the present available modalities for SIBO assessment in scleroderma and described the formal validity of those tests.
There is a need for more validation in SSc for most of the tests. We hope the updated data presented will serve the rheumatologic community to plan the future studies needed to improve the understanding the full extent of GI involvement in SSC and the future therapies.

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