

GASTROENTEROLOGY

Alternative investigations for irritable bowel syndromeHamish Philpott^{*,†} Sanjay Nandurkar^{*,†} John Lubel^{*,†} and Peter R Gibson^{*,†}^{*}Eastern Health Clinical School, Monash University, and [†]Department of Gastroenterology and Hepatology, Eastern Health, Box Hill, Melbourne, Victoria, Australia**Key words**

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Hamish.philpott@monash.edu**Abstract****Background and Aim:** Alternative and complementary medical practitioners have long advocated alternative treatments for irritable bowel syndrome. A more recent development has been the use of alternative investigations by these practitioners and, in the era of internet advertising, directly by patients themselves. The aim of the present study was to examine the alternative investigations that are advocated for the assessment of gastrointestinal disease and that are available through mainstream laboratories in Australia.**Methods:** A comprehensive literature review was undertaken for each investigation, which was then evaluated on the basis of ACCE criteria for diagnostic tests. The ACCE criteria consider the analytical and clinical validity, clinical utility and ethical implications of the test.**Results:** Serum immunoglobulin G (IgG) to food antigens, salivary IgA, intestinal permeability, fecal short-chain fatty acids and fecal microbial analysis were identified as readily available. None of the investigations satisfied the ACCE criteria. The tests were deficient in one or more areas of analytical validity, clinical application, validity and ethical usage standards.**Conclusion:** Alternative investigations lack reliability and direct clinical applications, and should not be recommended for the investigation of gastrointestinal symptoms.**Introduction**

Patients with irritable bowel syndrome frequently visit alternative medicine practitioners.¹ Aside from providing a variety of treatments, such as diet, phytotherapy and acupuncture, practitioners may offer their patients a range of alternative investigations (AIs). For the purpose of this review, AIs are defined as commercially available investigations that are marketed and provided by mainstream conventional diagnostic laboratories, and are used to support alternative medicine disease models. Many of these investigations appear to have their basis in medical research laboratories, but because of a lack of broader application or diagnostic utility, have subsequently been offered in the alternative arena. Both the popularity and availability of AIs appears to be increasing and, in many countries, individuals themselves may request these investigations directly via the internet. It is difficult to estimate the number of tests actually performed, although personal communication with the sales team of one major provider suggests that the number is "easily in the tens of thousands annually", and many of the tests cost between AU\$50 and AU\$200 (Genova Diagnostics NC, personal communication, 2011).

Given the expense and growing popularity of AIs, gastroenterologists and other physicians should be informed about and hence be capable of discussing these issues with their patients.

Methods

Investigations were identified according to current ready availability in Australia. A comprehensive literature review was undertaken for each investigation, using the online medical databases Medline, Pubmed and Embase. The tests were then analyzed using the theoretical framework for evaluating diagnostic tests as detailed in Table 1, (namely analytical validity, clinical validity, clinical utility and ethical implications^{2,3}). As the ethical implications for all of the investigations are similar, a general comment is made at the conclusion of the document.

Serum IgG antibodies to food antigens

Rationale and clinical validity. Immunoglobulin G (IgG) antibodies to food antigens may be detected in the serum of normal healthy individuals as well as patients with irritable bowel syndrome (IBS).⁴ Some groups have demonstrated elevated levels of IgG in patients with IBS suggesting that this may represent an "allergic" response to food.⁵ The pivotal randomized controlled trial used in support of this approach demonstrated benefit in using a diet excluding foods to which the patient had measurable circulating IgG antibodies over 12 weeks.⁶ The main problem with this study was that the differences in symptoms were only observed in

Table 1 Criteria used to assess alternative gastrointestinal investigations: (a) the ACCE model adopted from Haddow *et al.*² and (b) questions to ask on how to read a paper that report diagnostic or screening tests adopted from Greenhalgh³

(a) ACCE model for evaluation of a diagnostic test	Analytical validity: <i>the accuracy and precision by which a particular biomarker is identified by a test.</i> Clinical validity: <i>the accuracy with which a test predicts identifies or predicts a patient's clinical status.</i> Clinical utility: <i>an assessment of the risks and benefits, such as cost or patient outcome.</i> Ethical, legal or social implications resulting from using the test
(b) Does the paper validate the test?	<i>Question 1.</i> Is this test potentially relevant to my practice? <i>Question 2.</i> Has the test been compared with a true gold standard? <i>Question 3.</i> Did this validation study include an appropriate spectrum of subjects? <i>Question 4.</i> Has workup bias been avoided? <i>Question 5.</i> Has expectation bias been avoided? <i>Question 6.</i> Was the test shown to be reproducible? <i>Question 7.</i> What features of the test are as derived from the validation study? <i>Question 8.</i> Were the confidence intervals given? <i>Question 9.</i> Has a sensible "normal range" been defined? <i>Question 10.</i> Has this test been placed in the context of other potential tests in the diagnostic sequence?

a minority of patients who were deemed adherent to the exclusion diet. On an intention-to-treat basis, there were no differences between responses in the exclusion and sham diet groups. A second, smaller study also reported similar beneficial results but again did not correlate levels of IgG with symptom severity.⁵

The American Academy of Allergy, Asthma and Clinical Immunology (AAAAI) and the European Academy of Allergy, Asthma and Immunology (EAAACI) have published position statements that highlight the limitations of IgG antibody testing to food antigens,⁴ and conclude that the presence of IgG antibodies to food antigens may represent a normal physiological response to food ingestion.

Analytical validity. Irritable bowel syndrome is a diagnosis based on symptoms and hence there are no comparable "gold standard" tests to food antigen-specific IgG. The test itself is an enzyme-linked immunoassay (ELISA) and several different companies produce assays. A comparison of three different assays across several different North American laboratories found wide variations in the readings across both assays and laboratories⁷

Clinical utility. As the measurement of specific IgG in serum may be unreliable and the level of evidence of the efficacy of

exclusion diets is limited, treatment based on "abnormally" high antibodies cannot be recommended.

Salivary IgA

Rationale and clinical validity. Immunoglobulin A (IgA) is the major antibody isotype produced in gut-associated lymphoid tissue (GALT) and is the most plentiful antibody found in saliva and intestinal secretions. IgA is thought to possess properties of both a neutralizing agent (binding and immune exclusion) and of a mucosal immunopotentiator inducing effector immune responses against invading pathogens.⁸ Given the role of IgA in modulating gastrointestinal immunity, the measurement of salivary IgA is theoretically an appealing test. Patients with IBS are no more likely than healthy controls to have measurable abnormalities in their humoral immune function according to antibody assays of the serum or luminal aspirates.^{9,10} No studies have been performed to examine the relationship between IBS and salivary IgA levels.

Analytical validity. Salivary IgA may vary acutely with stress and with exercise,^{11,12} hence isolated measurements may not reflect any putative underlying disease process. What is also missing are data correlating the level of salivary IgA with that in the lower gastrointestinal system—arguably levels of IgA may not even be reflective of potential abnormalities elsewhere.

Clinical utility. Salivary IgA cannot be recommended as an investigation for gastrointestinal disease. Not only is the relationship between changes in IgA and gastrointestinal illness not established, but it is uncertain whether salivary IgA is reflective of IgA lower in the gastrointestinal system.

Intestinal permeability

Rationale and clinical validity. Some but by no means all studies have demonstrated increased intestinal permeability in a subset of patients with IBS, as recently reviewed.¹³ For example, a variable proportion of patients with post-infectious IBS and diarrhea have increased intestinal permeability.^{13–17} Animal studies, and a small number of recent human studies suggest that probiotics may decrease the abnormal intestinal permeability in patients with IBS and or abdominal pain in childhood and lead to clinical improvement.^{18–20}

Analytical utility. Several different methods may be used to measure intestinal permeability. The two most commonly performed investigations are the dual sugar tests with lactulose and either mannitol or rhamnose and the ⁵¹Cr-labeled ethylenediamine-tetraacetic acid (⁵¹Cr-EDTA) permeability test. Details of the construction and theoretical bases for the tests are reviewed elsewhere.¹³

The synthetic disaccharide, lactulose, is minimally absorbed via the paracellular route and then excreted unchanged in the urine, while the sugar alcohol, mannitol, or monosaccharide, rhamnose, being smaller molecules, are passively and, at the doses used,

usually completely absorbed in the small intestine, to be excreted unchanged in the urine. An elevated lactulose : mannitol (or lactulose : rhamnose) ratio indicates increased permeability. There are several interpretative problems with this test, including geographical variations in the intestinal permeability (a significant trend toward increased permeability in tropical locales has been noted) and intra-patient variability has been noted to occur dependent on a patient's hydration status.²¹

The ⁵¹Cr-EDTA test involves the ingestion of radio-labeled EDTA and the measurement of the percentage of the chemical excreted in the urine over a set time interval as determined by a gamma camera.²² Abnormalities in intestinal permeability using this technique have been noted in a range of intestinal conditions including inflammatory bowel disease and IBS (particularly the diarrhea subtype) as well as following the ingestion of non-steroidal anti-inflammatory drugs (NSAIDs) and following chemotherapy.¹³ As with other measures of intestinal permeability, marked day-to-day variability among normal healthy controls has been demonstrated and use beyond the research setting cannot be recommended.²²

Clinical utility. Measures of intestinal permeability are time consuming, expensive and reliability is influenced by factors such as a patient's hydration status and medications (such as NSAIDs). However, observations that intestinal permeability may be increased in the diarrhea-predominant subset of patients with IBS provide little useful diagnostic clinical information and do not guide management in any way. For such tests to be used in routine clinical use, standardized, clinical and outcome correlates of the findings are required, and the significance in terms of response to therapeutic interventions are required. There is some preliminary evidence that probiotics may alter the increased intestinal permeability of patients with IBS and children with abdominal pain.^{18,19} Until it has been conclusively established that measures of intestinal permeability are reliable, alter clinical management and until treatments that potentially modulate intestinal permeability have been proven to do so, this investigation cannot be advocated for routine clinical use.

Fecal short chain fatty acids

Rationale and clinical validity. Short-chain fatty acids (SCFAs) are the principle nutrient substrates of the colonic epithelium, and are themselves produced in the intestinal lumen by commensal anaerobic bacteria via carbohydrate fermentation.²³ Without an adequate supply of SCFAs, which may occur, for example, due to the surgical diversion of the fecal stream, the colonic epithelium atrophies and diversion colitis may occur. Both will reverse, at least to some extent, by the administration of SCFA enemas.²⁴ SCFAs, especially butyrate, are multi-talented molecules in that they promote epithelial differentiation (and thus, at least theoretically, induce a more mature and functionally better epithelium), are anti-inflammatory by inhibiting cytokine production, improve barrier function (at least *in vitro*) and inhibit carcinogenesis *in vivo* in rats.^{22,25} As a therapeutic modality, however, they have not been very successful due to the difficulty delivering the butyrate or other SCFAs to the site desired in sufficient dose. Nevertheless, given the protean beneficial effects, one might

suppose that higher levels in the feces are better for the bowel. This, together with the fact that increasing dietary fiber, particularly wheat bran, may increase fecal SCFAs, provide a rationale for measuring SCFA levels in the feces and using it, for example, to determine therapeutic measures to be applied.²⁶

Several problems compound this overly simplistic view. First, fecal SCFAs may not necessarily reflect levels in the more proximal colon. Second, the speed of transit through the colon will also influence the SCFA concentration. Third, there are no data to indicate in humans that different fecal levels are associated with clinical scenarios or outcomes (apart from gross deficiency as in diversion of the fecal stream). Fourth, neither the concentrations of SCFAs nor the relative proportions of different SCFAs have shown consistent associations with a disease state such as IBS.²⁷ The absolute quantity of fecal SCFAs in IBS compared to healthy controls has also delivered contradictory results.^{28,29}

Analytical validity. The measurement of fecal SCFAs involves the collection of stool, followed by stool treatment and liquid chromatography. Normal values for healthy individuals have not been established in large scale studies. Variations in diet greatly influence the amounts and types of SCFAs in the stool. Hence inter-patient and intra-patient variation can be expected.^{30,31} As intestinal commensal bacteria produce the SCFAs by fermentation, the quantity and species of colonic bacteria, along with the substrate (poorly absorbed carbohydrates) determine the SCFAs produced.³² Studies attempting to correlate particular bacterial strains to relative concentrations of individual SCFAs have once again shown contradictory results.³³

Clinical utility. Further research is needed to determine clinical implications and interpretation of (if any) fecal SCFAs in intestinal disease. The relative and absolute quantity of different stool SCFAs in health or disease has not been established—with contradictory reports in the literature. Furthermore, the efficacy of therapeutic interventions taken on the basis of the levels in disease states and of changing the levels with dietary or other means have not been studied. Measurement of SCFAs in the feces cannot, therefore, be recommended in clinical practice, but should be only performed in the research setting.

Fecal microbial analysis

Rationale and clinical validity. Instead of the culture of stool with a view of detecting pathogenic organisms, the relative and absolute quantities of commensal strains of bacteria are analyzed. Patients with IBS may have alterations in their intestinal microbiota as defined by stool culture or polymerase chain reaction (PCR).³⁴ Recent attention has focused on alteration in both small bowel bacteria (bacterial overgrowth causing IBS with diarrhea—IBS-D) and large bowel bacteria (in particular the ratio of potentially beneficial commensal bacteria such as lactobacillus and bifidobacteria, compared to others such as clostridium), which have in turn spurred the trial of the antibiotic rifaximin and a variety of probiotics respectively.^{35,36} A large randomized controlled trial supports the use of rifaximin in IBS-D, while a number of meta-analyses suggest a modest overall benefit of probiotics in

a diverse IBS population.^{35,37,38} What is lacking, however, is the demonstration of a precise cause and effect model whereby alterations in microbiota result in clinical improvement. Changes in large bowel commensal bacterial populations have not been correlated with symptomatic improvement in the trials of probiotic treatment for IBS (where symptom scores were the primary outcome measure), nor have changes in small bowel bacteria been shown to correlate with symptomatic improvement to rifaximin (again symptomatic improvement was the principal outcome of interest).^{39,40}

Analytical validity. Intestinal microbiota are notoriously hard to study, due to the predominance of anaerobic organisms that are by definition fastidious (difficult to culture), and by the potential geographical fluctuations that occur in both health and disease.⁴¹ These difficulties will in time be overcome with technological advances, and there has been a major shift in research away from microbial culture and towards genetic studies including PCR.³⁶ Indeed the majority of studies of patients with IBS that demonstrate an alteration in stool microbiota have used genetic rather than culture techniques. The culture of commensal bacteria in stool has not been validated rigorously in a large number of healthy subjects to define a robust normal range.³⁶

Clinical utility. The measurement of fecal microbiota by culture cannot be recommended. This method has proven difficult in dedicated research laboratories. Ongoing international efforts are now focused on improved genetic techniques to better delineate the intestinal microbiome. Moreover, there are no studies that address the question as to whether the measurement of potential alterations in the intestinal microbiome can influence treatment decisions, and the available treatments themselves (prebiotics, probiotics and antibiotics) are as yet generally unproven.

Other investigations

The number of commercially available investigations is growing rapidly. Human beta-defensin-2 (HBD2) is a protein produced by intestinal epithelial cells (Paneth cells) on contact with bacterial antigens and plays a role in innate immunity.⁴² HBD2 can be measured in the stool via ELISA and may be elevated in some patients with irritable bowel syndrome as well as ulcerative colitis according to some studies.⁴³ The HBD2 assay may be elevated in some normal individuals and inter-individual variation on repeated measurement is significant. This assay is already offered as a "functional" investigation in North America. Other stool tests such as eosinophilic cationic protein and tryptase for the assessment of putative food allergy are also available but, because of the lack of validation and studies of reliability, cannot be recommended for clinical practice.^{44,45}

Ethical implications

It is the opinion of the authors that for a test to be viewed as ethically sound it must confer benefit and not do harm to the patient. As the rationale, validity and reliability of all of the investigations are questionable, the benefit to the patient is hence

not readily apparent. Moreover as the investigations cost the patient money, the potential to do harm (in a fiscal sense) is apparent.

Conclusion

None of the investigations examined by the authors can be recommended for routine clinical practice. Not only are the scientific underpinnings for the individual investigations questionable (the validity and rationale for testing), but the reliability of the individual assays are poor. Clinical decisions made on the basis of the test results are not evidence-based. Alternative investigations for IBS should for now be limited to the research laboratory.

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