A NOVEL APPROACH TO CREATE AN IBS SPECIFIC IMMUNOASSAY FOR IDENTIFYING FOODS THAT CAUSE AN ELEVATED IgG IMMUNE RESPONSE IN IBS PATIENTS

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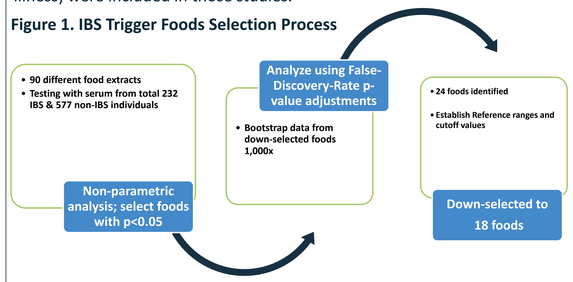
Introduction

To address an unmet need and develop a tool to help identify triggers of IBS patient symptoms, an immunoassay was developed to determine which dietary antigens (foods) were more likely to generate an elevated immune response in subjects diagnosed with IBS (Rome III) over healthy controls. IBS trigger foods were identified by comparing the IgG immunoreactivity between IBS and non-IBS patients (p-values ≤ 0.05). Individual IgG cut-offs were established for each IBS-specific food using a 95% confidence interval such that 95% of patients in a non-IBS healthy population would test negative.

Methods

The inFoods® IBS (ELISA) was developed by comparing the serum IgG levels against commonly ingested foods in cohorts of confirmed IBS and non-IBS patients. Three different cohorts with a total of 232 IBS patients (Rome III, all subclasses) and 577 non-IBS healthy control patients (no GI or other illness) were included in those studies.

Figure 1. IBS Trigger Foods Selection Process



90 foods were initially screened in a first cohort. Each food-specific dataset was bootstrap resampled 1,000 times. Within each bootstrap replicate, the 95th percentiles of the control signal scores were determined. From this data, 40 foods were selected and tested with additional patient cohorts and 50,000 bootstrapped re-samplings to differentiate residual signal scores between IBS subjects and healthy controls. Further, to account for any lack of homogeneity of variances, the Satterthwaite approximation was used for the denominator degrees of freedom. The p-values were then adjusted using the Benjamini-Hochberg[1] (1995) procedure to control for the False Discovery Rate (FDR). Foods were then ranked according to their 2-tailed FDR multiplicity-adjusted p-values and only foods with adjusted p-values equal to or lower than an FDR threshold (p≤ 0.05) were deemed significant. Figure 1 depicts the food selection process. Subsequent studies followed the same analysis using down-selected foods from the previous cohort. The final 18 foods (Table 1) were then selected in a reference range and cut-off study, and subsequently used in a clinical utility study.

ELISA assay: ELISA plates were prepared using food extracts following Biomerica Inc's. (Irvine, CA) instructions for extract preparation and ELISA manufacture. ELISA plates were subsequently blocked and stored until use. On the day of testing, plates were incubated with serum samples from individual patients and processed using standard ELISA testing protocols. Color intensity in the respective wells was measured in an ELISA plate reader and is directly proportional to food-specific IgG levels in the sample.

Results

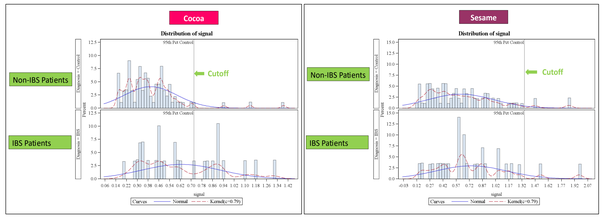
Reference ranges and cut-off values were determined following Clinical Laboratory Standards Institute guideline CLSI C28-A3[2].

1. For each healthy control (non-IBS) sample, the food specific IgG concentration was calculated.
2. Rank sum and Mann-Whitney tests (Figures 2-3) were employed to test the significance between non-IBS and IBS, using p<0.05 as the criterion and Box & Whisker plots were generated.
3. 18 different foods (Table 1) met the p-value criteria. Reference Ranges were established, and cutoff values calculated (Table 3).

Table 1: Final 18 foods associated with elevated IgG response in IBS

Food extract	P-value	Food extract	P-value
Cabbage	0.0002	Orange	0.0007
Cane Sugar	0.0001	Pineapple	<0.0001
Cocoa	<0.0001	Rye	0.0026
Corn	<0.0001	Soybean	0.0308
Cow's Milk	0.0128	Black Tea	0.0041
Grapefruit	0.0004	Walnut	<0.0001
Honey	<0.0001	Wheat	0.0002
Lemon	0.0013	Whole Egg	<0.0001
Oat	0.0001	Yeast	<0.0001

Figures 2-3: Histograms and Mann-Whitney tests for IBS positive foods (Cocoa) and food that is not linked to IBS (Sesame).



Data analysis also showed that IBS patients have elevated IgG levels to a Median of 5 (95% CI: 4-8) different foods in the panel, while non-IBS patients were found to have elevated IgG levels to only 1 food.

Table 2: Median IgG positive Foods in IBS vs. non-IBS Patients

Median Number of Positive Foods		
Group	Median # Foods Positive	95% CI for Median
IBS	5	4-8
Non-IBS	1	1-1

Results

The reference values for each food extract were calculated following the non-parametric percentile method. MedCalc (v18.2.1) was used to generate the reference values reported at 95% reference interval (U/mL). The 95th percentile reference interval, right-sided, was then used to establish the cut-off value for the 18 food extracts (Table 3).

Table 3: Cut-off values for 18 selected foods

Food extract	Cut-off values (IgG U/mL)	Food extract	Cut-off values (IgG U/mL)
Cabbage	46.7	Orange	19.6
Cane Sugar	30.3	Pineapple	145.9
Cocoa	93.4	Rye	62.5
Corn	141.5	Soybean	52.3
Cow's Milk	175.0	Black Tea	36.2
Grapefruit	19.3	Walnut	24.4
Honey	52.1	Wheat	43.2
Lemon	26.9	Whole Egg	74.2
Oat	40.7	Yeast	80.4

Clinical Utility:

The utility of the novel ELISA assay was tested in a double-blinded clinical study with IBS patients (Rome IV) at Mayo Clinic, Beth Israel Deaconess Medical Center Inc., Harvard Medical School Teaching Hospital, the University of Michigan, Houston Methodist Hospital, and others. Patients were randomized to either a treatment/true or a sham diet and instructed to eliminate specific foods identified by the ELISA at enrollment. Following a two-week washout period, various parameters were monitored for 8 weeks, including daily assessments of bowel habits, bloating, and Abdominal Pain Intensity (API), and weekly assessments for IBS Adequate Relief (AR), Subject Global Assessment of Relief (SGA), and Global Improvement Scale (GIS).

Linear mixed and logistic regression modeling of endpoints in the intent-to-treat (ITT) population for all IBS patients and for IBS C+M patients are summarized below (Table 4 and Figure 4).

Table 4: p-value Key Assessment Criteria (significant values in bold)

Baseline to 8 weeks p-value				
	All Subj	IBS-C	IBS-M	IBS-C+M
API (Lin. Mix. Model)	0.0718	**0.0256**	0.1200	**0.0139**
Bloating (LMM)	0.0827	0.1200	0.0900	**0.0214**
SGA (LMM)	**0.0093**	**0.0100**	**0.0154**	**0.0010**
GIS (LMM)	**0.0302**	**0.0090**	0.1200	**0.0020**
IBS-SSS (LMM)	0.1100	0.2300	0.3600	0.1400

Results

Figure 4: Bloating IBS C+M Population Change from Baseline

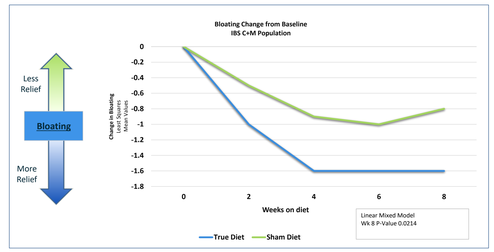


Table 5: Responder Analysis for IBS-C, IBS-D, and IBS-M

	True Diet Response	Sham Diet Response	True minus Sham Diet	p-values
All	58%	48%	10%	0.0246
Women	58%	43%	15%	0.0239

Table 5: For all subjects, there was 10% greater IBS-API Responder rate (IBS-API with ≥30% improvement) on the True diet vs the Sham diet. Whereas a 15% responder rate was noted in women between the True diet and the Sham diet groups.

Discussion

A novel ELISA to measure elevated IgG levels against 18 food extracts that are relevant in IBS patients has been developed. The 18 food extracts were selected using three different cohorts of IBS and non-IBS patients and applying rigorous statistical methods in the selection process. IgG reference ranges and cutoff values for each of the 18 foods were determined.

The utility of the assay was subsequently tested in a multicenter double-blinded placebo-controlled clinical study with IBS patients (Rome IV).

IBS-C+M patients in the true/treatment diet arm had significant individual symptom relief for abdominal pain (p=0.0139) and bloating (p=0.0214). The food-specific IgG based elimination diet in comparison to a sham diet significantly improved global endpoints for GIS (p=0.0302) and SGA (p=0.0093) while IBS-API (p=0.0718) and IBS-Bloating (p=0.0827) assessments in all IBS subtypes were not significant. For Abdominal Pain Intensity (API) responder endpoint of >30% improvement in pain, patients with IBS-Constipation and IBS-Mixed in the treatment diet arm had a statistically significant improvement over patients in the sham/placebo diet arm (p-value 0.0246 (all), 0.0239 (women)). The improvement for patients in the treatment arm versus the placebo arm is considered clinically significant.

The results indicate that food-specific IgG based elimination diets using this ELISA with 18 specific foods may offer benefits to patients with IBS and can help reduce IBS symptoms such as pain, bloating, diarrhea or constipation.

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References

1. Benjamini, Y. and Hochberg, Y. (1995) Controlling the False Discovery Rate—A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society Series B—Methodological, 57, 289-300.
2. CLSI C28-A3: Clinical Laboratory Standards Institute- Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory, 3rd Edition; October 2010

IgG-based Elimination Diets for Patients with IBS: Results From a Prospective, Multi-Center, Double-Blind, Placebo-Controlled Trial

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1: Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA; 2: Michigan Medicine, Ann Arbor, MI; 3: Mayo Clinic, Jacksonville, FL; 4: Gastroenterology Clinic of San Antonio, San Antonio, TX; 5: Mayo Clinic Arizona, Scottsdale, AZ; 6: Houston Methodist Hospital, Houston, TX; 7: University of Texas Health Science Center, Houston, TX; 8: Biomerica, Inc., Irvine, CA

Introduction

Diet modification can improve symptoms in patients with IBS, however, outcomes following self-directed elimination diets are poor. Food intolerances/ sensitivities are common in patients with IBS but the role of IgG antibodies in identifying patients with food sensitivities is controversial. This study was designed to evaluate the utility of a novel, proprietary IgG-based elimination diet to improve symptoms in IBS patients. ClinicalTrials.gov Identifier: NCT03459482

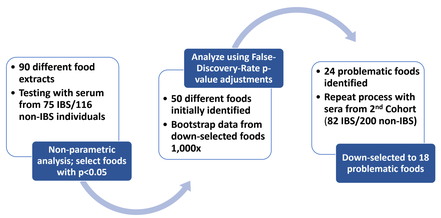
Methods and Materials

Adults with IBS (Rome IV), all subtypes, were enrolled from 6 centers into a 2-week baseline period. Patients who tested positive ≥1 food in an IgG panel (inFoods®, Biomerica, Irvine, CA) and who reported an average daily IBS abdominal pain intensity (API) score (0-10) between ≥3 and ≤7.5 were randomized to either a treatment diet arm or a sham (placebo) diet arm for 8 weeks. Patients in the treatment diet arm were instructed to eliminate foods to which they tested positive. Patients in the sham diet arm were instructed to eliminate foods to which they tested negative. The sham diet arm was balanced to the treatment diet arm with respect to the number of foods eliminated and self-reported frequency of consuming a particular food. Daily assessments included bowel habits, bloating, and API, as well as weekly assessments for IBS Adequate Relief (AR), Subject Global Assessment of Relief (SGA), and Global Improvement Scale (GIS). Linear mixed and logistic regression modeling of endpoints in the intent-to-treat (ITT) population is presented for all IBS patients and for non-IBS-D patients.

Background

InFoods® IBS is unique in that the panel of foods and cutoffs for positive and negative results are specific for IBS patients. (See Chart 1) Briefly, 90 different food extracts were tested with confirmed ROME III positive IBS patient samples and confirmed non-IBS, non-GI disease patient samples. Using non-parametric statistical analysis, 50 foods with unadjusted p-values of <0.05 for increased IgG values in IBS patients (vs. non-IBS patients) were identified. Data were then bootstrapped 1,000 times and further analyzed using False-Discovery-Rate (FDR) p-value adjustments. Multiple cohorts were then analyzed. As a result, 18 foods were selected for the assay. Cutoff values were set using a 95% confidence interval for each food.

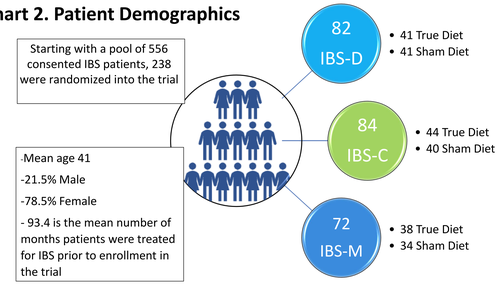
Chart 1. InFoods® IBS Trigger Foods Selection Process



Results

Results: Of the 556 patients with IBS (all subtypes) who entered the study, 222 met eligibility criteria and were randomized to the treatment diet or sham diet. IBS patients in the treatment diet arm had a greater decrease in IBS-API and IBS-Bloating scores from baseline compared to patients in the sham diet arm (IBS-API p=0.0718; IBS-Bloating p=0.0827, these p-values did not reach the threshold of p< 0.05). However, patients in the treatment diet arm did have a significant improvement in GIS and SGA (GIS p=0.0302; SGA p=0.0093) compared to the sham diet arm. Non-IBS-D patients (i.e., IBS-C + M) (n=149) had the greatest decrease from baseline (IBS-API p=0.0139; IBS-Bloating p=0.0214) as well as for global measures (GIS p=0.0020;SGA p=0.0010). No significant adverse events were noted during the study.

Chart 2. Patient Demographics



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Baseline to 8 weeks p-value	All Subj	IBS-C	IBS-M	IBS-C+M
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SGA (LMM)	0.0093	0.0100	0.0154	0.0010
GIS (LMM)	0.0302	0.0090	0.1200	0.0020
IBS-SSS (LMM)	0.1100	0.2300	0.3600	0.1400
AR logistic mixed model	0.0600			
API 30% improvement	0.0246 p-value	10% difference between treatment and placebo (15% female only)		
IBS-SSS 50pt improvement	0.13 P-value	IBS SSS Responder		

Results

Figure 1. API IBS non-D Population Change from Baseline

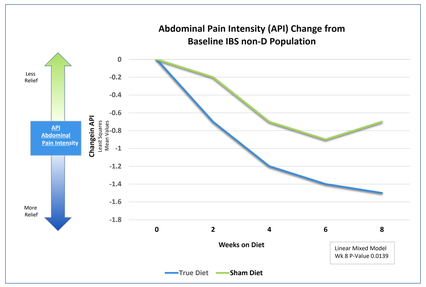


Figure 2. Bloating IBS non-D Population Change from Baseline

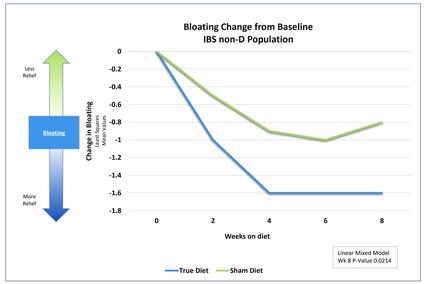
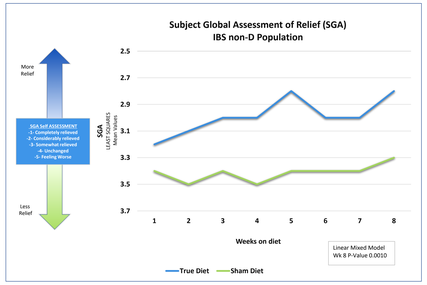


Figure 3. SGA Relief IBS non-D Population Change from Baseline



Results

Figure 4. GIS IBS non-D Population Change from Baseline

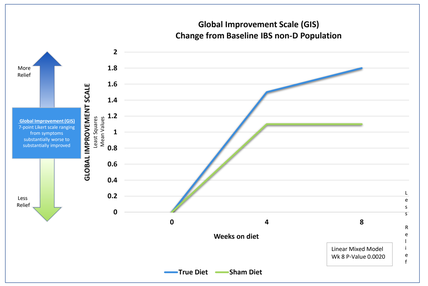


Figure 5. SGA IBS All Population Change from Baseline

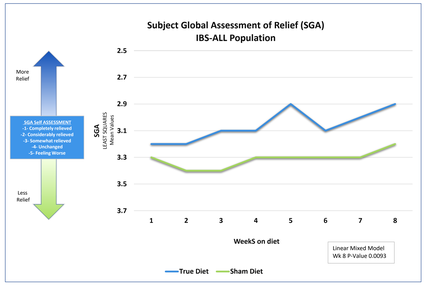
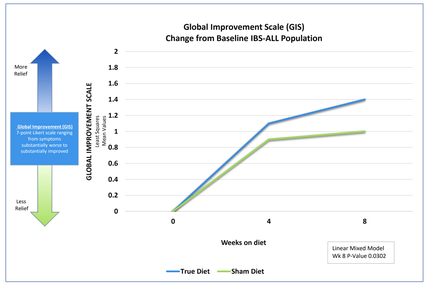
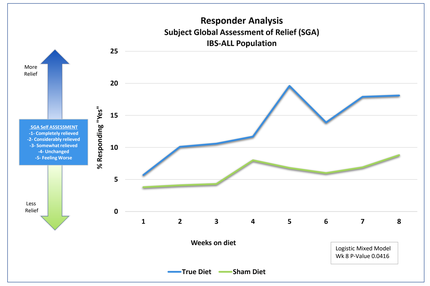


Figure 6. GIS IBS All Population Responder Relief



Results

Figure 7. SGA IBS All Population Responder Relief



Discussion

These results suggest that IgG based elimination diets using a novel, proprietary diagnostic (inFoods® IBS) with 18 specific foods to guide therapy may offer benefit to patients with IBS.

Results of this study warrant further study.

Conclusions

A novel IgG based elimination diet in comparison to a sham diet significantly improved global endpoints (both GIS and SGA) and showed a trend for improvement in IBS-API (p=0.0718) and IBS-Bloating (p=0.0827) in all IBS subtypes.

Non-IBS-D (IBS-C + M) patients in the treatment diet arm had significant individual symptom relief for abdominal pain (p=0.0139) and bloating (p=0.0214).

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References

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