Brief report

Lymphocyte stimulation test for diagnosing hen's egg yolk–induced enterocolitis syndrome

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Background: There is currently little research into factors predicting the results of an initial diagnostic oral food challenge (OFC) test for food protein–induced enterocolitis syndrome (FPIES).
Objective: The present study aimed to identify predictors of the diagnosis of hen's egg yolk-induced FPIES (HEY-FPIES).
Methods: The present monocentric study was performed at Tokyo Metropolitan Children's Medical Center and included patients who underwent hen's egg yolk OFC (HEY-OFC) between March 2018 and March 2023 to assess for HEY-FPIES. The baseline characteristics of the groups and HEY-OFC positivity or negativity were then compared. Univariate analysis was conducted by using the Mann-Whitney *U* test or Fisher exact test. Receiver operator characteristic analysis was used to create probability curves.
Results: In total, 35 patients were analyzed; of these, 17 were HEY-OFC–positive. No significant difference was observed between the HEY-OFC–positive and HEY-OFC–negative groups in terms of background factors except for the HEY-LST value, which was significantly higher in the HEY-LST group ($P = .027$). Receiver operator characteristic analysis demonstrated that the area under the curve for HEY-OFC positivity using the HEY-LST value was 0.719 (95% CI = 0.541-0.897). The statistically optimal cutoff value for the HEY-LST was 610%, which had a clinical sensitivity and specificity of 64.7% and 83.3%, respectively.
Conclusions: The present study demonstrated that the HEY-LST may be a useful predictor of the result of an initial OFC for HEY-FPIES. (J Allergy Clin Immunol Global 2023;2:100138.)

Key words: *Hen's egg yolk, FPIES, LST, oral food challenge*

Abbreviations used
AUC: Area under the curve
FPIES: Food protein–induced enterocolitis syndrome
HE: Hen's egg
HEW: Hen's egg white
HEY: Hen's egg yolk
IQR: Interquartile range
LST: Lymphocyte stimulation test
OFC: Oral food challenge
ROC: Receiver operator characteristic

TABLE I. Oral food challenge test and final dosing procedures

OFC characteristic	HEY dose	HEW dose
Setting		
Step 1	1 g	1.5 g
Step 2	4 g	6 g
Step 3	10 g	15 g
Final dose		
For patients aged <2 y	10 g	15 g
For patients aged ≥2 y	20 g	30 g

OFCs were conducted by using the open, single-dose method in a hospital setting. The attending physician determined the OFC setting after considering each patient's clinical course. After step 2, the dose was increased at home as follows: half of the final amount 3 times, followed by three-fourths of the final amount 3 times, followed by completion of the total amount 3 times. HEY and HEW that had been boiled for 20 minutes were used in each OFC.

INTRODUCTION

Food protein–induced enterocolitis syndrome (FPIES) is a type of non–IgE-mediated gastrointestinal food allergy. Most patients with acute FPIES present with recurrent vomiting within 1 to 4 hours and/or diarrhea within 24 hours after ingesting the triggering food.[1] The common triggers include cow's milk, grains, fish, meat, and vegetables.[1] Of late, the incidence of hen's egg (HE)–induced FPIES (HE-FPIES) has been increasing in Japan, with HE yolk (HEY) being the chief causative antigen in HE-FPIES.[2-4]

Historical diagnostic criteria based on the current international guidelines are widely used to diagnose FPIES.[5] However, a previous study reported that as many as 11 of 21 oral food challenges (OFCs) for diagnosing FPIES (52%) yielded a negative result,[6] casting some doubt on the accuracy of the data and suggesting that the false-positive rate might be high if the FPIES diagnosis is based solely on a history of multiple concurrent gastrointestinal symptoms. Therefore, a definitive diagnosis of FPIES should be based on the results of OFCs, even if they are somewhat invasive. However, there are currently no diagnostic biomarkers for FPIES, and studies reporting potentially useful predictors of OFC

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TABLE II. Patient characteristics and comparison of clinical factors between the HEY-OFC–positive group and HEY-OFC–negative group

Characteristic	Total (n = 35)	HEY-OFC result		*P* value
		Negative (n = 18)	Positive (n = 17)	
Male sex, no. (%)	18 (51.4)	9 (50.0)	8 (47.1)	1.000
Age of onset of HEY-FPIES (mo), median (IQR)	8.0 (7.0-9.0)	7.0 (7.0-8.0)	8.0 (7.0-9.0)	.241
Participants meeting international consensus diagnostic criteria, no. (%)†	17 (48.6)	7 (38.9)	10 (58.8)	.400
Age at HEY-OFC (mo), median (IQR)	14.0 (12.0-18.0)	14.5 (13.0-17.5)	14.0 (11.0-18.0)	.728
Duration to HEY-OFC from HEY-FPIES onset (mo), median (IQR)	6.0 (3.5-11.0)	6.5 (5.0-11.5)	5.0 (3.0-9.0)	.517
Atopic dermatitis, no. (%)	10 (28.6)	6 (33.3)	4 (23.5)	.789
Immediate food allergy, no. (%)	2 (5.7)	1 (5.6)	1 (5.9)	1.000
FPIES other than HEY, no. (%)	3 (8.6)	1 (5.6)	2 (11.8)	.959
History of asymptomatic ingestion of HEY, n (%)	33 (94.3)	17 (94.4)	14 (100.0)	1.000
Total IgE level (IU/mL), median (IQR)	13.3 (7.8-36.3)	15.0 (8.5-30.7)	11.3 (6.8-37.0)	.908
HEW-specific IgE level (kU_A/L), median (IQR)	0.34 (0.05-2.42)	0.52 (0.05-5.5)	0.33 (0.05-0.81)	.905
HEY-specific IgE level, (kU_A/L), median (IQR)	0.17 (0.05-0.73)	0.13 (0.05-0.86)	0.18 (0.05-0.36)	.837
OVM-specific IgE level, (kU_A/L), median (IQR)	0.05 (0.05-0.09)	0.05 (0.05-0.58)	0.05 (0.05-0.05)	.367
HEY-LST (%), median (IQR)	466 (264-754)	383 (209-481)	666 (378-878)	.027*
HEW-LST (%), median (IQR)	302 (190-492)	291 (191-371)	441 (191-533)	.373
Duration to LST measurement from HEY-FPIES onset (mo), median (IQR)	3.0 (2.0-7.5)	3.5 (2.0-7.0)	3.0(2.0-9.0)	.776
Duration to HEY-OFC from LST measurement (mo), median (IQR)	2.0 (1.0-3.0)	2.0 (1.0-3.0)	1.0(1.0-3.0)	.270

P value was calculated by using the Mann-Whitney *U* test for continuous variables and the Fisher exact test for percentages or proportions.
*Statistically significant.
†Meeting the international consensus diagnostic criteria based on clinical history (Nowak-Węgrzyn et al[5]).

outcomes for FPIES are scarce. Thus, the present study aimed to evaluate the clinical predictors of OFC results for HEY by analyzing the medical records of patients who visited Tokyo Metropolitan Children's Medical Center between March 2018 and March 2023 and underwent an OFC with HEY. This study was conducted in accordance with the principles of the Declaration of Helsinki and the ethical guidelines of Japan and was approved by the ethics committee at Tokyo Metropolitan Children's Medical Center (approval no. 2022b-130).

HEY-FPIES was diagnosed by using OFCs performed with a single dose of boiled HEY (1/20-1/5 of 1 HEY). Patients with a negative result received increasing doses of HEY at home or during OFCs (up to half of 1 heated HEY). Patients with a positive result or those who had never ingested HE white (HEW) received an OFC with HEW (1/40-1/2 of 1 heated HEW). Table I summarizes the OFC procedure.

Blood was drawn between the patients' first visit to our department and the OFC to conduct a sensitization and lymphocyte stimulation test (LST) (SRL, Tokyo, Japan). The serum levels of total IgE, HEW-specific IgE, HEY-specific IgE, and HE ovomucoid–specific IgE were measured (ImmunoCAP, Thermo Fisher Scientific/Phadia), and an LST for HEY and HEW was performed. The HEY-LST and HEW-LST results were analyzed as described previously.[7] In the LST, lymphocytes are centrifuged and cultured with mitogen and ^{3}H-thymidine to measure thymidine uptake during DNA synthesis. The stimulation index is calculated by dividing the number of cells stimulated using mitogen per minute by the number of cells per minute that were not stimulated by mitogen in a negative control from the same subject. In the present study, HEY and HEW were used as the mitogen source (allergen) after boiling for 20 minutes.

Demographic and clinical data were expressed as medians (interquartile ranges [IQRs]). Univariate analysis of the 2 groups was conducted by using the Mann-Whitney *U* test for continuous variables. The Fisher exact test was used to compare percentages or proportions. *P* values less than .05 were considered statistically significant. Receiver operating characteristic (ROC) curves were generated for the HEY-LST. Diagnostic performance was evaluated by using the area under the curve (AUC). The optimal cutoff for a test was estimated by the minimum distance between the corresponding point on the ROC curve and the point 1, 0. The ROC analysis demonstrated sensitivity, specificity, and positive predictive value for the optimal cutoff point and drug allergy cutoff of 180%.[8] R for Mac, version 2022.02.3 (R Foundation for Statistical Computing, Vienna, Austria), was used for all statistical analyses.

RESULTS AND DISCUSSION

In total, 35 patients were analyzed. Table II summarizes the patient characteristics. Of the 35 patients, 18 (51.4%) were male. The median age at HE-FPIES onset was 8.0 months (IQR = 7.0-9.0 months). HEY-OFC yielded positive results for 17 patients (48.6%). All of the patients were able to ingest HEW without an allergic reaction during HEW-OFC or when at home. There was no statistically significant difference in the characteristics of patients in terms of the HEY-OFC results except for the HEY-LST value (Table II). Fig 1 shows that the median (IQR) HEY-LST (%) value was significantly higher in the HEY-OFC–positive group (666 [range 78-878]) than in the HEY-OFC–negative group (383 [range 209-481]) (P = .027). The median durations from HEY-FPIES onset to LST measurement and from LST measurement to HEY-OFC were 3.0 months and 2.0 months, respectively.

ROC analysis using the HEY-LST value and the HEY-OFC–positive results (Fig 2) demonstrated an AUC of 0.719 (95% CI = 0.541-0.897) (Table III). The statistically optimal cutoff for the HEY-LST value was 610%, which had a clinical sensitivity and specificity of 64.7% and 83.3%, respectively. On the other hand, when a drug allergy cutoff value of 180% was used,[8] the

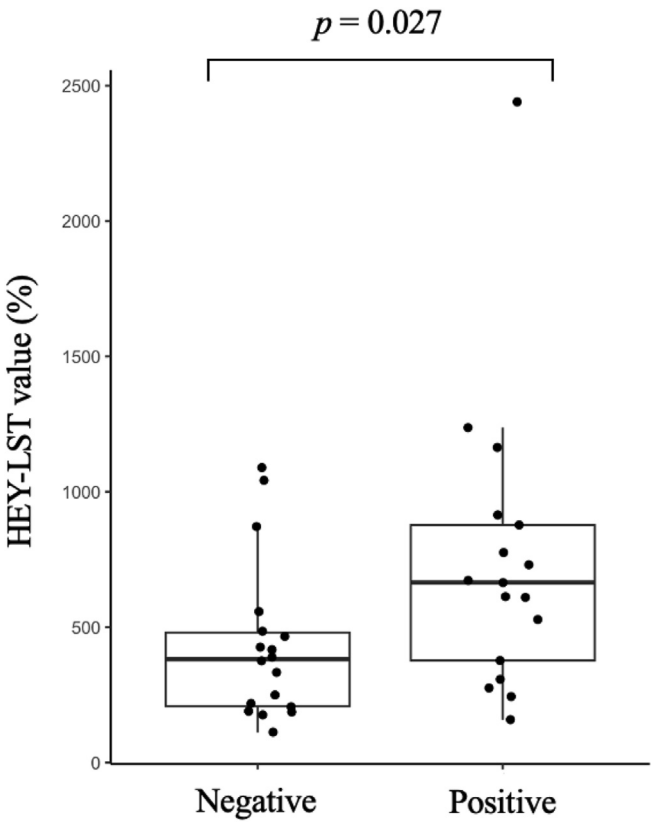

FIG 1. Comparison of the HEY-LST value between the HEY-OFC–positive and the HEY-OFC–negative groups.

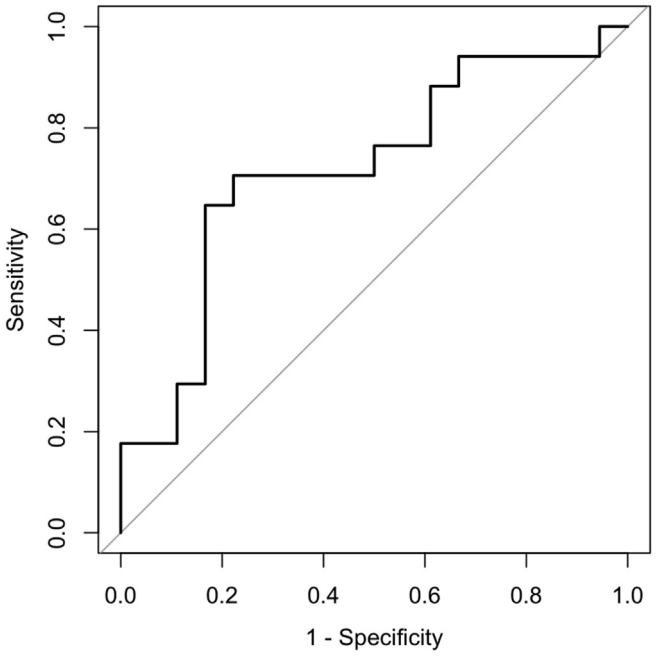

FIG 2. ROC curves for predicting HEY-OFC results using the HEY-LST.

sensitivity and specificity were 94.1% and 0.06%, respectively (Table III).

The LST, especially for κ-casein, is known to be useful for diagnosing intestinal cow's milk allergy.[9] Although several previous case reports have indicated the utility of the LST in evaluating acute solid FPIES for non–cow's milk antigen,[7,10,11] as of yet there are no studies investigating its use for predicting OFC results in patients with suspected acute solid FPIES. Therefore, the present study investigated the utility of the LST for diagnosing HEY-FPIES, which has been on the rise in Japan.[2] Our study found a statistically higher HEY-LST value in the HEY-OFC–positive group than in the HEY-OFC–negative group, with an AUC of 0.719. These findings indicate that the HEY-LST has the potential to be a useful predictor of HEY-OFC result in patients with suspected HEY-FPIES. At the very least, our study demonstrated that the drug allergy cutoff of 180% is inappropriate for use in predicting HEY-OFC result. The strength of the LST is that it can be performed easily even in daily clinical practice regardless of the patient's clinical condition. However, there are some limitations to its use in children: it uses antigens that are not yet standardized, large amounts of peripheral blood are required for the test, and the culture time is relatively long (5-7 days).[12] Further research is needed to establish the clinical utility of the LST for diagnosing FPIES. Moreover, the LST is not intended specifically for FPIES. Thus, exploration of the utility of other antigen-specific tests targeting neutrophils and natural killer cells, which are thought to be involved in the FPIES mechanism,[13] is warranted.

TABLE III. Diagnostic performance of the HEY-LST

Parameter	HEY-LST	
AUC	0.719	
95% CI	0.541-0.897	
Cutoff value	610	180
Sensitivity (%)	64.7	94.1
Specificity (%)	83.3	0.06
Positive predictive value (%)	78.6	48.5

The absence of an asymptomatic ingestion history is considered a negative predictor of the initial FPIES OFC result,[14] but this is controversial because an asymptomatic ingestion history of HEY is common among patients with HEY-FPIES, as described in previous reports.[15,16] Indeed, almost all of the patients in our study had a history of asymptomatic ingestion of HEY, as shown in Table II; thus, the absence of this factor had no predictive value for the HEY-OFC results in our study.

The present study has several limitations. First, the number of cases included was too small. A larger number of cases is needed for a definitive conclusion regarding utility of the HEY-LST. However, despite the small sample size, our analysis of the diagnostic performance of the HEY-LST yielded a statistically significant result. Second, partly because of the impact of coronavirus disease 2019 (COVID-19), the interval between the HEY-OFCs and HEY-FPIES onset differed in each patient. The timing of the HEY-OFC was not standardized and might therefore have been affected by restrictions on hospitalization and hospital visits as a result of the COVID-19 pandemic. To analyze the utility of the HEY-LST accurately, a cohort with consistent intervals between HEY-OFC and HEY-LST administration is necessary. Third, the HEY-LST and HEW-LST values in subjects with no allergies were not evaluated. These values in healthy infants must be determined to establish reference values.

In conclusion, the present study demonstrated that the HEY-LST has the potential to be a useful predictor of the initial OFC results for HEY-FPIES. Further research studies with larger

cohorts are needed to confirm the utility of the LST in identifying biomarkers of FPIES.

DISCLOSURE STATEMENT

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

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Clinical implications: Although no biomarkers useful for diagnosing FPIES have been identified as of yet, the LST may be able to predict the results of the initial OFC for HEY-FPIES.

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