ORIGINAL RESEARCH

Frequency of Abnormal Fecal Biomarkers in Irritable Bowel Syndrome

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ABSTRACT

Primary Study Objective: Determine the frequency of abnormal fecal biomarker test results in patients with 13 irritable bowel syndrome (IBS)–related ICD-9 (International Statistical Classification of Diseases and Related Health Problems) codes.

Study Design: Quantitative review of de-identified records from patients in whom IBS was a possible diagnosis.

Methods: Records were selected for analysis if they included any of 13 IBS-related diagnostic codes and laboratory test results of fecal testing for all biomarkers of interest. Data collection was restricted to one 12-month period. Frequency distributions were calculated to identify rates of abnormal results for each biomarker within the total number of tests conducted in the eligible population.

Results: Two thousand, two hundred fifty-six records were included in the study, of which 1867 (82.8%) included at least one abnormal value. Quantitative stool culture for beneficial bacteria (*Lactobacillus* and *Bifidobacterium*) indicated low growth suggestive of intestinal dysbiosis in 73.1% of records, followed by abnormally elevated eosinophil protein X (suggestive of food allergy) in 14.3%, elevated calprotectin (suggestive of inflammation) in 12.1%, detection of parasites in 7.5%, and low pancreatic elastase (suggestive of exocrine pancreatic insufficiency) in 7.1%.

Conclusions: Abnormal fecal biomarkers are prevalent in patients with diagnoses suggestive of IBS. Abnormal fecal biomarker testing, if confirmed in additional independent clinical trials, could substantially reduce the economic costs associated with diagnosis and management of IBS.

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BACKGROUND

It is estimated that 10% to 20% of Americans in their most productive years are afflicted with irritable bowel syndrome (IBS).[1-3] IBS imposes a social burden estimated to cost approximately $20 billion a year.[4]

Despite the existence of guidelines to the contrary, many primary care physicians continue to view IBS as a "diagnosis of exclusion" and pursue costly and often invasive diagnostic studies.[5-7] The conditions to be excluded (such as inflammatory bowel disease, malignancy, and infectious colitis), while carrying potentially grave prognoses, are rarely discovered during evaluation of patients who have IBS or other functional bowel disorders.[5,8-10]

Conversely, evidence is emerging that the syndromic symptoms that define IBS according to the Rome III clinical criteria (recurrent abdominal pain or discomfort, improvement with defecation, change in frequency or in form/appearance of stool) may in fact have protean causes, often arising from one or more specific gastrointestinal (GI) conditions.[11] The advent of relatively inexpensive tests based on identification of selected fecal biomarkers now makes it possible to identify or exclude several of these underlying conditions, with the potential for a positive clinical and economic impact.[12]

GI conditions capable of producing manifestations of IBS include exocrine pancreatic insufficiency, which has an estimated prevalence of 6.1% in subjects with IBS symptomatology, and may be suggested by low levels of fecal pancreatic elastase (PE).[13] Inflammatory disorders such as inflammatory bowel disease may be discriminated from IBS with the use of the neutrophil-derived protein calprotectin in stool.[14-17] Food allergies, which have a reported prevalence rate of about 25% in IBS patients,[18] may be suggested by the presence of elevated fecal levels of eosinophil protein X, which may also be elevated in inflammatory bowel disorders and parasitic infections.[19-25] Pathogenic infections such as *Clostridium difficile* and parasites such as *Giardia lamblia* are reported in 5.7% and 6.5%, respectively, of people with symptoms attributable to IBS[26,27] and are readily detected on fecal specimens using established techniques such as culture and light microscopy. *Blastocystis hominis*, the most common human intestinal parasite, was long thought to be non-pathogenic.[28,29] Some (but not all) recent studies, however, have demonstrated a significant increased prevalence of *Blastocystis hominis* in IBS patients compared with controls, and at least one authority has recommended treatment with metronidazole in the face of a positive identification of the organism and a symptomatic patient.[29-35]

Even in the absence of known pathogens, close study of the microbiome reveals differences in fecal bacterial populations (dysbiosis) between IBS patients and healthy controls. While a clear-cut "IBS microbiotype" has not been identified, studies have described relative increases in detrimental groups of commensal bacteria and decreases in beneficial groups, most specifically a decrease in Bifidobacteria and an increase in

Table 1 Biomarkers of Interest

Biomarker	Definition of Abnormal Result	Interpretation of Abnormal Result in Context of IBS
Stool Culture, Beneficial Bacteria: (Lactobacillus, Bifidobacterium)	Growth in 1 or fewer quadrants (Lacto)/2 or fewer (Bifido)	Reduced numbers of beneficial symbionts (dysbiosis)
Eosinophil Protein X	>7 µg/g	Suggestive of food allergy or parasites (causes of eosinophilic inflammation)
Pancreatic Elastase	<200 µg/g	Suggestive of exocrine pancreatic insufficiency
Calprotectin	>50 µg/g	Suggestive of neutrophilic inflammation, eg, IBD
Occult Blood	Present	Suggestive of inflammation, malignancy, enteric infection
H pylori	Present	Suggestive of gastritis
C difficile	Positive	Suggestive of *C difficile* colitis
Parasites	Entamoeba histolytica/dispar, *Giardia lamblia*, Cryptosporidium: EIA[a] positive *Blastocystis hominis*: present on microscopic exam All other parasites: present on microscopic exam	Evidence of parasitic infection

[a] Detection by enzyme-linked immunosorbent assay (EIA).
Abbreviations: IBD, inflammatory bowel disease; IBS, irritable bowel syndrome.

enterobacteriaceae.[36-43] Additionally, IBS patients are known to have a reduced diversity and stability of populations of bacterial organisms compared with controls.[36,44] The emergence of rapid means of detecting intestinal dysbiosis (eg, through 16S ribosomal DNA polymerase chain reaction [PCR] amplification) in patients suspected of having IBS adds an additional potentially powerful biomarker to the list.[36,41,44,45]

Publications to date, however, have typically focused on the identification or exclusion of one suspected condition potentially capable of producing IBS-like symptoms, such as bile acid abnormalities, exocrine pancreatic insufficiency, or inflammatory bowel diseases.[11,13-17,46-48] Identification of these individual disorders has proved useful at containing diagnostic and therapeutic costs.[12,49]

Unlike older, invasive diagnostic tests that are used in a serial fashion as each condition is excluded, fecal biomarker testing is relatively inexpensive and suited to parallel testing on a single fecal sample. If this approach is validated, it may permit clinicians and patients to arrive at a treatable diagnosis associated with the symptoms of IBS in a rapid and cost-effective manner. It may also suggest further targeted evaluations. A comprehensive study of the use of parallel testing in the context of IBS needs to be performed.

We report here a retrospective, administrative database review study of patients in whom multiple fecal biomarker testing had been performed, seeking to produce a descriptive but quantitative account of the various conditions capable of being evaluated by such testing.

METHODS

Objectives

Determine the frequency of abnormal fecal biomarker test results in patients with 13 IBS-related ICD-9 codes.

The objective of this study was to identify the frequency of abnormal fecal biomarkers in patients with diagnoses consistent with IBS. The presence of abnormal fecal biomarkers may be suggestive of a potentially treatable source of GI symptomatology.

Design

We conducted a quantitative review of administrative records from patients in whom IBS was a possible diagnosis and who had undergone fecal testing for all biomarkers of interest over a 12-month period and then generated frequency distributions of abnormal test results. For this study, all data were de-identified prior to analysis, and no protected health information was recorded. It was not possible to correlate this dataset with clinical criteria such as Rome III.

Setting

We examined the computerized database of Genova Diagnostics, Inc (Asheville, NC, www.gdx.net), the Clinical Laboratory Improvement Amendments (CLIA)–certified clinical laboratory where the biomarker testing was conducted.

Patient Population

Adult records (18 years and older) were eligible for inclusion in the study if they contained results for all of the biomarkers of interest (Table 1) and if the ordering requisition listed at least one of the 13 ICD-9 codes commonly used by clinicians when evaluating patients with functional bowel disorders including IBS (Table 2).

Performance characteristics of these biomarkers for diagnoses that may present as IBS have been published elsewhere for pancreatic elastase,[50-53] calprotectin,[54-56] eosinophil protein X,[57] *Clostridium difficile*,[58,59] and parasitology exam,[60] with sensitivities and specificities for such diagnoses ranging from 83% to 96% and specificities in the range of 82% to 96%. The precise relationship of gut microbiota patterns to human health and disease is not yet sufficiently clear to provide specific performance characteristics.

Table 2 Diagnostic Codes Used To Define Eligible Records

ICD-9 Code	Diagnosis	Frequency (%)
789	Abdominal pain	47.61
787.91	Diarrhea	14.14
564.1	Irritable bowel syndrome	13.92
787.3	Flatulence, eructation, and gas pain	7.89
564.01	Slow-transit constipation	6.47
564	Constipation, unspecified	4.83
579.9	Unspecified intestinal malabsorption	1.99
558.9	Other noninfectious gastroenteritis and colitis	1.33
789.07	Abdominal pain, generalized	0.93
536.8	Dyspepsia and other disorders of stomach function	0.40
789.06	Abdominal pain, epigastric	0.22
536.9	Unspecified functional disorder of stomach	0.18
564.9	Functional intestinal disorder, unspecified	0.09

Intervention

The intervention in this retrospective, descriptive study was the ordering of fecal biomarker tests at the discretion of the referring physician.

Main Outcome Measure

The study's main outcome measure was a frequency distribution representing the proportion of abnormal results (as defined in Figure 2) within the total number of tests conducted in the eligible population.

RESULTS

A total of 2256 records were associated with one of the pre-selected IBS-related ICD-9 codes and had data available for all biomarkers of interest (Table 1). ICD-9 codes 789 (abdominal pain), 787.91 (diarrhea), and 564.1 (IBS) accounted for the majority (75.5%) of records; no other code represented more than 8% of records.

The gender distribution of the 2256 records was 73% female and 27% male, a ratio consistent with published data on gender distribution in IBS.[61,62] Of that group, 1867 records (82.2%) included at least one abnormal value. A frequency distribution of records with at least one abnormal test result is shown in Figure 1.

Figure 2 shows the distribution, by fecal biomarker, of total abnormal results among the 2256 records analyzed. Several biomarkers could be divided into subcategories. The 7.5% of all abnormal labeled as "parasites" represented 73 instances (3.2%) positive for Blastocystis hominis by light microscopy, 8 each (0.4%) for *Giardia lamblia* and *Entamoeba histolytica/dispar* (by enzyme immunoassay [EIA]; similar testing for *Cryptosporidium* revealed no positive results), and 78 (3.5%) for all other parasites by microscopic examination.

For calprotectin, the 12.1% of results with abnormal values represents 102 specimens (4.5%) with values greater than 119 ²g/g; lowering that threshold to include specimens with values in the range of 51 to 119 ²g/g added an additional 171 cases (7.6%) with abnormal values.

LIMITATIONS

This study had certain limitations. This retrospective, data review study did not use clinical Rome III criteria for inclusion of records. Rather, it included patients whose ICD-9 codes suggested the presence of

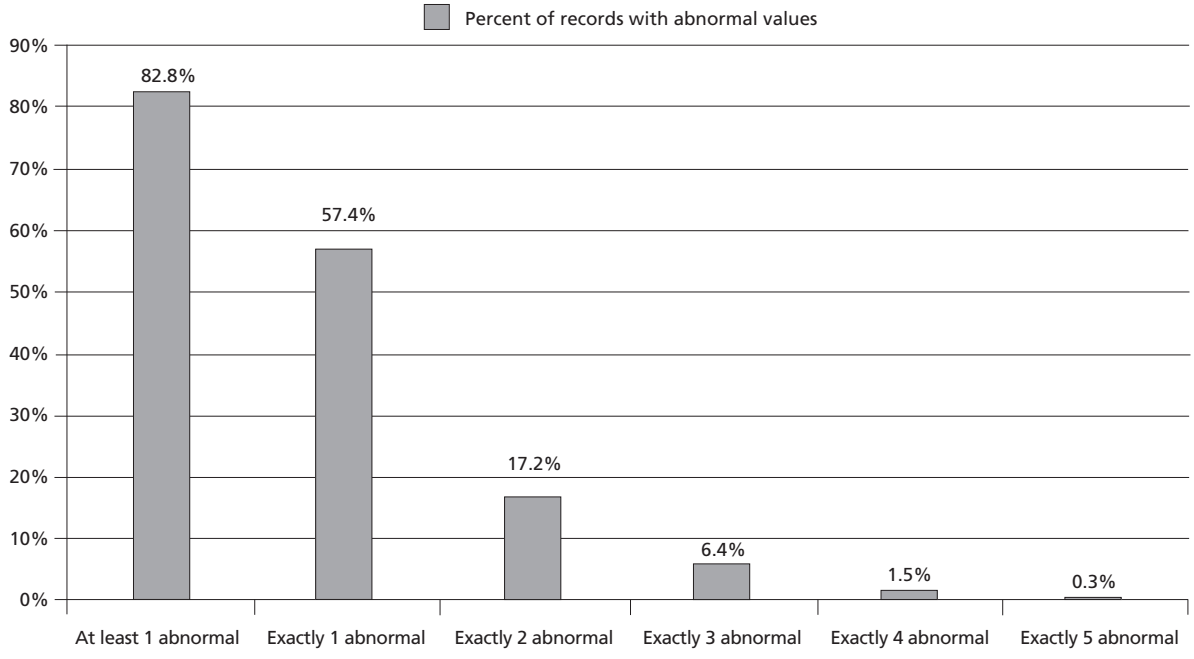

Figure 1 Distribution of records with abnormal results as a proportion of all records (N=2256).

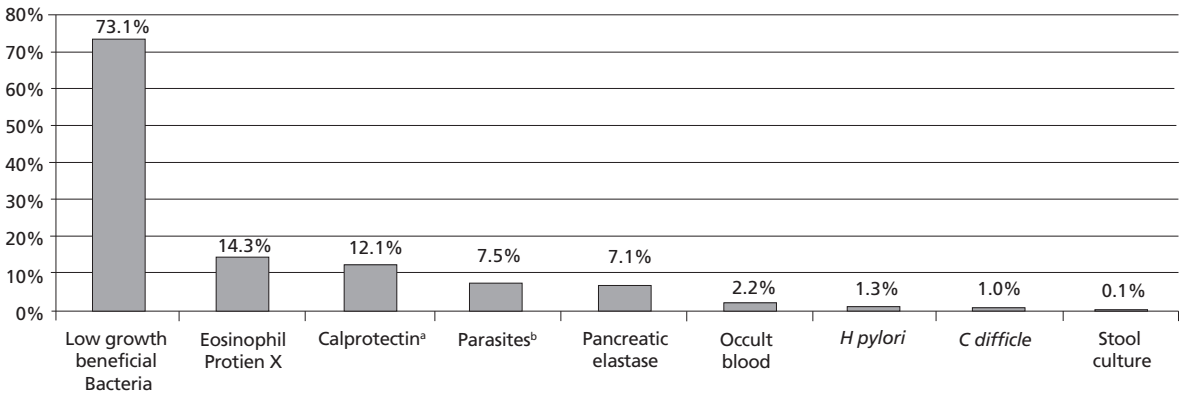

Figure 2 Frequency distribution of abnormal test results for each biomarker studied, as a proportion of all records (N = 2256). Percentages add to more than 100% because some records had more than one abnormal value.

a Calprotectin value = 4.5% greater than 119 + 7.6% in range 51-119 µg/g.
b Parasites value = Blastocystis hominis at 3.2% + entamoeba 0.4% + giardia 0.4% + other parasites 3.5%.

GI symptoms commonly manifested by patients undergoing evaluation for IBS, including abdominal discomfort associated with changes in fecal frequency or appearance. We argue, however, that these patients may in fact represent the real situation faced by practicing clinicians, namely, patients whose symptoms have no immediately obvious cause.

The population studied here is representative of primary care physicians who submitted samples for comprehensive stool profiles to one CLIA-certified clinical laboratory. These physicians and their patients may represent a unique community of providers and patients not representative of the general population. Study requisition forms to capture diagnoses from ICD-9 codes are unlikely to be precise and clinicians use variable codes. These figures may represent an over- or under-estimations of the true prevalence of these conditions in this popultation and the general population.

We believe, nonetheless, that this study provides valuable descriptive information about the potential occurrence of treatable conditions within a pool of subjects in whom IBS may have been a consideration. This study is hypothesis-generating, and additional rigorously designed studies will enhance our understanding of the role of fecal biomarker testing in evaluation of patients who have symptoms consistent with IBS.

DISCUSSION

IBS has recently been proposed to be an "umbrella diagnosis," representing a collection of different clinical conditions capable of causing symptomatology associated with the syndrome.[11] Individual studies have been published focused on one of the many conditions that may produce IBS symptoms,[11,13,63] but no study, to our knowledge, has yet attempted to characterize the frequency with which such multiple conditions may occur within a single population. Such information would be of use in developing cost-effective screening strategies aimed at suggesting the presence or absence of treatable conditions in patients manifesting IBS symptoms.

We chose the biomarkers for this preliminary study based on their known utility in establishing or excluding the more common disease processes that can produce symptoms consistent with IBS. Several of the biomarkers, eg, calprotectin and *C difficile* EIA, are also FDA cleared. Newer fecal biomarkers of relevance, such as 16S ribosomal DNA PCR amplification, may emerge as practical additions to the biomarker toolkit for evaluation of patients with IBS-like symptoms.

We studied the frequency of abnormal test results on fecal biomarker testing conducted on a group of patients whose ICD-9 codes indicated the potential for IBS. We identified at least one abnormality among the biomarkers tested in more than 80% of cases. While this may appear to be a large proportion, it is consistent with previous work by Habba et al,[11] who found that 98% of patients with initial presentation of diarrhea-predominant IBS had a different diagnosis after testing and that 68% had conditions related to a treatable condition; of that group, 98% had a favorable response to therapy.

In our study, five biomarkers (beneficial bacteria, eosinophil protein X [EPX], calprotectin, parasites, and pancreatic elastase) accounted for the bulk of abnormal results. Each of these biomarker abnormalities is potentially useful as a screening test, suggesting the possible presence of a treatable condition whose eradication would reduce or eliminate symptoms compatible with IBS.

A low growth of beneficial bacteria (lactobacillus or bifidobacteria) was found in 73.1% of our samples. This is consistent with the type of beneficial bacteria insufficiency, or dysbiosis, that has been associated with IBS symptomatology.[43] Dysbiosis of this kind in IBS patients has been found to respond favorably to probiotic therapy.[64,65]

An elevation in fecal EPX was identified in approximately 14% of fecal samples. Elevated fecal EPX has been reported in patients with inflammatory bowel disorders (including Crohn's disease, ulcerative colitis, and microscopic colitis), in which concentrations in

stool are especially high, in those with parasitic infections, and also in patients with known food allergies; EPX levels fall significantly when specific treatment is provided.[19,21,22,24,25,66,67] Patients who present with IBS symptoms have been found to have EPX levels that do not differ from healthy controls when such patients do not have an associated eosinophilia-mediated condition.[67]

Fecal calprotectin is known to be present in stool in neutrophil-mediated inflammation of the intestinal mucosa.[68] Conversely, in functional disorders such as IBS, calprotectin levels are typically much lower than those found in inflammatory bowel disease (IBD) and not significantly different from those found in healthy controls.[69,70] Van Rheenen et al, in a meta-analysis of 13 studies from the primary literature, found that in adults being evaluated for IBD, screening by measuring calprotectin levels would produce a 67% reduction in the number of adults undergoing endoscopy, while only 3 of 33 adults in every 100 who do undergo endoscopy will not have IBD (but would likely have a different condition for which endoscopy is nonetheless inevitable).[14] Conversely, 6% of adults would have a delay in diagnosis of IBD because of a false negative result.

In the present study, fecal calprotectin levels were elevated in 12.1% of all specimens with use of a screening cutoff of >50 μg/g of stool. This figure represents 4.5% with values greater than 119 μg/g and an additional 7.6% of the total data set with values in the range 51 to 119 μg/g. In most clinical studies, cutoff levels of 50 or 100 μg/g are used[71,72]; however, when values from healthy controls are reported, levels of fecal calprotectin are well below 50 μg/g, typically in the range less than 10 to 20 μg/g.[73-75]

The clinical implication of using the lower cutoff level is clearly that more potential cases of inflammatory conditions will be identified, with a secondary increase in false-positive results. In a previous study aimed at predicting relapse in IBD patients, however, the 50 μg/g cutoff produced a sensitivity and specificity of 90% and 83%, respectively, indicating an acceptable false-positive rate.[73] While the higher cutoff level of 100 μg/g has been shown to produce greater overall diagnostic accuracy,[76] in a test aimed at screening for treatable conditions in IBS, the lower cutoff (and resulting higher false-positive rate) may be preferable.

In the present study, parasites as a whole accounted for 7.5% of abnormal values. The single most commonly-identified organism was *Blastocystis hominis*, which until recently was regarded as a non-pathogenic organism.[28,35] Several recent studies, however, point to a moderately strong association between *B hominis* and symptomatic IBS, with some variation between geographic areas.[29,31,35] Certain genotype 1 of the organism shows the closest correlation with IBS.[32,77] In light of growing evidence for an etiologic role for the organism,28 it appears reasonable to include *B hominis* in a screening test seeking treatable underlying conditions capable of producing IBS symptoms, particularly because treatment with metronidazole is curative.[34]

In the present study, abnormally low levels of pancreatic elastase (<200 μg/g of stool) were identified in 7.1% of all specimens; this figure represents the sum of the 2.2% of records with a value <100 μg/g and the 4.9% in the range 100 to 199 μg/g. Low pancreatic elastase is a reliable indicator of exocrine pancreatic insufficiency, comparing favorably with the secretin-cerulein test (the "gold standard"),[78] as well as several other commonly used tests for detecting pancreatic exocrine impairment. Various estimates of sensitivity, specificity, and negative and positive predictive values of fecal pancreatic elastase have been published, depending on the test used and the specific pancreatic pathology detected. A cutoff of 200 μg/g is generally accepted as the lower limit of normal[79-82]; using this value, Loser et al[78] found fecal PE1 to correlate well with the secretin-cerulein test, to outperform fecal chymotrypsin, and to have an overall sensitivity and specificity of 93% for diagnosing exocrine pancreatic insufficiency. When patients in that study were classified by disease severity, the sensitivity was 100% in moderate-to-severe cases but only 63% in mild cases. The lower cutoff value (<100 μg/g) may assist in identifying patients with more severe pancreatic exocrine insufficiency.

In conclusion, in this retrospective database review study of subjects with common GI disorders compatible with manifestations of IBS, a large proportion (more than 80%) were found to have evidence of a potentially treatable condition capable of producing IBS-like symptoms. Abnormal values suggesting intestinal dysbiosis, food allergy, parasite infection, exocrine pancreatic insufficiency, or inflammatory processes in the gastrointestinal tract were the most common findings. In clinical practice, these patients might then have undergone further, focused evaluation in order to arrive at a firm organic diagnosis and an effective treatment regimen; in short, these individuals might prove to have diagnoses other than "IBS."

A structured, parallel fecal biomarker testing panel may represent a relatively inexpensive screening method for underlying, treatable causes of IBS symptoms. Future prospective studies focusing on patients meeting current clinical criteria such as the Rome III should be conducted, including a rigorous follow-up of all abnormal findings to evaluate the utility of a structured fecal biomarker testing panel in patients with IBS symptomatology.

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