

# A Cytologic Assay for Diagnosis of Food Hypersensitivity in Patients With Irritable Bowel Syndrome

ANTONIO CARROCCIO,\* IGNAZIO BRUSCA,<sup>‡</sup> PASQUALE MANSUETO,<sup>§</sup> GIUSEPPE PIRRONE,<sup>§</sup> MARIA BARRALE,<sup>‡</sup> LIDIA DI PRIMA,<sup>§</sup> GIUSEPPE AMBROSIANO,<sup>§</sup> GIUSEPPE IACONO,<sup>||</sup> MARIA LETIZIA LOSPALLUTI,<sup>||</sup> STELLA M. LA CHIUSA,<sup>‡</sup> and GAETANA DI FEDE<sup>¶</sup>

**AQ:2-3** \*Internal Medicine, Ospedali Civili Riuniti, Sciacca (AG), Sciacca, Italy; <sup>§</sup>Internal Medicine, Department of Clinical Medicine and Emerging Diseases, <sup>¶</sup>Department of Medical Oncology, University of Palermo, Italy; <sup>‡</sup>Clinical Chemistry, "Buccheri La Ferla" Hospital, Palermo, Italy; <sup>||</sup>Pediatric Gastroenterology, "Di Cristina" Hospital, Palermo, Italy

**AQ:7** **BACKGROUND & AIMS:** A percentage of patients with symptoms of irritable bowel syndrome (IBS) suffer from food hypersensitivity (FH) and improve on a food-elimination diet. No assays have satisfactory levels of sensitivity for identifying patients with FH. We evaluated the efficacy of an in vitro basophil activation assay in the diagnosis of FH in IBS-like patients. **METHODS:** Blood samples were collected from 120 consecutive patients diagnosed with IBS according to Rome II criteria. We analyzed in vitro activation of basophils by food allergens (based on levels of CD63 expression), as well as total and food-specific immunoglobulin (Ig)E levels in serum. Effects of elimination diets and double-blind food challenges were used as standards for FH diagnosis. **RESULTS:** Twenty-four of the patients (20%) had FH (cow's milk and/or wheat hypersensitivity); their symptom scores improved significantly when they were placed on an elimination diet. Patients with FH differed from other IBS patients in that they had a longer duration of clinical history, a history of FH as children, and an increased frequency of self-reported FH; they also had hypersensitivities to other antigens (eg, egg or soy). The basophil activation assay diagnosed FH with 86% sensitivity, 88% specificity, and 87% accuracy; this level of sensitivity was significantly higher than that of serum total IgE or food-specific IgE assays. **CONCLUSIONS:** A cytometric assay that quantifies basophils after stimulation with food antigens based on cell-surface expression of CD63 had high levels of sensitivity, specificity, and accuracy in diagnosing FH. This assay might be used to diagnose FH in patients with IBS-like symptoms.

*Keywords:* Irritable bowel syndrome; Food hypersensitivity; Flow-CAST; IgE; Diagnosis

**AQ:8-9** Irritable bowel syndrome (IBS) is a common gastrointestinal disorder in which a disturbed brain-gut axis has been thought to have a mandatory role.<sup>1</sup> However, many patients suffering from IBS report an association of symptoms with specific food ingestion, referred to as self-perceived food hypersensitivity (FH),<sup>2</sup> and recent clinical studies imply that dietary factors might be more important in the pathogenesis of IBS than was earlier anticipated.<sup>3</sup>

Furthermore, approximately 20% of the population alter their diet owing to self-perceived FH, but the application of a double-blind, placebo-controlled, oral food challenge, considered as the gold standard for FH diagnosis, shows that questionnaire-based studies overestimate the prevalence of this disease.<sup>4</sup>

Because both IBS and FH with gastrointestinal symptoms often have the same clinical presentation, with patients suffering from mild to severe abdominal pain, abdominal discomfort, bloating, and alteration of bowel habits,<sup>5</sup> differential diagnosis between these 2 conditions may be difficult and essentially based on elimination diets and double-blind, placebo-controlled challenges. Unfortunately, none of the available in vivo and in vitro allergy tests (ie, skin prick test and serum total immunoglobulin [Ig]E and specific IgE assays) have shown a good diagnostic reliability.<sup>6</sup>

More recently, the flow cytometric basophil activation test, based on the demonstration of altered membrane phenotypes on allergen-activated basophils, with up-regulation, surface expression, and cytofluorometric detection of CD63 protein, has been applied to allergy diagnosis.<sup>7</sup> However, no studies have evaluated the diagnostic accuracy of the test in FH diagnosis in patients with IBS-like clinical symptoms. **AQ:10**

In this study, we evaluated the diagnostic reliability of the flow cytometric allergen stimulation test to discriminate IBS from FH in a group of diagnosed IBS patients, some of whom referring a self-perceived FH. **AQ:11**

## Patients and Methods

A total of 120 patients (97 women, 23 men; age range, 18–56 y; median age, 36 y), who had been consecutively referred as outpatients to the Department of Internal Medicine of the University of Palermo from January 2005 to December 2006 for IBS, completed this study.

The inclusion criteria were as follows: (1) age older than 17 years, (2) no previous referral to our clinic, and (3) diagnosis of IBS. Patients with a diagnosis of organic gastrointestinal disease were excluded.

IBS diagnosis was based on the Rome II criteria for functional gastrointestinal disorders<sup>8</sup> (see Supplementary Document; see Supplementary material online at [www.cghjournal.org](http://www.cghjournal.org)). Furthermore, organic gastrointestinal disorders were excluded by an accurate work-up (described later). **AQ:12**

After inclusion, the patients underwent a clinical evaluation that included a detailed family and personal clinical history and

**Abbreviations used in this paper:** DBPC, double-blind, placebo-controlled; FH, food hypersensitivity; IBS, irritable bowel syndrome; Ig, immunoglobulin.

a physical examination. Afterward, 2 predesigned questionnaires were administered to all the patients: the first was regarding the type and severity of the symptoms, the second was regarding any possible self-perceived FH.

AQ: 13 None of the enrolled patients were on any medication or were on an elimination diet at the time of the study. In fact, they were asked to suspend medications and/or diet at least 3 weeks before the beginning of the study protocol.

The study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the University Hospital of Palermo and all patients gave their written informed consent to participate.

### Healthy and Disease Control Groups

Two control groups were selected. One was composed of 40 patients with various gastrointestinal diseases (28 women, 12 men; age range, 18–62 y; median age, 34 y): celiac disease (n = 16), active ileum-colon Crohn's disease (n = 15), and ulcerative colitis (n = 9), diagnosed according to standard serologic, endoscopic, and histologic criteria. These patients were selected at random from those diagnosed in our hospital during the period of the present study. The other group was composed of 40 healthy volunteers chosen from the students attending the University Hospital (28 women, 12 men; age range, 18–63 y; median age, 30 y).

### Work-Up for Irritable Bowel Syndrome Diagnosis

All patients underwent first-step hematology and chemistry tests (including erythrocyte sedimentation rate; serum C-reactive protein level; blood cell counts; electrolytes; and thyroid, liver, and renal function); stool examination for occult blood, ova, and parasites; and a lactose-H<sub>2</sub> breath test after oral load of 50 g of lactose. Only the patients showing symptoms after lactose load were excluded, the simple evidence of malabsorption was not considered a reason for exclusion. Particular care was taken to exclude a diagnosis of celiac disease because some patients had been on a diet with a reduced content of wheat owing to self-perceived wheat-intolerance (see Supporting Document; see Supplementary material online at [www.cghjournal.org](http://www.cghjournal.org)). However, no patients were following a strict gluten-free diet. Patients also underwent sigmoidoscopy with biopsy if younger than age 40 or underwent a colonoscopy with biopsy if older than 40 years of age. Patients with negative results for all of the examinations described earlier and with a clinical history indicating IBS, according to the Rome II criteria, were considered to be suffering from IBS and were enrolled in the study. All medications and food restrictions were suspended at least 3 weeks before the beginning of the study.

### Study Protocol

AQ: 14 After enrollment in the study, the patients completed the Symptoms Severity and Food Hypersensitivity questionnaires (see Supporting Document; see Supplementary material online at [www.cghjournal.org](http://www.cghjournal.org)), and underwent serum total and food-allergen-specific IgE determination, together with the flow cytometric basophil activation test. The diagnostic tests were performed by different operators, unaware of the clinical history of the patients and the results of the other tests.

The study patients then were observed for a 4-week run-in period. After this they underwent an elimination diet without cow's milk and derivatives, wheat and derivatives, egg, tomato, and chocolate for 4 weeks. Patients self-reporting FH also were asked to avoid ingestion and/or contact with the food(s) causing symptoms. The patients wrote a dietary diary, and adherence to the elimination diet was evaluated by trained dietitians. Patients who specified a symptom/sign improvement after the elimination diet period underwent a double-blind, placebo-controlled, oral food challenge (DBPC) first with cow's milk proteins and then with wheat proteins.<sup>9</sup>

After FH had been excluded or confirmed, all IBS patients were invited to continue the follow-up evaluation with regular visits every 6 months for 2 years. During the follow-up visits the patients again underwent a physical examination, clinical history, and routine hemochemical assays, and when considered opportune some instrumental examinations were repeated.

### Symptoms Questionnaire

Severity of the symptoms was assessed in the study both at the end of the run-in period and at the end of the elimination diet period. Symptoms were assessed using a questionnaire scoring system validated for use in IBS, including an IBS symptom severity score (range, 0–500). This is a system for scoring pain, distension, bowel dysfunction, and general well-being, with mild, moderate, and severe cases indicated by scores of 75 to 175, 175 to 300, and greater than 300, respectively. A reduction in score of 50 or more was regarded as a clinically significant improvement, whereas an increase in score of 50 or more was considered as a clinically significant worsening.<sup>10,11</sup>

### Double-Blind, Placebo-Controlled Challenges

DBPC for cow's milk was performed by administering capsules coded as A or B containing milk proteins (casein from bovine milk Sigma C7078, lactoalbumin Sigma L7252, lactoglobulin Sigma L2506) or xylose (Aldrich 245321), respectively. DBPC for wheat proteins was performed with capsules coded as C or D containing wheat (Fluka, BCR563) or xylose, respectively. Capsules A or B were given for 2 consecutive weeks, and then after 1 week of washout the patients received the other capsules for another 2 weeks. After 1 week of washout, capsules C or D were given for 2 consecutive weeks, then after another week of washout, the patients received the other capsules for 2 weeks. The challenges were stopped when a clinical reaction occurred (ie, the onset of abdominal discomfort or pain) associated with a change in stool frequency and/or appearance.

Figure 1 summarizes the study design.

### Serum Total Immunoglobulin E and Food Allergen-Specific Immunoglobulin E Antibodies

Serum samples from all patients were collected and analyzed for serum total IgE and food allergen-specific IgE antibodies by using the Phadia CAP-system (Phadia, Uppsala, Sweden), according to the manufacturer's instructions. The following 6 common food allergens were tested: egg, cow's milk, soy, peanut, wheat, tomato, and fish. Levels of 0.35 kU/L or greater (level 1 on the specific IgE scale) were considered positive. Total IgE also was determined by the same method with a detection limit of 2 kU/L and an upper limit of 5000 kU/L. Normal limit for total IgE was 100 kU/L.

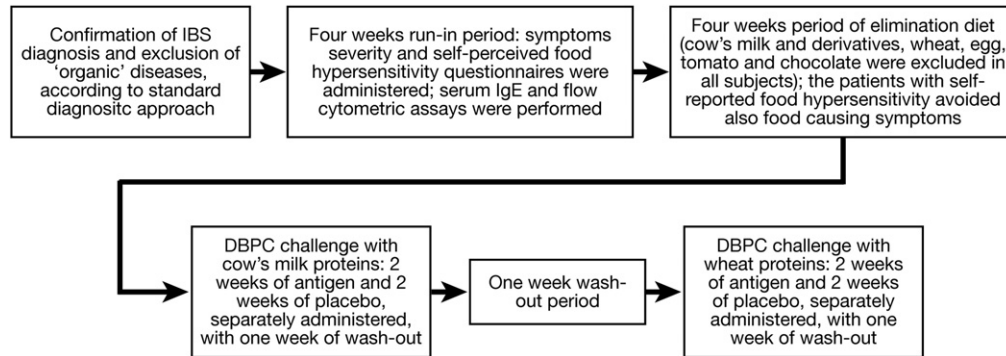


Figure 1. Study design.

### Flow Cytometric Allergen Stimulation Test

Patients were instructed to avoid systematically administered antiallergic drugs, such as corticosteroids, chromoglycic acid, indomethacin, or similar, for at least 7 days before blood sampling.

Flow cytometric allergen stimulation test was performed with Flow-CAST (Bühlmann Laboratories AG, Schönenbuch, Switzerland), according to the manufacturer's instructions (see Supporting Document; see Supplementary material online at [www.cghjournal.org](http://www.cghjournal.org)).

The manufacturer's allergens used in all patients were  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin, casein, egg white, egg yolk, wheat, soybean, fish, tomato, plus other food allergens specifically suggested by each individual clinical history. As a positive control, we used a monoclonal Le27 anti-IgE antibody solution (Bühlmann Laboratories AG). As a negative control, only the stimulation buffer containing interleukin-3 was used.

To define a result as positive, we considered that the percentage of basophils activated after incubation with antigen should be at least 3-fold the percentage of basophils activated in the background tube. When the percentage of basophils activated spontaneously was less than 2.5%, we required an additional condition, namely that the percentage of basophils activated after contact with the antigen should be equal to or greater than 5%. These cut-off values were chosen on the basis of receiver operating characteristic curves plotted in our laboratory, enabling us to achieve the highest possible sensitivity with an optimal specificity.

The intra-assay variation of the test was 2.5% and was calculated from 4 blood samples taken from 2 healthy controls and 2 patients suffering from food allergy stimulated with Stimulation Buffer and anti-Fc $\epsilon$ RI Ab and consecutively analyzed 10 times by flow cytometry. The interassay variation of the test was 6.8% and was calculated from the blood samples of 3 healthy controls and 3 patients suffering from food allergy stimulated with Stimulation Buffer and anti-Fc $\epsilon$ RI Ab, analyzed 10 times by flow cytometry and retested after 2 weeks.

The reproducibility of the test was evaluated in 20 patients (14 suffering from IBS unrelated to FH, and 6 with IBS related to FH), and all determinations were performed by 3 expert operators (I.G., M.B., S.L.C.). The correlation coefficient was excellent, with a K value of 0.92 ( $P < .001$ ).

### Statistical Analysis

Frequency analysis was performed using the chi-square test or the Fisher exact test. Means comparison was performed

by the Student  $t$  test or the Mann-Whitney test where appropriate.

The Wilcoxon rank-sum test was used to compare the symptoms score before and after the diet treatment. Tables were constructed for frequency and percentage. The sensitivity and specificity of the immunologic assays and their positive and negative predictive values, along with their 95% confidence intervals, were calculated by standard statistical methods.<sup>12</sup>

To minimize type I errors, a  $P$  value of less than .05 was considered statistically significant. All statistical analyses were performed using the SPSS 11.0 statistical package (Systat Software Inc., CA).

### Results

During the study period, a total of 173 patients fulfilled the Rome II criteria for IBS diagnosis, but the clinical work-up showed that 53 of them suffered from organic diseases (10 with celiac disease, 21 with lactose intolerance, 8 with small-bowel bacterial overgrowth, 6 with large colon adenoma, 6 with colon cancer, and 2 with intestinal giardiasis).

After the 4 weeks of run-in observation period, the patients of both groups underwent an elimination diet. Patients self-reporting FH also were asked to avoid ingestion and/or contact with the food(s) causing symptoms. Table 1 summarizes the number of patients with an improved (score reduction,  $>50$ ), unchanged, or worsened (score increase,  $>50$ ) symptom score on an elimination diet. In the 76 patients with unchanged or worsened symptoms on an elimination diet, a diagnosis of IBS

Table 1. Number and Percentage of IBS Patients With Scores Improved, Unchanged, or Worsened During a Four-Week Period of Elimination Diet

	Patients improved	Patients unchanged	Patients worsened
Number and percentage	N = 44 (36%)	N = 50 (42%)	N = 26 (22%)
Score at baseline	300 $\pm$ 70 <sup>a</sup>	320 $\pm$ 85	305 $\pm$ 65
Score during the diet	160 $\pm$ 55 <sup>a</sup>	295 $\pm$ 80	310 $\pm$ 70

NOTE. Symptom severity scores before and after elimination diet also are shown. Cow's milk and derivatives, wheat, egg, tomato, and chocolate were excluded in all subjects. The patients with self-reported food hypersensitivity also avoided food-causing symptoms. <sup>a</sup> $P < .0001$ , Wilcoxon rank-sum test.

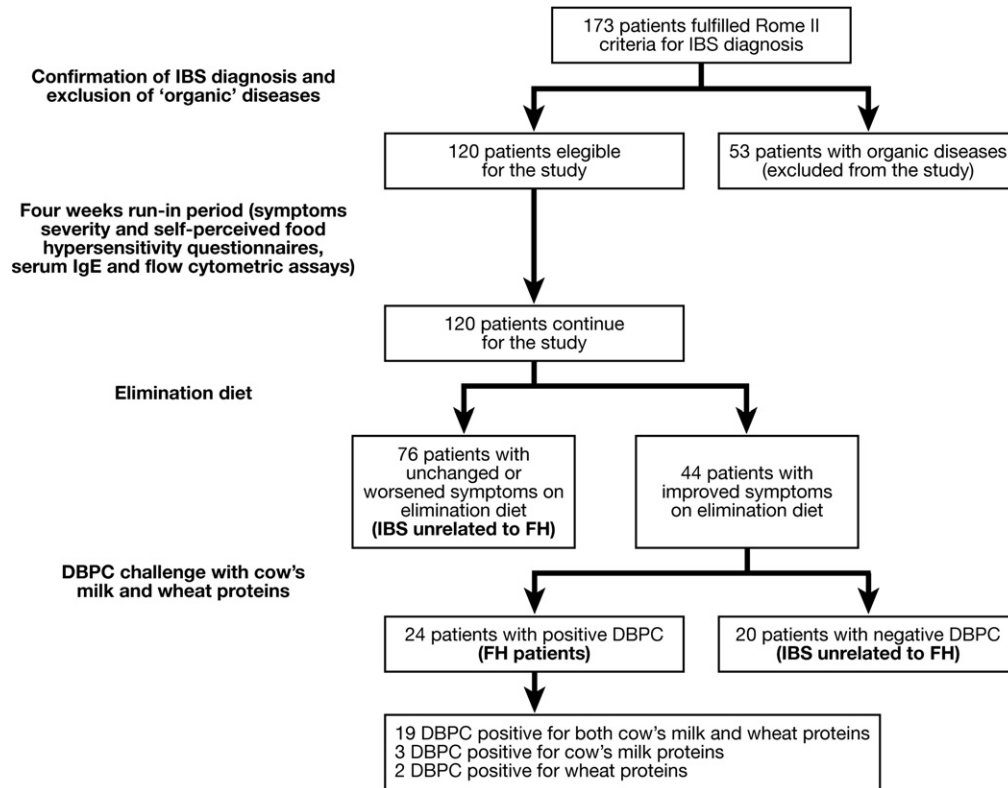


Figure 2. Number of patients included/excluded during the different phases of the study.

unrelated to FH was made. The other 44 patients showing a significant reduction in symptom score on an elimination diet underwent DBPC challenges.

Nineteen patients were positive for both cow's milk and wheat protein challenges, 3 were positive exclusively for cow's milk challenge, and 2 were positive exclusively for wheat challenge. In total, 24 patients were positive for DBPC food challenges. In all DBPC-positive patients, the symptoms (abdominal pain, bloating, diarrhea, constipation, and so forth) reappeared after a median period of 3 days (range, 1–8 d) after commencing the challenge with cow's milk or wheat proteins. Twelve of these 24 patients did not complete the challenge period with the active food owing to the severity of symptoms. None of these patients reacted on placebo administration.

The 24 patients with positive DBPC challenges to cow's milk and/or wheat proteins fulfilled the Rome II criteria for IBS diagnosis and were diagnosed with FH.

The other 20 patients, who improved on elimination diet, did not react to the DBPC challenges; these 20 subjects underwent open challenges also with egg, tomato, and chocolate and did not react. Consequently, they were diagnosed with IBS not related to FH. Figure 2 summarizes the results, according to the study design.

According to the results of the elimination diet and the subsequent DBPC challenges, the patients then were divided into 2 subgroups: group A (IBS not related to FH), including the subjects with scores unchanged or worsened on elimination diet and the patients with negative DBPC challenge for cow's milk and wheat proteins ( $n = 96$ ; 80% of the total patients included); and group B (FH), including the subjects with scores improved on elimination diet and positive to DBPC challenge

with cow's milk and/or wheat proteins ( $n = 24$ ; 20% of the total patient number).

Table 2 shows the demographic and clinical characteristics of these patient groups. IBS severity score at entry to the study was identical in both groups. However, the duration of IBS symptoms was significantly higher in patients with FH ( $P < .001$ ). Furthermore, the FH patients showed a higher frequency of self-perceived food intolerance (chi-square = 7;  $P = .01$ ) and of history of food allergy during infancy (chi-square = 5.9;  $P = .02$ ).

Table 3 shows the results of the immunologic assays in the IBS patients and in the healthy and disease controls. According to these results, Table 4 shows the sensitivity, specificity, and diagnostic accuracy of the immunologic assays in the diagnosis of FH in patients with a clinical presentation of IBS. The in vitro basophil stimulation assay showed a higher sensitivity than serum total IgE and serum food-specific IgE in the diagnosis of cow's milk hypersensitivity ( $P < .0001$  and  $.01$ , respectively) and in the diagnosis of wheat protein hypersensitivity ( $P < .0001$  and  $.01$ , respectively). Diagnostic accuracy also was higher for in vitro basophil stimulation assay:  $P$  less than  $.01$  versus serum total IgE and  $P$  less than  $.05$  versus serum food-specific IgE, both for cow's milk hypersensitivity and for wheat protein hypersensitivity diagnoses. Specificity of the in vitro basophil stimulation assay was 86% in the IBS subjects, but no false positives were found in the healthy controls, whereas in the group of the disease controls 4 patients with Crohn's disease and 5 patients with celiac disease were false positive for the in vitro basophil stimulation assay.

The patients then underwent a 2-year follow-up period, during which they were reassessed at 6-month intervals. FH pa-

**Table 2.** Demographic and Clinical Characteristics of the Patients With IBS Not Related to Food Hypersensitivity (Group A, n = 96) and of the Patients With IBS Related to Food Hypersensitivity (Group B, n = 24)

	Group A	Group B	P value
Demographic characteristics			
Age, y, mean + SD	35.2 ± 9.6	34.0 ± 9.4	NS
Sex, M/F	20/76	3/21	NS
Clinical characteristics			
Predominant diarrhea/constipation/alternating, %	60/20/20	50/26/24	NS
Median IBS symptom duration, y (range)	5.5 (1–18)	12 (1–35)	.001
Coexistent atopic diseases	28 (29%)	7 (29%)	NS
History of food hypersensitivity in infancy	6 (6%)	6 (25%)	.02
Family history of atopic disease	38 (40%)	9 (38%)	NS
Self-reported food hypersensitivity	20 (21%)	12 (50%)	.01
IBS symptom severity score at entry to the study, mean ± SD	310 ± 80	315 ± 75	NS

tients continued to follow an elimination diet with the exclusion of the foods causing the IBS symptoms and all reported the persistent disappearance of or a consistent improvement in the symptoms. Symptom score at entry to the study was 350 ± 80, at 6 months was 180 ± 50, at 12 months was 140 ± 40, at 18 months was 130 ± 50, and at 12 months was 140 ± 40. Occasional ingestion of the eliminated foods always caused the recurrence of the IBS symptoms, which was always associated with abdominal pain. Furthermore, all patients reported multiple hypersensitivity to other foods, which we evaluated with elimination diets and open challenges. Apart from cow's milk and wheat, the following foods caused IBS-like symptoms: egg (16 cases), tomato (12 cases), soy (5 cases), yeast (4 cases), pork (3 cases), prawn (3 cases), fish (3 cases), celery (3 cases), olive (2 cases), nuts (2 cases), carrot (2 cases), and meat (2 cases).

During the follow-up period, some patients underwent further examinations: colonoscopy (3 cases), esophogastrosocopy and duodenal biopsies (6 cases, which were negative). Serum assays for celiac disease (anti-tTG and EmA) were repeated in all 20 patients and were always negative.

Seventy-nine of the IBS patients without FH completed the 2-year follow-up evaluation and 17 were lost to follow-up evaluation. In most of these subjects IBS symptoms persisted periodically, despite several treatments. None of them were found to be suffering from an organic cause of symptoms.

## Discussion

IBS is a highly prevalent disorder, characterized by recurrent abdominal pain and altered bowel habits, which is associated with a marked reduction in quality of life and causes a considerable financial burden.<sup>13,14</sup>

The origin and development of IBS are unclear and genetic, neurobiological and psychosocial factors seem to be involved in the pathogenesis of the disease.<sup>15</sup> Furthermore, IBS appears to result from an interplay between susceptibility genes and impaired gut barrier functions, immunologic dysregulation, as well as bacterial and viral infections and other environmental factors.<sup>16</sup> However, given the high frequency with which patients report an association of symptoms/signs appearing or worsening with food(s) ingestion, it is relevant to systematically explore the evidence for an association of FH with the clinical manifestations of IBS.

In this study, we investigated the diagnostic reliability of the flow cytometric allergen stimulation test to discriminate IBS from FH in a group of diagnosed IBS patients, some of whom reported a self-perceived FH. To make the diagnosis of true FH, all of the patients, whether reporting or not reporting a self-perceived FH, underwent an elimination diet and then patients reporting a symptom/sign improvement after the elimination diet period underwent a double-blind, placebo-controlled oral food challenge with cow's milk proteins and then with wheat proteins.

Our results showed that approximately 20% of the IBS patients were suffering from multiple FH and the elimination diet significantly reduced symptom severity. The results of the elimination diet and double-blind, placebo-controlled oral food

**Table 3.** Results of the Immunologic Assays in the Patients With IBS Unrelated to FH (n = 96), in Those Related to Cow's Milk Protein Hypersensitivity (n = 22), in Those Related to Wheat Protein Hypersensitivity (n = 21), in the Healthy Controls (n = 40) and in the Disease Controls (n = 40)

	Increased serum total IgE	Increased serum specific IgE	Basophil stimulation test
IBS unrelated to FH			
Positive	15	14	12
Negative	81	82	84
IBS related to cow's milk protein hypersensitivity			
Positive	4	10	19
Negative	18	12	3
IBS related to wheat protein hypersensitivity			
Positive	4	9	18
Negative	17	12	3
Healthy control group			
Positive	5	4	0
Negative	35	36	40
Disease control group			
Positive	8	6	9
Negative	32	34	31

NOTE. Reference values were as follows: total serum IgE less than 100 kU/L; food allergen-specific IgE antibodies less than 0.35 kU/L; basophil stimulation test the percentage of basophils activated after incubation with antigen lower than double the percentage of basophils activated spontaneously. For CMP hypersensitivity the following antigens were used in the basophil stimulation assay: α-lactalbumin, β-lactoglobulin, casein; for wheat hypersensitivity wheat proteins were used.

**Table 4.** Sensitivity, Specificity, and Diagnostic Accuracy ( $\pm 95$  CI) of the Immunologic Assays in the Diagnosis of FH in Patients With Clinical Presentation of IBS

All IBS patients (n = 120)	Total serum IgE	Food-specific IgE	Basophil stimulation
Sensitivity in cow's milk hypersensitivity	18% (2%–34%)	45% (25%–66%)	86% (72%–100%)
Sensitivity in wheat protein hypersensitivity	19% (2%–36%)	43% (22%–64%)	86% (76%–100%)
Specificity	84% (77%–92%)	85% (78%–92%)	88% (81%–94%)
Diagnostic accuracy	72% (64%–80%)	78% (70%–86%)	87% (81%–93%)

NOTE. Statistics: sensitivity of basophil stimulation assay versus serum total IgE ( $P < 0.0001$ ), versus serum food-specific IgE ( $P < .01$ ) both in the diagnosis of cow's milk hypersensitivity and of wheat protein hypersensitivity. Diagnostic accuracy of basophil stimulation assay:  $P < .01$  versus serum total IgE and  $P < .05$  versus serum food-specific IgE, both for cow's milk hypersensitivity and for wheat protein hypersensitivity diagnoses.

challenge, considered as the gold standard in the diagnosis of FH,<sup>17</sup> show that the questionnaire-based diagnosis of FH overestimated the real disease prevalence in IBS patients. In fact, only 12 of the 32 patients self-reporting FH improved on elimination diet and reacted to the DBPC challenges. However, it also is interesting to note that a percentage of IBS patients, not self-reporting gastrointestinal symptoms specifically caused or worsened by food ingestion, had a true misunderstood FH. In fact, in our study only 50% of the FH patients were able to indicate the foods causing the symptoms. This probably is caused by 2 factors: (1) the most frequent foods causing FH and IBS symptoms were wheat and cow's milk, which are consumed daily by almost everyone in Western countries, and a prolonged period of elimination of these foods from the diet was never attempted by the patients; and (2) all patients with documented FH were suffering from multiple FH and in these cases a clinical improvement can be obtained only by following a very restricted diet.

Furthermore, the reaction to the DBPC food challenges confirmed that the relationship between food(s) ingestion and the development or worsening of gastrointestinal symptoms might not be clearly evident because the reaction often is delayed or very delayed after ingestion, thus causing underdiagnosis of the disease. This pattern of delayed reaction after food ingestion has been described previously<sup>18,19</sup> and is in agreement with the evidence that IgE does not seem to play a fundamental role in the pathogenesis of FH causing IBS-like symptoms.<sup>20</sup> For the same reason, serum total and food allergen-specific IgE antibodies assays have a low diagnostic sensitivity. Among the assays alternative to those based on IgE, serum IgG seemed useful in patients with IBS-like symptoms.<sup>10</sup> Although we have had similar experience in pediatric patients,<sup>21</sup> several investigators have noted that physiologic circulating food-specific Igs, especially IgG food-specific antibodies, can be present in apparently healthy individuals.<sup>22–24</sup>

However, an assay indicating suspected FH is of paramount importance because the elimination diet and the DBPC challenge are cumbersome and time-consuming procedures to be used only in selected patients. This is also because, in our experience, an undue elimination diet can worsen the psychological condition of IBS patients and lead to anorexia-like pictures.

In the early 1990s, the discovery of the basophile activation marker CD63 induced the development of a flow cytometric technique to analyze allergen-specific, IgE-mediated in vitro activation of basophils.<sup>25</sup> Basophils, on encountering specific allergens recognized by surface receptor Fc $\epsilon$ RI-bound IgE, up-

regulate the expression of different markers (eg, CD63 and CD203c), which can be detected by flow cytometry using specific monoclonal antibodies. At present, the most commonly used marker in basophile activation studies is CD63. In resting basophils, CD63 is expressed weakly on the surface membrane both in normal subjects as well as in patients with allergies. In contrast, CD63 is expressed with a high density on activated basophils and mirrors histamine release.

During recent years, the technique has proved to be an easily accessible method that allows the simultaneous testing of several putative allergens with a minimum amount of blood. In previous studies in vitro basophil activation has been shown to be useful in detecting "classic IgE-mediated" allergic disorders to various allergens, such as aeroallergens (cypress pollen, house dust mites), foods, hymenoptera venoms, natural rubber latex,  $\beta$ -lactam antibiotics, and muscle relaxants.<sup>7,26–29</sup> On the contrary, in our study we showed the high sensitivity and diagnostic accuracy of the detection of basophil activation in the diagnosis of FH in IBS patients, compared with a much lower sensitivity of the other IgE-mediated assays.

The clinical evidence of delayed reaction in our patients could seem absolutely independent from an IgE-mediated mechanism. However, because serum IgE participates in the activation of the basophils and in the expression of the CD63 marker on their surface, the complex pathogenetic mechanism of the IBS-like gastrointestinal manifestation of FH should be reviewed and a possible role for IgE not totally excluded.

Finally, it is noteworthy that we found a high frequency of false-positive results when patients with chronic intestinal inflammatory diseases (eg, celiac disease, Crohn's disease) were tested. This could be owing to the impaired intestinal permeability, which could favor a nonspecific basophil activation; however, the hypothesis of concomitant FH in these diseases cannot be excluded completely.

In our study, cytometric detection of basophil CD63 surface expression after stimulation with several antigens (cow's milk, egg white, egg yolk, wheat, soybean, fish, and tomato, and so forth) showed a good sensitivity, specificity, and diagnostic accuracy.

In conclusion, we suggest that in patients with IBS-like symptoms this method is feasible for making a differential diagnosis of true FH. For these patients, this test might supplement or better replace routine allergy diagnostic tests such as the skin prick test and serum total and allergen-specific IgE assay.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cgh.2009.11.010.

## References

1. Mayer EA. Clinical practice. Irritable bowel syndrome. *N Engl J Med* 2008;358:1692–1699.
2. Park MI, Camilleri M. Is there a role of food allergy in irritable bowel syndrome and functional dyspepsia? A systematic review. *Neurogastroenterol Motil* 2006;18:595–607.
3. Shepherd SJ, Parker FC, Muir JG, et al. Dietary triggers of abdominal symptoms in patients with irritable bowel syndrome: randomized placebo-controlled evidence. *Clin Gastroenterol Hepatol* 2008;6:765–771.
4. Mansueto P, Montalto G, Pacor ML, et al. Food allergy in gastroenterologic diseases: review of literature. *World J Gastroenterol* 2006;12:7744–7752.
5. Bischoff S, Crowe SE. Gastrointestinal food allergy: new insights into pathophysiology and clinical perspectives. *Gastroenterology* 2005;128:1089–1113.
6. Sampson HA, Sicherer SH, Birnbaum AH. AGA technical review on the evaluation of food allergy in gastrointestinal disorders. American Gastroenterological Association. *Gastroenterology* 2001;120:1026–1040.
7. Ebo DG, Hagendorens MM, Bridts CH, et al. In vitro allergy diagnosis: should we follow the flow? *Clin Exp Allergy* 2004;34:332–339.
8. Thompson WG, Longstreth GF, Drossman DA, et al. Functional bowel disorders and functional abdominal pain. *Gut* 1999;45(Suppl 2):43–47.
9. Iacono G, Cavataio F, Montalto G, et al. Intolerance of cow's milk and chronic constipation in children. *N Engl J Med* 1998;339:1100–1104.
10. Atkinson W, Sheldon TA, Shaath N, et al. Food elimination based on IgG antibodies in irritable bowel syndrome: a randomised controlled trial. *Gut* 2004;53:1459–1464.
11. Francis CY, Morris J, Whorwell PJ. The irritable bowel scoring system: a simple method of monitoring IBS and its progress. *Aliment Pharmacol Ther* 1997;11:395–402.
12. Feinstein A. On the sensitivity, specificity and discrimination of diagnostic tests. *Clin Pharmacol Ther* 1975;17:104–110.
13. Badia X, Mearin F, Balboa A, et al. Burden of illness in irritable bowel syndrome comparing Rome I and Rome II criteria. *Pharmacoeconomics* 2002;20:749–758.
14. Gralnek IM, Hays RD, Kilbourne A, et al. The impact of irritable bowel syndrome on health-related quality of life. *Gastroenterology* 2000;119:655–660.
15. Gwee KA, Leong YL, Graham C, et al. The role of psychological and biological factors in post-infective gut dysfunction. *Gut* 1999;44:400–406.
16. De Giorgio R, Barbara G. Is irritable bowel syndrome an inflammatory disorder? *Curr Gastroenterol Rep* 2008;10:385–390.
17. Sicherer SH, Sampson HA. Food allergy. *J Allergy Clin Immunol* 2006;117(Suppl):S470–S475.
18. Iacono G, Cavataio F, Montalto G, et al. Persistent cow's milk protein intolerance in infants: the changing faces of the same disease. *Clin Exp Allergy* 1998;28:817–823.
19. Carroccio A, Montalto G, Custro N, et al. Evidence of very delayed clinical reactions to cow's milk in cow's milk-intolerant patients. *Allergy* 2000;55:574–579.
20. Zuo XL, Li YQ, Li WJ, et al. Alterations of food antigen-specific serum immunoglobulins G and E antibodies in patients with irritable bowel syndrome and functional dyspepsia. *Clin Exp Allergy* 2007;37:823–830.
21. Carroccio A, Scalici C, Di Prima L, et al. IgG anti-betalactoglobulin in children with IBS symptoms: a valid aid to decide for the elimination diet. *Gut* 2005;53:1459–1464.
22. Haddad ZH, Vetter M, Friedmann J, et al. Detection and kinetics of antigen-specific IgE and IgG immune complexes in food allergy. *Ann Allergy* 1983;51:255.
23. Husby S, Oxelius VA, Teisner B, et al. Humoral immunity to dietary antigens in healthy adults. Occurrence, isotype and IgG subclass distribution of serum antibodies to protein antigens. *Int Arch Allergy Appl Immunol* 1985;77:416–422.
24. Kruszewski J, Raczka A, Klos M, et al. High serum levels of allergen specific IgG-4 (as IgG-4) for common food allergens in healthy blood donors. *Arch Immunol Ther Exp* 1994;42:259–261.
25. Bochner BS. Systemic activation of basophils and eosinophil markers and consequences. *J Allergy Clin Immunol* 2000;106(Suppl):S292–S302.
26. Moneret-Vautrin DA, Sainte-Laudy J, Kanny G, et al. Human basophil activation as measured by CD63 expression and LTC4 release in IgE mediated food allergy. *Ann Allergy Asthma Immunol* 1999;82:33–40.
27. Eberlein-König B, Varga R, Mempel M, et al. Comparison of basophil activation tests using CD63 or CD203c expression in patients with insect venom allergy. *Allergy* 2006;61:1084–1085.
28. Nettis E, Colanardi MC, Dambra PP, et al. Flow cytometric basophil activation test: detection of CD63 expression as a useful aid to diagnosis of latex allergy. *Ann Allergy Asthma Immunol* 2006;97:715–716.
29. Sanz ML, Gamboa PM, Antepara I. Flow cytometric basophil activation test by detection of CD 63 expression in patients with immediate type reactions to beta lactam antibiotics. *Clin Exp Allergy* 2002;32:277–286.

### Reprint requests

Address requests for reprints to: Professor Antonio Carroccio, Ospedale Civili Riuniti, Sciacca (AG), Italy. e-mail: acarroccio@hotmail.com; fax: (39) 0925-84757.

### Acknowledgments

Antonio Carroccio was responsible for the study design, collection and interpretation of the data, and writing the manuscript; Ignazio Brusca was responsible for the study design, immunology assays, statistics, collection and interpretation of the data, and revising the manuscript; Pasquale Mansueto was responsible for patient recruitment and collection and interpretation of the data; Giuseppe Pirrone was responsible for patient recruitment and collection and interpretation of the data; Maria Barrale was responsible for patient recruitment, immunology assays, and collection and interpretation of the data; Lidia Di Prima, Giuseppe Ambrosiano, Giuseppe Iacono, and Maria Letizia Lo Spalluti were responsible for patient recruitment and collection and interpretation of the data; Maria Stella La Chiusa was responsible for immunology assays and collection and interpretation of the data; and Gaetana Di Fede was responsible for patient recruitment and collection and interpretation of the data. All of the authors declare that they have seen and approved the final version of the manuscript.

### Conflicts of interest

The authors disclose no conflicts.