

Accepted Manuscript

Urinary PGDM, a prostaglandin D₂ metabolite, is a novel biomarker for objectively detecting allergic reactions of food allergy

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PII: S0091-6749(18)30995-3

DOI: [10.1016/j.jaci.2018.06.032](https://doi.org/10.1016/j.jaci.2018.06.032)

Reference: YMAI 13513

To appear in: *Journal of Allergy and Clinical Immunology*

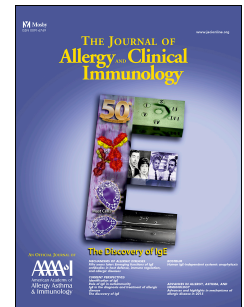
Received Date: 28 December 2017

Revised Date: 13 June 2018

Accepted Date: 27 June 2018

Please cite this article as: Inagaki S, Maeda S, Narita M, Nakamura T, Shimosawa T, Murata T, Ohya Y, Urinary PGDM, a prostaglandin D₂ metabolite, is a novel biomarker for objectively detecting allergic reactions of food allergy, *Journal of Allergy and Clinical Immunology* (2018), doi: 10.1016/j.jaci.2018.06.032.

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TITLE PAGE

Letter to the Editor

**Urinary PGDM, a prostaglandin D₂ metabolite, is a novel biomarker for
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40 Funding

41 This study was supported by a Grant-in-Aid for Scientific Research A from the Japan

42 Society for the Promotion of Science (grant number: 17921666) and Medical Research

43 and Development Programs Focused on Technology Transfers: Development of

44 Advanced Measurement and Analysis Systems from Japan Agency for Medical

45 Research and development, AMED (grant number: 17935758).

46

47 **Conflicts of interest:** Y. Ohya has been supported by the Japan Agency for Medical

48 Research and development (AMED), during the conduct of the study; grants from

AMED, grants and personal fees from National center for child health and development, grants from Ministry of Health, Labor and Welfare, grants from Environmental Restoration and Conservation Agency, grants from National Institute for Environmental Studies, personal fees from Shiseido, personal fees from Maruho, personal fees from MDD (Merck Sharp and Dohme), personal fees from Kyorin Pharmaceutical, personal fees from Taiho Pharma, personal fees from Glaxo Smith Kline, personal fees from Sysmex, personal fees from Kyowa Hakko Kirin, personal fees from Mitsubishi Tanabe Pharma.

T. Murata has been supported by AMED, and the Japan Foundation for Pediatric Research.

S. Inagaki, S. Maeda, M. Narita, T. Nakamura and T. Shimosawa have no relevant conflicts of interest.

Word count: 999

Capsule Summary

The concentration of urinary PGDM, a prostaglandin D₂ metabolite, can be used as a novel biomarker for detecting allergic reactions caused by food allergy objectively in a non-invasive manner.

73 **Key words**74 urinary PGDM, prostaglandin D₂, food allergy, oral food challenge, biomarker

To the Editor:

In recent years, the number of patients with food allergies (FA) has been increasing and the prevalence in children is now estimated to be between 6.5% and 8%.^{1,2} A diagnosis of FA based on their clinical history and/or the values of food-specific IgE is not accurate. An oral food challenge (OFC), especially double-blind placebo-controlled oral food challenge (DBPCFC), is said to be the most reliable test for the accurate diagnosis of FA, however, it is inevitably accompanied by ambiguous subjective symptoms and false-positive reactions. In addition to them, interpretations of these symptoms can be influenced by observer bias³. It is essential to identify biomarkers that can detect allergic reactions caused by an OFC objectively. The serum concentrations of mast cell mediators, including platelet-activated factor, histamine and tryptase, tend to be elevated during anaphylaxis.^{4,5} Especially, the serum tryptase seems to be a useful biomarker and was reported to peak within one hour after onset of anaphylaxis.⁵ From practical points of view, blood sampling from children when they receive OFCs is not easy to be carried out in routine clinical settings. Recently, we discovered that 11,15-dioxo-9 α -hydroxy-,2,3,4,5-tetranorprostan-1,20-dioic acid (PGDM), major prostaglandin D₂ metabolite levels in urine, were correlated with the severity of symptoms in an oral allergen-induced FA mouse model.⁶ The aim of the present study was to verify the usefulness of urinary PGDM as a practical biomarker for assessing the severity of acute allergic reactions in human children with FA during the OFC.

Children with suspected FA who were scheduled to undergo OFCs at the Division of Allergy, National Center for Child Health and Development, were recruited. OFCs were applied to them as described in detail in the Methods section in online repository. The

severity of the immediate allergic reactions was classified into Grades 1-5 by using the clinical criteria for the diagnosis of anaphylaxis published by Sampson.⁷ Patients without clinical symptoms were categorized as Grade 0. Urine samples were collected before the OFC and at 2, 4, 6, 8 and 18 hours after the OFC. The urinary PGDM levels were measured using LC-MS/MS, and the concentration of urinary PGDM was expressed as ng/mg Cre.⁶ This study received ethical approval from the Institutional Ethics Committee before patient recruitment.

In total, 157 children underwent OFCs, and 112 of them were assessed as positive based on their clinical signs and symptoms caused by OFC (Table E1, Table E2). There were no significant differences in pre-OFC urinary PGDM levels between the patients' with negative OFC results (Grade 0) and those with positive OFC results (Grade 1-4) (mean $\pm$ SD: 1.25 ± 0.72 vs. 1.32 ± 0.82 ng/mg Cre, respectively; $P > 0.05$, Mann-Whitney test) (Figure 1). The urinary PGDM levels of the patients with positive OFC results began to increase at 2 hours after the start of the OFC and peaked at 4 hours ($P < .001$, Mann-Whitney test) (Figure 1, Figure E1), while the urinary PGDM levels of the patients with negative OFC results did not increase ($P > .05$, Mann-Whitney test) (Figure 1, Figure E1). The Jonckheere-Terpstra trend test demonstrated that the urinary PGDM levels at 4 hours after OFC were positively correlated with the grade of severity ($P < .001$) (Figure 2A). As for the types of food, there were no significant differences in the urinary PGDM level in each Grade between egg white and milk, the majority of foods in this study (Table E3). Noteworthy are the elevated urinary PGDM levels in patients who only presented slight subjective symptoms without any objective sign. Of the 45 patients with Grade 1 reactions, 19 patients had only oral pruritus or

discomfort. The urinary PGDM levels in these patients (3.66 ± 3.01 ng/mg Cre) were significantly higher than those with Grade 0 reactions (1.48 ± 1.11 ng/mg Cre) ($P < .01$, Mann-Whitney test) (Figure 2B). To establish the best cutoff value for the prediction of allergic reactions based on the urinary PGDM level, a receiver-operating characteristic (ROC) curves were created (Figure 2C and Figure E2). Especially in regards to the ability to predict some reactions, an ROC curve analysis for patients with Grade 0 versus Grade 1-5 showed that when the cutoff value for urinary PGDM was optimally set at 2.25 ng/mg Cre, the area under the curve, the sensitivity, and the specificity were 0.812, 0.705 and 0.946, respectively (Figure 2C). Regardless of the types of foods, the levels of urinary PGDM were not correlated with those of allergen specific IgE (sIgE) (Figure E3 and Table E4) while sIgE is used to assess likelihood of clinical reactivity. Moreover, we followed two Grade 0 patients whose urinary PGDM levels were the highest and the second highest among the samples collected from Grade 0 patients. One patient (6.21 ng/mg Cre) with a very slight abdominal discomfort during the OFC experienced a continuous abdominal pain after challenging the same dose at home. Another patient (4.52 ng/mg Cre) with no symptoms during the OFC developed a cough, rhinorrhea and general urticaria after a half-dose of the same food. The other remaining patients with Grade 0 did not have any medical record of symptoms experienced at home.

Our data demonstrated that the urinary PGDM concentration at 4 hours after OFC was associated with the severity of immediate allergic reactions. Although an elevated urinary PGDM level was reported in adult patients with systematic mastocytosis, in which mast cells are activated,⁸ the present report indicated for the first time that the urinary PGDM level increased after an immediate allergic reaction in children with FA. Our study revealed that the urinary PGDM concentration was useful not only for

146 assessing the severity of allergic reactions in patients who received an OFC, but also for
147 detecting subtle allergic reactions objectively in patients who received threshold doses
148 of offending foods at their OFCs. van der Valk et.al. reported that 23% of children with
149 negative OFCs failed reintroduction at home because of allergic reactions to the food.⁹
150 Threshold of the offending food might be influenced by many factors in daily life and
151 vary in a certain range after an OFC. The urinary PGDM level can objectively detect
152 allergic reactions in intaking offending food in a non-invasive manner even if caregivers
153 overlook subtle signs of children caused by an OFC. Although the open challenge
154 design of OFC is our study limitation, the study suggests that the urinary PGDM level
155 can be used to detect allergic reactions followed by subtle signs or symptoms.

156
157 In summary, the urinary PGDM is a novel biomarker to detect immediate allergic
158 reactions in children with FA objectively in a non-invasive manner and its main uses are
159 to distinguish non-reactors from false-positive reactions and confirm allergic reactions
160 of patients who visit the emergency department setting.

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Acknowledgment

We thank Osamu Natsume, Miyuki Ohta, Mayako Saitoh, Akio Yoshida, Tomohisa Andoh, Aki Gen, Kiwako Yamamoto and Yumiko Miyaji for their clinical support in oral food challenges in our hospital.

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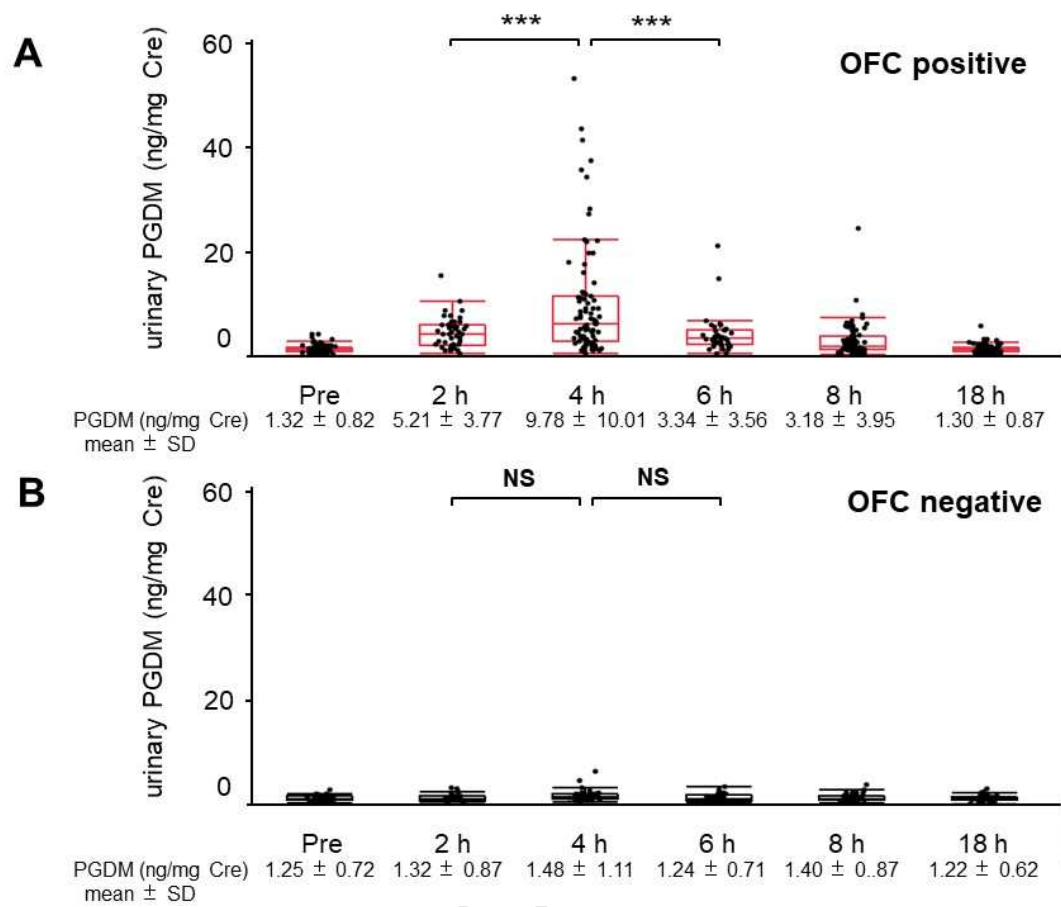
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Figure Legends**Figure 1. Urinary PGDM concentration before and after an OFC.**

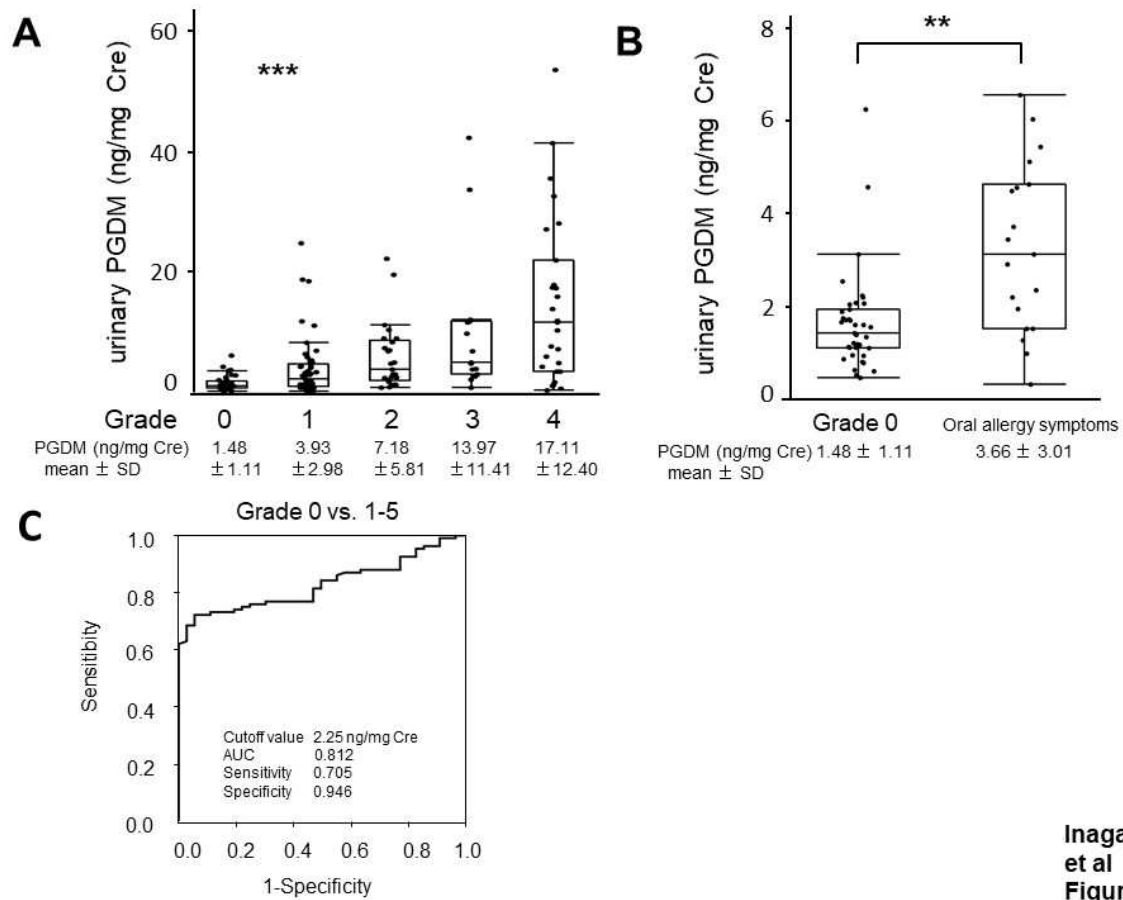
A, Time-dependent changes in patients with positive OFC results. **B**, Time-dependent changes in patients with negative OFC results. *** $P < .001$, Mann-Whitney U test. NS, not significant.

Figure 2. Relationship between urinary PGDM concentration and mild or severe immediate allergic reactions.

A, Comparison of urinary PGDM concentrations at 4 hours after an OFC according to each grade. *** $P < .001$, Jonckheere-Terpstra trend test. **B**, Relationship of urinary PGDM concentration at 4 hours after an OFC between patients with Grade 0 versus those with only oral allergy symptoms. ** $P < .01$, Mann-Whitney U test. **C**, ROC curve for urinary PGDM levels in patients with Grade 0 (OFC negative) versus Grade 1-5 (OFC positive).



Inagaki
et al
Figure 1



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Letter to the Editor

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METHODS

Study population

One hundred and fifty-seven patients (boys, 88; mean age, 7.4 years; range, 3-14 years) underwent urinary PGDM measurements during OFCs performed between December 2014 and August 2015. All the children were suspected FA or had already been diagnosed as FA by a physician based on a positive history of reactions to foods and/or detectable specific IgE or skin prick tests.

Measurement of urinary PGDM (urinary tetranor-PGDM)

Urinary PGDM was measured by LC-MS/MS, as described previously.^{E1} Briefly, the urine (0.4 mL) was diluted up to 1 mL with water acidified by 1 mol/l HCl (final pH ~ 3), and then 5 ng of d6-tetranor-PGDM (Cayman Chemical) were added as the internal standards. The samples were loaded on a Sep-Pak Vac 3 cc cartridge (Waters, Milford, MA) for solid-phase extraction. The analyte was eluted from the cartridge with 3 mL of ethyl acetate, dried under vacuum, dissolved in 100 µl of 10% acetonitrile in water, and injected for LC-MS/MS analysis. LC separation was carried out using a mobile phase consisting of 0.02% acetic acid (solvent A) and acetonitrile (solvent B).

Chromatographic elution was performed using a gradient of 5%–30% solvent B for the first 10 min followed by 5 min at 95% solvent B at a flow rate 0.4 mL/min. The LC-MS/MS was operated in the negative ion mode. Transitions monitored were m/z 327 → 309 for the endogenous materials (tetranor-PGDM) and m/z 333 → 315 for the internal standards (d6-tetranor-PGDM). Urinary creatinine concentration was measured using a kit (Wako, Tokyo, Japan), and the urinary tetranor-PGDM was expressed as ng/mg creatinine. The stability of urinary PGDM was examined by using normal urine

with exogenous PGDM and urine from patients with food allergies (Table E65 and E76).

Oral food challenge (OFC)

OFCs were performed as open food challenges. Before starting the OFCs, children were examined by physicians to evaluate symptoms of atopic dermatitis, bronchial asthma, urticaria, and allergic rhinitis. If a child exhibited exacerbated or unstable allergic symptoms, the OFC was postponed to ensure the safety and accuracy of the challenge. We carried out open OFCs based on the PRACTALL consensus^{E2} to try to demonstrate objective symptoms by increasing doses if open OFCs elicited only subjective symptoms. Children were asked to discontinue the use of antihistamines and leukotriene antagonists for 3 days and the use of oral steroids for 7 days before OFCs to ensure the accuracy of the OFCs. Before the administration of each dose, each child was examined by a physician. Skin and lung examinations, vital signs, and any treatment administered during the OFC were recorded in the clinical record. Foods were administered under the supervision of allergists at 20-min-intervals for up to five times during an OFC. The challenges were terminated if persistent objective symptoms or recurrent severe subjective symptoms appeared, but the challenges were continued if the patient only showed oral allergy symptoms. Children were observed by physicians for 2 hours after the last food challenge. The most frequently administered foods in the OFCs were egg white (n = 50), milk (n = 43), wheat (n = 17), and peanuts (n = 14). The grams of protein administered in this challenge consisted of egg white: 188.7 ± 170.3 mg (mean $\pm$ SD); milk: 545.8 ± 573.4 mg; wheat: 1115.9 ± 822.6 mg; and peanut: 676.6 ± 412.9 mg. This study received ethical approval from the Institutional Ethics Committee before

patient recruitment. All the participants provided verbal assent, and their parents provided written informed consent.

Severity of allergic reactions

The severities of the immediate allergic reactions were classified from Grade 1 to 5, based on the clinical criteria for the diagnosis of anaphylaxis published by Sampson.^{E3} Children with no clinical symptoms were categorized into Grade 0. Briefly, Grade 1 patients had acute allergic reactions with local cutaneous involvement (pruritus, flushing, urticaria, angioedema, or oral pruritus) and no other organ involvement. Those with Grade 2 reactions had acute manifestations of generalized cutaneous involvement, gastrointestinal symptoms (nausea and/or single emesis) or respiratory symptoms (nasal congestion and/or sneezing). Patients with Grade 3 reactions had severe manifestations with gastrointestinal symptoms (repetitive vomiting), respiratory symptoms (rhinorrhea, marked congestion, and/or sensation of throat pruritus or tightness), or cardiovascular symptoms (tachycardia: increase > 15 beats/min). Grade 4 patients had more severe symptoms with hoarseness, a “barky” cough, difficulty swallowing, dyspnea, wheezing, cyanosis, or dysrhythmia and/or mild hypotension. Those with Grade 5 reactions had the most severe symptoms with respiratory arrest, severe bradycardia and/or hypotension or cardiac arrest, or loss of consciousness. The severity score was based on the organ system most affected.

Statistical analysis

Analyses of the urinary PGDM concentration according to each grade or the timing of urine collection were performed out using a Mann-Whitney *U*-test.

Comparisons of the urinary PGDM concentrations at 4 hours after OFC among each grade were performed using the Jonckheere-Terpstra trend test. *P* values of less than .05 were considered statistically significant for all the tests. All the analyses were performed using JMP® 10 (SAS Institute Inc., Cary, NC, USA).

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Table E1. Characteristics and clinical features of the patients according to each grade.

	All	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
No. of subjects, n (%)	157	45 (28.7)	45 (28.7)	25 (15.9)	15 (9.6)	27 (17.2)	0 (0)
Sex, boys, n (%)	88	27 (60.0)	32 (71.1)	13 (52.0)	5 (33.3)	11 (40.7)	-
Age(y), mean	7.4	8.3	6.7	7.2	8.1	6.9	-
Previous adverse reactions related to challenged foods, n (%)	105	26 (57.8)	30 (66.7)	20 (90.9)	7 (46.7)	22 (81.5)	-
Atopic dermatitis, n (%)	97	25 (55.6)	31 (68.9)	16 (64.0)	5 (33.3)	20 (74.1)	-
Bronchial asthma, n (%)	67	13 (28.9)	28 (62.2)	7 (28.0)	4 (33.3)	15 (53.6)	-
Allergic rhinitis, n (%)	60	17 (37.7)	17 (37.7)	12 (48.0)	7 (46.7)	7 (25.9)	-
Total serum IgE level within 1 year (kUa/L), mean	1010	1123	1047	899	972	984	-
Uses of antihistamine drugs p.o. during OFC, n (%)	54	0 (0)	8 (17.7)	12 (48.0)	9 (60.0)	25 (92.6)	-
Uses of corticosteroid p.o. or i.v. during OFC, n (%)	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	-
Uses of salbutamol inhalation during OFC, n (%)	28	0 (0)	0 (0)	3 (12.0)	3 (20.0)	22 (81.5)	-
Uses of epinephrine i.m. during OFC, n (%)	5	0 (0)	0 (0)	0 (0)	0 (0)	5 (18.6)	-

Table E2. Subjective symptoms and objective symptoms in OFCs.

	Grade 0 (45)		Grade 1 (45)		Grade 2 (25)		Grade 3 (15)		Grade 4 (27)	
Only subjective symptom	Abdominal discomfort or pain	3	Oral allergy symptom	19	Nausea	5	-	-	-	-
	Non-continuous cough	5								
Most severe objective symptom	-	-	Localized urticaria	22	General urticaria	15	Repetitive vomiting	11	Wheezing	22
			Localized flushing	3	Nasal sneezing or congestion	3	Continuous cough without wheezing	3	Mild hypotention	4
			Localized angioedema	1	Single emesis	2	Rhinorrhea	1	Hoarseness with "barkly cough"	1

Table E3. Comparison of urinary PGDM between Egg white and Milk in each Grade.

Grade	Grade 1			Grade 2		
Food	Egg white (n=11)	Milk (n=15)	<i>P</i> value	Egg white (n=11)	Milk (n=8)	<i>P</i> value
Urinary PGDM, mean $\pm$ SD (ng/mg Cre)	4.34 $\pm$ 3.57	3.59 $\pm$ 2.21	NS	7.12 $\pm$ 4.93	6.65 $\pm$ 5.22	NS
Grade	Grade 3			Grade 4		
Food	Egg white (n=6)	Milk (n=5)	<i>P</i> value	Egg white (n=9)	Milk (n=5)	<i>P</i> value
Urinary PGDM, mean $\pm$ SD (ng/mg Cre)	11.19 $\pm$ 10.98	15.14 $\pm$ 12.79	NS	20.01 $\pm$ 11.29	17.9 $\pm$ 13.75	NS

P value was calculated with the use of the Mann-Whitney *U* test.

NS, not significant.

Table E4. The levels of urinary PGDM and allergen-specific IgE in patients with egg white or milk allergies in each Grade.

Egg white (n=50)	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
N	13	11	11	6	9
Urinary PGDM, mean $\pm$ SD (ng/mg Cre)	1.34 $\pm$ 0.44	4.34 $\pm$ 3.57	5.86 $\pm$ 4.01	11.19 $\pm$ 10.98	20.01 $\pm$ 11.29
Total serum IgE level within 1 year, mean (kUa/L)	863	835	839	1008	767
Allergen-specific IgE level within 1 year, mean (kUa/L)	23.14	29.01	31.39	25.18	43.6

Milk (n=43)	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
N	10	15	8	5	5
Urinary PGDM, mean $\pm$ SD (ng/mg Cre)	1.47 $\pm$ 0.66	3.59 $\pm$ 2.21	4.14 $\pm$ 4.9	15.14 $\pm$ 12.79	17.9 $\pm$ 13.75
Total serum IgE level within 1 year, mean (kUa/L)	1083	1904	1246	999	802
Allergen-specific IgE level within 1 year, mean (kUa/L)	17.24	31.02	49.01	21.05	33.01

Table E5. Stability of urinary PGDM added in normal urine.

	0 h (control) PGDM (ng/mL)	24 h at 4°C		72 h at 4°C		7 days at 4°C		7 days at -20°C	
		ng/mL	% of control	ng/mL	% of control	ng/ml	% of control	ng/mL	% of control
Urine #1	40.90	42.73	104.47	39.85	97.43	38.69	94.58	41.91	102.46
Urine #2	35.46	36.53	103.04	34.34	96.86	35.99	101.49	36.62	103.28

Table E6. Stability of urinary PGDM in patients with food allergy.

	0 h (control)	24 h at 4°C		72 h at 4°C		7 days at 4°C		7 days at -20°C	
	PGDM (ng/mL)	ng/mL	% of control	ng/mL	% of control	ng/mL	% of control	ng/mL	% of control
Patient #1	0.80	0.88	109.63	0.88	109.91	0.90	111.82	0.94	116.95
Patient #2	0.49	0.58	118.57	0.58	117.98	0.71	145.69	0.70	143.39
Patient #3	6.12	8.06	131.83	7.72	126.25	6.87	112.33	6.54	106.85
Patient #4	8.27	6.30	76.18	8.10	97.95	5.93	71.68	6.99	84.61
Patient #5	5.33			5.21	97.72	5.05	94.75	5.72	107.21
Patient #6	78.72			65.91	83.74	50.1	63.65	71.24	90.5

Figure Legends**Figure E1. Difference of urinary PGDM concentration from baseline (pre-OFC).**

A, Time-dependent changes in patients with positive OFC results. **B**, Time-dependent changes in patients with negative OFC results. *** $P < .001$, Mann-Whitney U test. NS, not significant.

Figure E2. ROC curves for urinary PGDM levels in patients with allergic reactions.

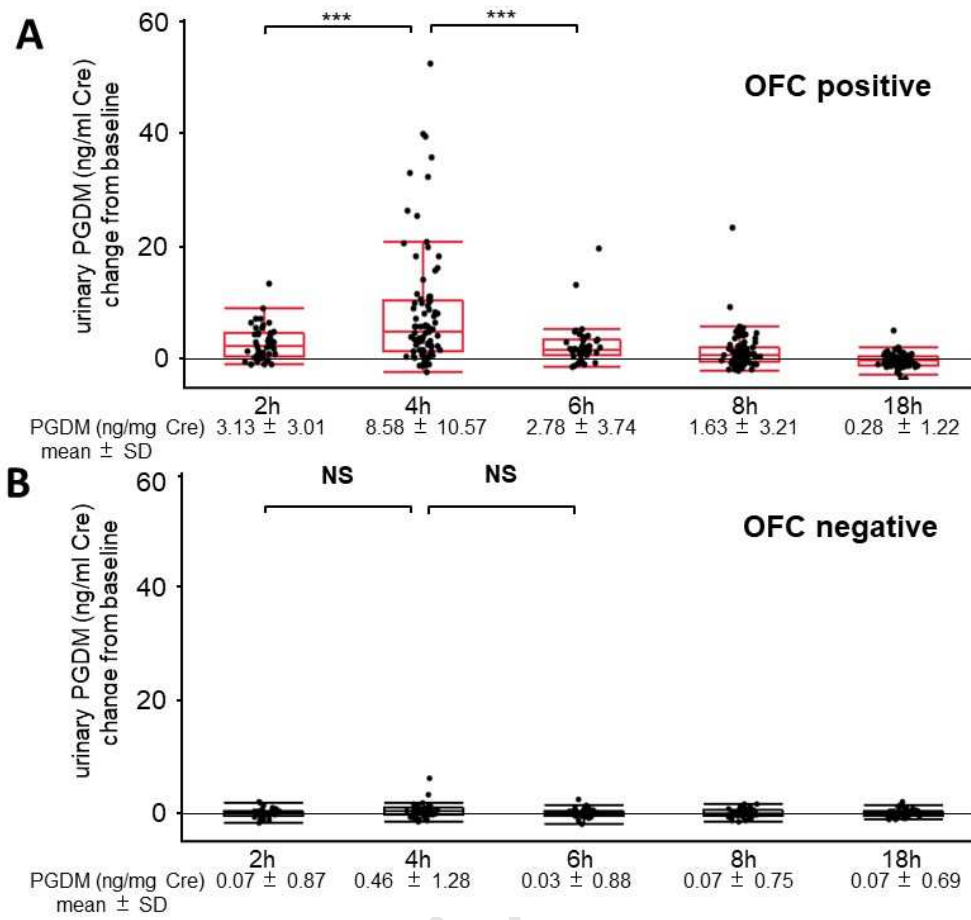
A, ROC curve for urinary PGDM levels in patients with Grade 0-2 (non-reactors or mild reactions) versus Grade 3-5 (anaphylaxis).

B, ROC curve for urinary PGDM levels in patients with Grade 1-2 (mild reactions) versus Grade 3-5 (anaphylaxis).

C, ROC curve for urinary PGDM levels in patients with Grade 0 (OFC negative) versus only oral allergy symptoms.

Figure E3. Association of urinary PGDM and allergen-specific IgE.

Correlation of urinary PGDM and allergen-specific IgE in patients with egg white or milk allergies. The Spearman coefficient was calculated for correlation.



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Figure E1

