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

ANTONIO CARROCCIO,[*,‡] IGNAZIO BRUSCA,[§] PASQUALE MANSUETO,[‡] GIUSEPPE PIRRONE,[‡] MARIA BARRALE,[§] LIDIA DI PRIMA,[‡] GIUSEPPE AMBROSIANO,[‡] GIUSEPPE IACONO,[‖] MARIA LETIZIA LOSPALLUTI,[‖] STELLA M. LA CHIUSA,[§] and GAETANA DI FEDE[¶]

[*]Internal Medicine, Ospedali Civili Riuniti, Sciacca (Agrigento), Sciacca, Italy; [‡]Internal Medicine, Department of Clinical Medicine and Emerging Diseases, [¶]Department of Medical Oncology, University of Palermo, Italy; [§]Clinical Chemistry, "Buccheri La Ferla" Hospital, Palermo, Italy; [‖]Pediatric Gastroenterology, "Di Cristina" Hospital, Palermo, Italy

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.

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

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.[4]

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,[5] 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) has shown a good diagnostic reliability.[6]

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.[7] However, no studies have evaluated the diagnostic accuracy of the test in FH diagnosis in patients with IBS-like clinical symptoms.

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.

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[8] (see Supplementary Materials and Methods). Furthermore, organic gastrointestinal disorders were excluded by an accurate work-up (described later).

After inclusion, the patients underwent a clinical evaluation that included a detailed family and personal clinical history and a physical examination. Afterward, 2 predesigned questionnaires were administered to all the patients: the first was re-

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

1542-3565/10/$36.00
doi:10.1016/j.cgh.2009.11.010

garding the type and severity of the symptoms, the second was regarding any possible self-perceived FH.

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 was 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-H2 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 Supplementary Materials and Methods). 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

After enrollment in the study, the patients completed the Symptoms Severity and Food Hypersensitivity questionnaires (see Supplementary Materials and Methods), 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 physicians, 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 dieticians. Patients who specified a symptom/sign improvement after the elimination diet period underwent a double-blind, placebo-controlled (DBPC), oral food challenge first with cow's milk proteins and then with wheat proteins.[9]

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 hematochemical 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.[10,11]

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; Sigma-Aldrich, St. Louis, MO) or xylose (Aldrich 245321; Sigma-Aldrich), respectively. DBPC for wheat proteins was performed with capsules coded as C or D containing wheat (Fluka, BCR563; Sigma-Aldrich) or xylose, respectively. Capsule A or B was given for 2 consecutive weeks, and then after 1 week of washout the patients received the other capsule for another 2 weeks. After 1 week of washout, capsule C or D was given for 2 consecutive weeks, then after another week of washout, the patients received the other capsule 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 7 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.