Title: Novel Blood Test to Predict Safe Foods for Infants and Toddlers With Food Protein-induced Enterocolitis Syndrome (FPIES)

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A NOVEL BLOOD TEST TO PREDICT SAFE (NON-TRIGGER) FOODS FOR INFANTS AND TODDLERS WITH FOOD PROTEIN-INDUCED ENTEROCOLITIS SYNDROME (FPIES).

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VERSION 2; *.**

I. PROTECTION OF HUMAN SUBJECTS

1. *Risks to Human Subjects*

A. Human Subjects Involvement, Characteristics, and Design

Describe and justify proposed human involvement

The **objective** of this project is to validate a blood test that can distinguish safe from trigger foods for protein-induced enterocolitis syndrome (FPIES).

Background: FPIES is an allergic disease that leads to repetitive profuse vomiting in response to solid food. In severe cases, when patients fail to thrive, FPIES requires nasogastric tube installment or total parenteral nutrition (TPN). The major challenge in managing FPIES is the identification of safe (non-trigger) foods in a timely manner, while avoiding repetitive allergic reactions and recovery periods. This study proposes a solution to the problem by developing a new blood assay that screens a large number of foods (more than 20) in a culture plate. Its purpose is to rapidly predict what the safe foods are. The outcome will overcome the time-lapse required to identify such safe foods, which could normally extend past a year or longer, by which time the child may have failed to thrive or developed food aversions due to association with negative experiences.

FPIES leads to repetitive hospital and ER visits with 2,064 encounters occurring at the University of Michigan Health System (UMHS) in the past year (from 11/01/17 until 11/01/18; data obtained using Datadirect for cohort discovery results; FPIES ICD10 K52.2). The latter number of encounters only comprises newly diagnosed patients.

In this study, FPIES patients will be recruited from the UMHS patient cohort. From 11/01/17 – 11/01/18, this cohort comprised 223 newly diagnosed patients at UMHS. Two sub-groups will be identified as follows: *i)* children exhibiting FPIES reactions to 2-3 foods, *ii)* children exhibiting FPIES reactions to 4 or more foods. The reason for separating patients into these two groups is due the existence of two separate groups [one with 2-3 food triggers, and another with more than 4 food triggers] as previously described by the UMHS Allergists who care for FPIES patients (Volertas S., Hudson E., McMorris G., and G. Sanders*. Annals of Allergy, Asthma & Immunology* 121:S119 2018).

The plan is to enroll up to 20 patients with FPIES: up to10 exhibiting reactions to 2-3 foods, and up to 10 exhibiting FPIES reactions to 4 or more foods. Recruitment will take place at the University of Michigan Allergy Clinics, and University of Michigan Pediatric outpatient clinics.

Hypotheses:

1. Our blood test that we developed will distinguish safe from trigger foods for FPIES patients.
2. The mechanism of FPIES involves innate immunity and the identified heatmap pattern for trigger foods will remain after foods are tolerated.

Specific aims:

1, Assess the assay's precision by its predictive accuracy in distinguishing safe foods from trigger foods.
2. Evaluate the reliability of the test when using a panel of standardized, frozen foods vs freshly prepared foods
3. Demonstrate persistence of the immune reaction to trigger foods by comparing results of the test after trigger foods are tolerated to the initial results

Exploratory aim:

1. Gain further insight into the pathophysiology of FPIES through single cell RNASeq

Statistical Design: A random-effects logit model will be used to model the binary outcome (safe or trigger food) as a function of the 9 biomarker measurements in the assay. The random effect in the logit model will take into consideration of the correlated data measured within the same subject. A cluster Receiver operating characteristic (ROC) curve analysis will be used to assess the precision of the assay. Specifically, we will compute the area under the cluster ROC curve (AUC), along with a 95% confidence interval (CI). The assay is predictive if the lower limit of the 95% CI is above 0.5, which is the null value indicating no predictive ability.

Brief description of the proposed study

FPIES is a severe food allergy to a wide variety of foods in babies and toddlers. Usually, the symptoms resolve between the ages of 3 to 5 years old. However, the children are at risk of failing to thrive, and requiring a nasogastric tube or TPN. The objective of the study is to validate a newly developed blood assay that will rapidly predict safe (non-trigger) foods for the affected children. In our preliminary data (Case Study HUM00155216), the blood assay safely predicted more than 10 consecutive safe foods without triggering an allergic response. Prior to the assay, allergic reactions and ER visits were repeatedly occurring.

Subject Recruitment: Subjects will be recruited through the University of Michigan Allergy Clinics, and University of Michigan Pediatric outpatient clinics:

1. Patients may be referred by the clinician caring for the patient.
2. Pre-screening by reviewing FPIES visits in the past 2 years will be performed. Data Direct, EMERSE and billing codes may be used to identify potential subjects. The patient's primary allergist or pediatrician will be informed about study team's contact with the identified patients.
    - Patients will then be contacted by email and/or telephone by the clinical members of the study team to explain the study and determine whether they are interested in participating. Opt out information will be provided in the email and by phone contact.
3. Patients may self-refer:
    - The study will be listed in UMHealthResearch.org
    - Flyers describing the study, with study team contact information, will be posted in the participating clinics.

Inclusion Criteria:

1. Patients aged 1 month – 7 years old with a physician diagnosis of FPIES:
    a. Group 1: Documented reactions to 2-3 trigger foods with recurrent delayed vomiting.
    b. Group 2: Documented reactions to 4 or more trigger foods with recurrent delayed vomiting.

Exclusion Criteria:

1. Patients without a physician diagnosis of FPIES.
2. Patients who are currently on medications that suppress the immune system.

3. Patients who do not have at least 2 trigger foods identified.
4. Patients who have a history of an organic GI disease (e.g., inflammatory bowel disease, celiac disease, biliary disorders, bowel resection), cardiac, pulmonary, neurologic, renal, endocrine, or gynecological pathology
5. Lack of parental or guardian informed consent.

Study Design

1. Potential participants will be contacted by email and / or telephone after pre-screening of physician-diagnosed FPIES patients who have visited the UM allergy clinics within the past three years, or who are currently in contact with the UM allergy clinic nutritionist. An option for the patients to opt out will be provided. The patient's primary allergist or pediatrician will be informed about study team's contact with the identified patients. Patients may be referred by their primary allergist during a clinic visit.
2. If a parent/guardian expresses interest in the study, they will be sent/given a copy of the informed consent to review.
3. An appointment will be scheduled with a study team member at the UM allergy clinics.
4. The protocol will be discussed face-to-face with parent/guardian with an opportunity to ask questions and review the informed consent document. After written informed consent is signed by one parent/guardian, study procedures can begin. Parent/guardian will be given a copy of the signed informed consent and it will be imaged into the chart.
5. Parent/Guardian will be asked to fill out Questionnaire One at the time of entry into the study.
6. Parent/Guardian will be instructed regarding scheduling the blood draw for analysis:
    a. Only known "safe foods" (foods that do not induce repetitive vomiting) are to be consumed for one week before blood draw.
    b. Blood draw will be performed at the MLabs Blood Draw Stations at Michigan Medicine outpatient clinics.
7. Patient will have 6 ml of blood drawn for analysis:
    a. 5.5 ml of blood for testing of potential food reactions
    b. 0.5 ml of blood for RNA/DNA analysis, which will be stored.
8. After blood draw, diet will be managed per the primary clinician and nutritionist.
    a. Parents will be given Questionnaire Two to fill out detailed information about all foods introduced until the results of the assay are received.
    b. During that time, food introduction will continue as directed by primary allergist and/or nutritionist
9. Blood will be analyzed by the PI in a research lab at the University of Michigan to determine differences in the PI's assay between potentially tolerated and not tolerated foods (see description of laboratory test lines 216-232)
10. After identification of potentially "safe foods" to be introduced in the future, the information will be given to the clinical study team and child's clinician, who will contact parents by phone or email (parental preference).
    a. Parents will be asked to detail current nutritional status, including foods tolerated or not tolerated since the initial blood draw. Results will be recorded by the study team and the parents will be asked to return Questionnaire Two.
    b. After discussion within the clinical team (including clinical research team and child's clinicians), parents will be instructed to introduce new foods identified as potentially safe into the diet, one at a time for seven days.
    c. Results of these trials will be recorded on Questionnaire Three and returned to the study team 3 month intervals.

11. A second blood draw will be performed, at least 2 weeks after the initial blood draw. 6 cc will be drawn:
    a. To explore the consistency of a standard panel of foods vs individually prepared foods
    b. To perform additional analyses including single cell RNASeq (scRNA-seq) on WBCs following treatment with foods.
12. Subjects will be followed at 3 month intervals with review of tolerated foods, number and types of known triggers assessed. When a subject is tolerating two or more triggers foods for 3 or more months:
    a. Questionnaire Four will be given to fill out and return
    b. Subject will have 6 mL drawn for analysis
        i. 5.5 mL to rerun the initial assays
        ii. 0.5 mL for RNASeq on WBCs following treatment with foods.

Study Grid

	Baseline visit - Day 0	Lab Day 0-14	Lab ≥2 weeks after initial blood draw	At home 1-3 months	3 up to 36 months	At home every 3 months up to 36 months	≥ 2 trigger foods tolerated
Consent	X						
Questionnaire 1	X						
Blood Draw		X	x				X
Phone/email/in-person to inform of potentially tolerated foods.				X			
Questionnaire 2				X			
Testing Foods Found as tolerable					X		
Questionnaire 3						X	
Questionnaire 4						X	X

B. Sources of Materials

The research material obtained from the human subjects

1. 6ml of blood in a K2 EDTA (lavender top) blood tube withdrawn by specialized phlebotomists in the blood draw unit (0.5 ml of this will be used to isolate DNA for potential future sequencing under a separate IRB). White blood cells (WBCs) will be isolated from 5.5 ml of blood using ACK buffer lysis of red blood cells, within 1 hour of blood collection, as performed previously for the Case Study (HUM00155216). Blood will be kept on ice in the K2 EDTA tubes during the waiting period (< 1 hour). WBCs will be plated in 24 well plates, and exposed to different food homogenates for 3 hours. Total RNA will be extracted, and RT-qPCR for our gene panel will be used to generate the heatmap.

A second blood draw may be requested from a limited number of patients, at least 2-4 weeks after the initial blood draw, for scRNA-seq. In this case, the blood will be prepared in the same manner as for the initial draw above, but instead of extracting RNA, samples will be submitted to the UofM Advanced Genomics Core for them to perform scRNA-seq on the samples. The data will be analyzed using the cloupe software by 10x Genomics.

A third blood draw may occur (or second blood draw if scRNA were not performed for that patient) after the patient recovers from several triggers. The patient will be screened at 3 month intervals, so the third (or second if no scRNA-seq) will be performed for this purpose at least 3 months after the initial blood draw. In this case, the cells will be prepared in the same manner as the initial blood draw, but treated with

only a limited number of safe, trigger foods, and outgrown trigger foods.

2. At the termination of the protocol samples and data will cease to be accessed, or otherwise accessed again after IRB approval transferred to another existing protocol. There are no plans to destroy records or samples.

Protection of Information:

All data will be kept on REDCap, a password-protected, HIPAA compliant, web-based application developed by Vanderbilt University to capture data for clinical research and create databases and projects. The databases use instruments such as surveys and forms as research capture tools. Projects are self-sufficient and secure databases that can be used for normal data entry or for surveys across multiple distinct time points. Only study team members will have access to this data.

1. A master list that includes identifiable data will be kept in a REDCap "Study Housekeeping" database that includes subject number, patient name, parent names, MRN, contact information including address, email, phone number, documentation of blood draw, samples saved and filling out of questionnaires.
2. All data variables will be kept in a separate "Research Data" REDCap database. Subjects will be identified only by subject number in this database. Data will include:
    a. Results of blood tests
    b. Questionnaire answers
    c. Age
    d. Number of food reactions
    e. Identified trigger foods
    f. Identified tolerated foods.
3. Data downloaded for analysis will have only subject number included.
4. Stored RNA, DNA or cDNA samples will contain only the subject number without personal identifiers.
5. Parents/guardians may request that stored blood samples be destroyed at the end of the study.

C. Potential Risks

Describe potential risks

The risk in this study arises due to incorrect predictions by the test. The current test being validated is not an FDA-approved or cleared test. However, current standard of care recommends which foods to trial in a random fashion, and incorrect predictions frequently occur leading to reactions. Hence this study will not pose an added risk to the current standard of care. The indication of what to trial will be communicated to the patients by the allergist and nutritionist as currently occurs in the standard of care. However, the only difference is that the allergist and nutritionist will in this study use this assay for indication regarding what foods to trial. If a food is falsely identified as safe by the assay but triggers a reaction, then these reactions will be managed by the nutritionist and allergist as per current standard of care for failed trials.

Blood draw from infants and toddlers is a safe procedure with minimal risks. Trained and experienced phlebotomists at UMHS perform blood draws on infants and toddlers routinely. The procedure may cause anxiety for the infant/toddler. The test can result in a small bruise or mild soreness for the child at the site of blood draw. The bruise can last for a few days.

A risk of breach of confidentiality always exists in all studies.

Describe alternate treatments and procedures

The parent/child can proceed with introduction of new foods per guidance of the nutritionist. This is based on historically better tolerated foods in the general FPIES population and may or may not apply to the patient.

2. Adequacy of protection against risks

A. Recruitment and Informed Consent

Plan for recruitment

All FPIES patients will be recruited from the University of Michigan Allergy Clinics, and University of Michigan Pediatric outpatient clinics. The patients will be identified by pre-screening of the UM FPIES patient cohort. Pre-screening will be performed by members of the Division of Allergy and Immunology at Michigan Medicine. Patients will not be contacted by the study team until pre-approval is obtained from the primary allergist or pediatric clinician. Suitable FPIES patients who fulfill the inclusion and exclusion criteria will be contacted by email and / or phone. An option to opt out will be provided. If patients are interested in participating, a meeting with the study team will be scheduled at the UM allergy clinics. The patients will be presented with the consent form by the study team member to consider consenting for the study and the blood draw procedure. Patients will be told that their decision whether to participate in the study will not affect their clinical care.

Describe the circumstances of consent

A study coordinator or clinical study team member familiar with the study protocol will obtain informed consent for all participants. Consent/assent will be obtained prior to any study measures/questionnaires. Each participant will receive a verbal and written explanation of the purposes, procedures, risks, and potential benefits of the study in language appropriate for the individual.

B. Protections Against Risk

Planned procedures for protecting against risk

1. The risk of a false result will be managed by the allergist and nutritionist as per current standard of care in managing reactions to failed trials.

2. The risk from blood draw is minimal and routinely performed by the phlebotomists at UMHS.

3. All data variables will be kept in a separate “Research Data” REDCap database. Subjects will be identified only by subject number in this database.

4. A separate “Housekeeping” REDCap database will be used to link the patient identifiable information and the study number.

3. Potential benefit of the proposed research to the research subjects and others

Potential benefits

There may be direct benefit to the patient by the potential of identifying tolerated foods, thus avoiding the adverse reactions of frequent emesis and potential dehydration, as well as the trauma parents encounter when giving their infant a food that causes such reactions. There may be benefit to the larger community of FPIES patients if this protocol indicates the new assay can identify tolerated vs non-tolerated foods.

Discuss why risks to subjects are reasonable in relation to benefits

Patients already encounter false predictions and failed trials with allergic reactions regularly. The indication from the assay is expected to predict safe foods at a higher rate than the guesswork utilized in the current standard of care.

4. Importance of the knowledge to be gained

Discuss importance of the knowledge to be gained

Validation of this test has the potential to modify patient care strategy for patients with FPIES. As such, random guessing of safe foods would be replaced by a scientific “guide” to what might be hypoallergenic to the body’s immune cells using this test.

Discuss why the risks are reasonable in relation to the importance of the knowledge to be gained

Currently, trialing food is random. Obtaining a guide for what might be a safe food, by utilizing our proposed assay, would be invaluable. Incorrect predictions cannot pose more risk than random selection of foods to trial. Hence, there is only benefit to be gained if the test is predictive or partially predictive.

II. INCLUSION OF WOMEN AND MINORITIES

Inclusion of Women

This study will include children of both genders.

Inclusion of Minorities

The disease is overwhelmingly predominant in White Caucasian children (Based on personal communication with allergists caring for FPIES patients at UofM clinics, and the following published abstract: R. Tarrant and A. and Byrne. Clinical Presentation and Food Allergens Associated with Food Protein-Induced Enterocolitis Syndrome – a frequently misdiagnosed rare form of gastrointestinal food hypersensitivity. *European Society of Pediatric Gastroenterology, Hepatology and Nutrition* 62(1); DOI: 10.13140/RG.2.1.1621.2087). Therefore, for the purpose of feasibility of meeting the required proposed patient numbers for statistical analyses, we have chosen a majority of White Caucasian subjects for this initial study. If patients from other ethnicities – who do not usually have FPIES enroll – they will not be excluded from the study.

Inclusion of Children

Children with FPIES will be included in this study.

III. PLANNED ENROLLMENT TABLE

Total Planned Enrollment: 20

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Females**	**Males**	**Total**
Hispanic or Latino	1	1	2
Not Hispanic or Latino	9	9	18
Ethnic Category: Total of All Subjects *	10	10	20
Racial Categories			
American Indian/Alaska Native			
Asian	1	1	2
Native Hawaiian or Other Pacific Islander			
Black or African American	1	1	2
White	8	8	16
Racial Categories: Total of All Subjects *	10	10	20