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Original article

Increased ratio of pollock roe-specific IgE to salmon roe-specific IgE levels is associated with a positive reaction to cooked pollock roe oral food challenge

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AUC, Area under the curve; CIs, confidence

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IgE, Immunoglobulin E; OFC, Oral food

challenge; PR, Pollock roe; ROC

curves, Receiver operating characteristic

curves; SR, Salmon roe

ABSTRACT

Background: Anaphylaxis and immediate-type fish roe allergies have been reported worldwide, and, in Japan, fish roe is the sixth most common food allergen. No oral food challenges (OFCs) have used pollock roe (PR), which is reported to have high cross-reactivity with salmon roe (SR). Therefore, we administered an OFC using cooked PR to evaluate PR- and SR-specific immunoglobulin E (IgE) levels and allergic reactions in patients with PR sensitivity.

Methods: This retrospective study evaluating patient characteristics and responses to OFCs was conducted with 10–20 g of cooked PR, between April 2006 and November 2016.

Results: We assessed 51 patients (median age: 6.8 years). All had PR sensitization, 6 (12%) with a history of immediate reactions to PR, and 18 (35%) of immediate reactions to SR. Median PR-specific and SR-specific IgE values were 3.4 kUA/L and 9.9 kUA/L, respectively. Seven patients (14%) had a positive OFC. There was no anaphylaxis. Induced symptoms were mild and included localized urticaria, throat pruritus, intermittent cough, and mild abdominal pain. We treated one patient with mild abdominal pain with oral antihistamines. There were no significant differences in history of immediate reaction to PR and PR-specific IgE titers between OFC-positive and OFC-negative patients, although significant differences were found for PR-specific IgE titers adjusted for SR-specific IgE ($p = 0.025$) and PR-specific IgE/SR-specific IgE ratio ($p = 0.009$).

Conclusions: Increased PR-specific IgE/SR-specific IgE ratio or PR-specific IgE levels adjusted for SR-specific IgE levels were risk factors for OFC positivity.

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Introduction

In Japan, fish roe is the sixth most common food allergen.¹ Moreover, among new-onset immediate-type food allergies, fish roe is the most common food allergen of 2- to 3-year-old children.¹

Fish roe used in cooking includes caviar, herring roe, salmon roe (SR), pollock roe (PR), and flying fish roe. In Japan, SR is often ingested in the form of sushi rolls. PR is a popular food in Japan and Korea, and is often an ingredient in rice balls and pasta sauces,

which are frequent components of Japanese food. In Sweden, processed roe of Pacific cod, which belongs to the same family as pollock, is commonly served.²

Cases of immediate-type allergy and anaphylaxis in reaction to caviar and SR have been reported worldwide.^{3–5} In Japan, the majority of allergic reactions to fish roe are to SR, and there has been one report of an SR oral food challenge (OFC).⁶ However, there have been no reports of cross-reactivity between PR and SR on an OFC.⁷

PR is often served cooked in school meals and in meals eaten out in Japan; therefore, if children with suspected PR allergy could eat cooked PR, their quality of life would improve. Thus, the aim of our study was to evaluate the safety of an OFC with cooked PR and to evaluate the risk factors for OFC positivity.

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Methods

Subjects

We retrospectively evaluated PR-sensitized patients who had undergone an OFC with 10–20 g of cooked PR to diagnose a PR allergy or confirm their tolerance, between April 2006 and November 2016. Sensitization to PR was defined as PR-specific immunoglobulin E (IgE) > 0.35 (kUA/L) observed within one year prior to the OFC.

Methods

The total amount of challenge food was 10–20 g of cooked PR (protein: 2830–5660 mg), which was cooked either at the Sagami-hara National Hospital or in the patients' homes. Patients who had been prescribed anti-allergy drugs (e.g., antihistamines) were excluded from the study because they could influence the results of the OFC. This OFC involving PR was an open challenge, because PR is usually eaten in a simple cooked form. We employed three different protocols in our study. For the first, the dosing interval was 15 min, and five doses were administered, starting with 1/16 of the total load, followed by 1/8, 1/4, and 1/2. For the second protocol, the dosing interval was 30 min, and three doses were administered, starting with 1/8 of the total load, followed by 3/8 and 1/2. For the third protocol, the dosing interval was 60 min, and two doses were administered, starting with 1/4 of the total load, followed by 3/4. Until March 2014, we used the first protocol in our inpatient department and the second in our outpatient department. However, from April 2014, we used the third protocol for all patients. Follow-up of patients for 3 h from the beginning of the OFC.

Positive symptoms of OFC were defined as skin symptoms (urticaria, exanthema, and pruritus), oral mucosal symptoms (pruritus of the throat or oral cavity), respiratory symptoms (cough, wheezing, hoarseness, and dyspnea), gastrointestinal symptoms (emesis, diarrhea, and persistent abdominal pain), cardiovascular symptoms (hypotension), and neurological symptoms (confusion), as reported by Sampson *et al.*⁸ The severity of symptoms was evaluated using the Japanese anaphylaxis guidelines (Supplementary Table 1).⁹ When adverse symptoms occurred, treatments such as antihistamines, steroids, beta-2 stimulant inhalation, or adrenaline injection were administered on the basis of the European Academy of Allergy and Clinical Immunology (EAACI) food allergy and anaphylaxis guidelines.¹⁰ If the patient had no symptoms or only mild symptoms that did not require treatment, they were asked to consume cooked PR at home. We then judged the results of OFC based on symptoms shown at home.

Measurement of serum-specific IgE

Patients' PR, SR, hens' egg white, and codfish-specific IgE titers were measured using the Immuno-CAP assay system (Thermo Fisher Scientific, Uppsala, Sweden). Specific IgE titers >0.35 kUA/L were considered positive.

Statistical analysis

Patients' characteristics and immunological parameters were evaluated, and the results were expressed as the median and interquartile range. For statistical comparisons between the two groups, we used Mann–Whitney U tests for continuous variables and Fisher's exact tests for categorical variables. *P*-values <0.05 were considered significant.

Receiver operating characteristic (ROC) curves were generated for PR-specific IgE levels and PR-specific IgE/SR-specific IgE ratios. Performance of the model was assessed using the area under the curve (AUC). Specificity and sensitivity were calculated for each cut-off value. We analyzed the relationship between OFC results and PR or SR-specific IgE levels using logistic regression.

Probability curves and 95% confidence intervals (CIs) were estimated from PR-specific IgE values and PR-specific IgE/SR-specific IgE ratios using logistic regression according to the methods used by Soderstro *et al.*¹¹ and Grabenhenrich *et al.*¹² All data were statistically analyzed using SPSS version 24 (IBM, Armonk, NY, USA).

Ethical considerations

In accordance with the tenets of the Declaration of Helsinki, the study design and the risks of symptom provocation were fully explained to the patients and the children's guardians both verbally and in writing. Written informed consent was obtained from the guardians prior to participation. The study design was approved by the institutional review board of Sagami-hara National Hospital.

Results

Baseline subject characteristics

Sixty-five patients underwent OFCs with 10–20 g of cooked PR. The following 14 patients were excluded: 13 patients who had no sensitization and no history of immediate reaction, and 1 patient for whom PR-specific IgE had not been measured. Therefore, we analyzed 51 patients in total (Fig. 1).

Table 1 shows patients' characteristics and immunological parameters. The median age of all patients was 6.8 years, and 59% were male. Six patients (12%) had a history of immediate reactions to PR, and 1 patient (2%) had a history of anaphylaxis to PR. Forty-five patients (88%) were already avoiding SR due to possible cross-reactivity with PR, and 18 patients (35%) had a history of immediate reactions to SR. Twenty-nine patients (57%) had a history of immediate reactions to hens' egg whites. Complications from other allergic diseases included atopic dermatitis (24 patients, 47%), bronchial asthma (9 patients, 18%), and allergic rhinitis (6 patients, 12%).

The median total IgE value was 1460 kIU/L, the PR-specific IgE value was 3.4 kUA/L, the SR-specific IgE value was 9.9 kUA/L, the hens' egg white-specific IgE value was 11.7 kUA/L, and the codfish-specific IgE value was 0.18 kUA/L.

Relationship of specific IgE values

Figure 2 shows the relationship-specific IgE values between PR and SR, PR and hens' egg whites, and PR and codfish. We found moderate positive correlations between PR and SR ($R = 0.583$, p -value <0.001) (Fig. 2 A). However, there were no significant correlations found between PR and hens' egg whites ($R = 0.254$, p -value = 0.088), and PR and codfish ($R = 0.262$, p -value = 0.147) (Fig. 2 B, C).

OFC results

Seven patients (14%) had positive OFC results to cooked PR; Table 2 shows the induced symptoms, severity, and treatments for these patients. The most common positive symptoms were oral mucosal symptoms, followed by skin, gastrointestinal, and respiratory symptoms. All positive patients ($n = 7$) had Grade-1 (mild) symptoms. One patient who presented with pruritus of

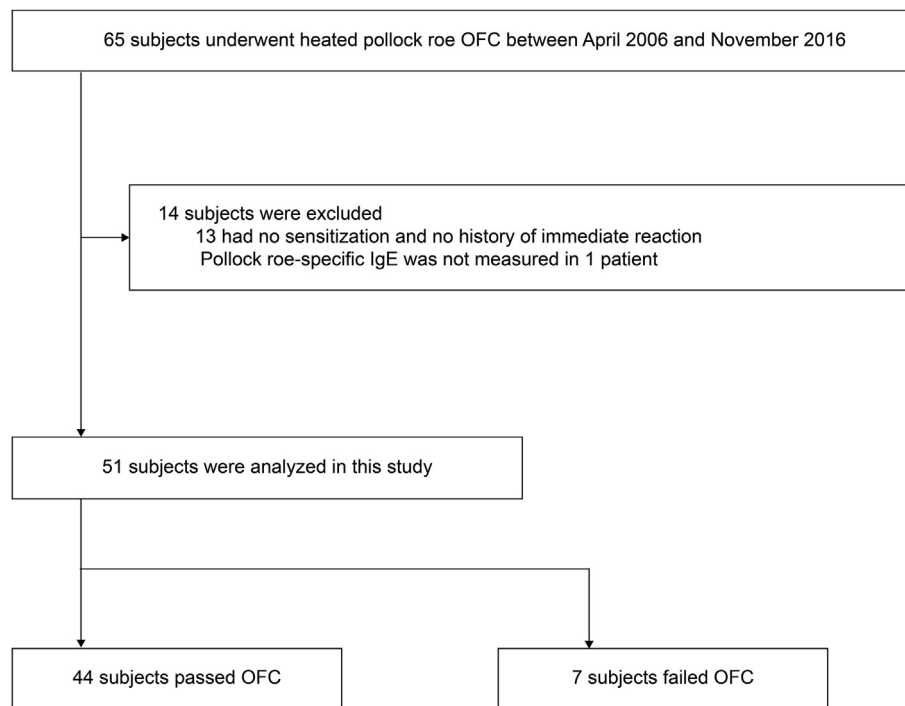


Fig. 1. Study enrollment and outcomes. The cooked pollock roe oral food challenge was conducted with 10–20 g of cooked pollock roe.

Table 1
Patient characteristics.

Characteristics	Total (n = 51)
Male sex (%)	30 (59)
Median age at challenge (years)	6.8 (4.6–9.0)
History of immediate reactions to PR (%)	6 (12)
History of anaphylaxis to PR (%)	1 (2)
Avoiding SR (%)	45 (88)
History of immediate reactions to SR (%)	18 (35)
History of immediate reactions to hens' eggs (%)	29 (57)
Atopic dermatitis, current (%)	24 (47)
Bronchial asthma, current (%)	9 (18)
Allergic rhinitis, current (%)	6 (12)
Total IgE (kIU/L)	1460 (529–2470)
PR-specific IgE (kUA/L)	3.45 (1.36–6.50)
SR-specific IgE (kUA/L) [†]	9.95 (4.90–28.80)
PR-specific IgE/SR-specific IgE [‡]	0.26 (0.12–0.61)
Egg white-specific IgE (kUA/L) [‡]	11.70 (1.23–41.30)
Codfish-specific IgE (kUA/L) [§]	0.185 (0.05–0.562)

Data are presented as medians (with interquartile range: 25–75 percentiles) or n (%).

[†] n = 49.

[‡] n = 46.

[§] n = 32.

the throat and mild abdominal pain was treated with an oral antihistamine. Although 1 in 7 OFC-positive patients had no symptoms in the hospital setting, 1 patient had mild abdominal pain and emesis due to consuming cooked PR at home (Table 2, Supplementary Table 2).

We examined patients' characteristics and immunological parameters in relation to positive and negative results (Table 3). No significant differences were observed for age, sex, history of immediate reactions to PR and SR, complications of other allergic diseases, or values of specific IgE (PR, SR, hens' egg whites, and codfish). However, OFC-positive patients had significantly higher PR-specific IgE/SR-specific IgE ratios than did negative patients ($p = 0.009$) (Table 3, Supplementary Fig. 1).

One of the negative OFC patients underwent an OFC with raw PR after the OFC with cooked PR, and had positive OFC results with the raw PR. Other patients did not request an OFC with raw PR. Subsequently, we were not able to confirm whether or not those patients could eat raw PR at home.

ROC analysis

The AUC for PR-specific IgE was 0.636, whereas the AUC for PR-specific IgE/SR-specific IgE ratio was 0.803 (Fig. 3). The AUC for the PR-specific IgE/SR-specific IgE ratio was higher than that for PR-specific IgE. For the PR-specific IgE/SR-specific IgE ratio at an optimal cutoff of 0.47 (Youden index), we obtained values of 73.8% (specificity), 71.4% (sensitivity), 31.2% (positive predictive value, PPV), and 93.9% (negative predictive value, NPV). At a cutoff of 0.2, we obtained values of 45.2% (specificity), 100% (sensitivity), 23.3% (PPV), and 100% (NPV). At a cutoff of 1.3, we obtained values of 97.6% (specificity), 42.9% (sensitivity), 75.0% (PPV), and 91.1% (NPV) (Supplementary Table 3).

Multivariate analysis and probability curves

When OFC-positive and OFC-negative patients were compared, no significant differences were observed for PR-specific IgE as determined by using univariate analysis. Nevertheless, results from multivariate logistic regression analysis indicated that high PR-specific IgE levels were significant risk factors for a positive OFC adjusted for SR-specific IgE (adjusted odds ratio: 20.2 per 10-fold increase [95% CI, 1.45–283.0]; $p = 0.025$) (Table 4).

Fitted probability curves for the relationships between OFC outcomes and PR-specific IgE values or PR-specific IgE/SR-specific IgE ratios are presented in Figure 4. The curve shows that a PR-specific IgE/SR-specific IgE ratio of 0.16 indicates a 5% PPV, a ratio of 0.31 indicates a 10% PPV, a ratio of 0.61 indicates a 20% PPV, and a ratio of 1.94 indicates a 50% PPV. We were unable to calculate the value for more than 70% PPV, because there were no applicable patients.

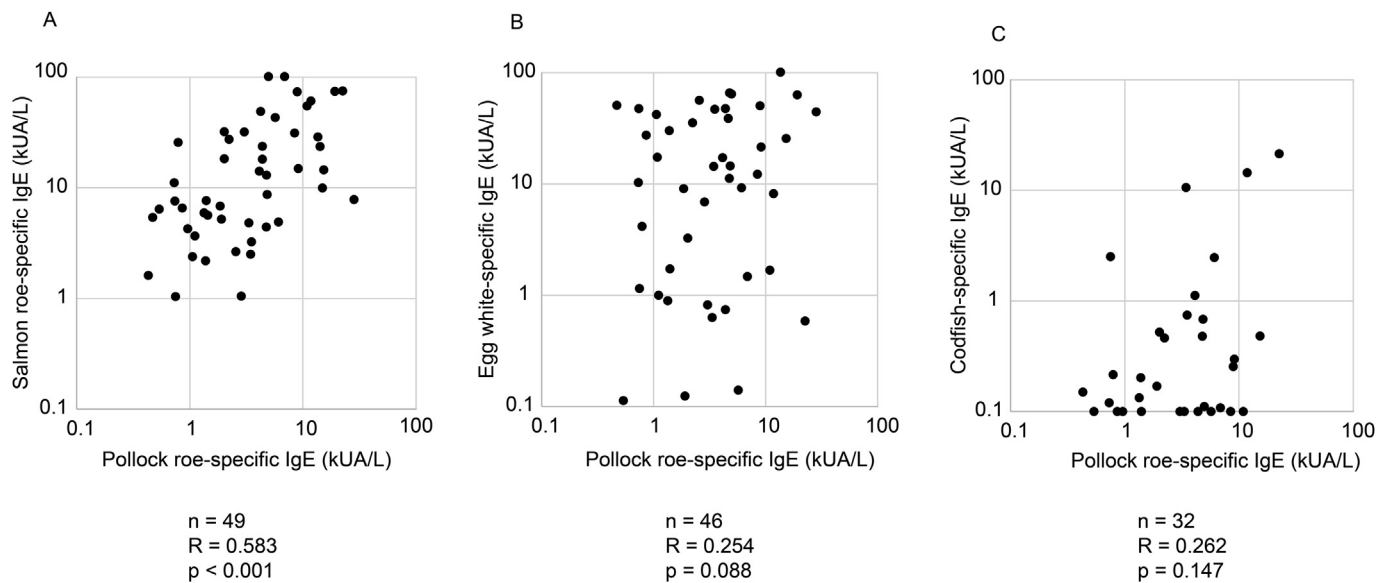


Fig. 2. Relationship of the specific IgE value between pollock roe and salmon roe (A), pollock roe and egg whites (B), and pollock roe and codfish (C).

Table 2

Induced symptoms, severity, and treatments for patients with positive PR OFC test results.

Characteristics		Total
Symptoms	Oral mucosal symptoms	6 (86%)
	Skin symptoms	3 (43%)
	Gastrointestinal symptoms	2 (29%) [†]
	Respiratory symptoms	1 (14%)
Severity	Grade 1 (mild)	7 (100%) [‡]
	Grade 2 (moderate)	0 (0%)
	Grade 3 (severe)	0 (0%)
Treatment	Antihistamine (oral)	1 (14%)
	No treatment	6 (86%) [‡]

The definitions of severity grade were based on anaphylaxis guidelines.

[†] Although 1 of the 7 OFC-positive patients showed no symptoms in the hospital setting, that patient had mild abdominal symptoms later from consuming cooked PR at home.

Discussion

Our study is the first report of an OFC using cooked PR. PR-sensitized patients, who underwent an OFC with 10–20 g of cooked PR in order to diagnose a PR allergy or confirm their tolerance, were evaluated. At 14%, the positivity rate for the OFC was low. An increased PR-specific IgE/SR-specific IgE ratio or increased PR-specific IgE levels adjusted for SR-specific IgE levels were shown to be risk factors for OFC positivity.

PR is the salted ovary of Alaskan pollock (*Theragra chalcogramma*). In Japan, PR is commonly served as an ingredient in rice balls, traditional Japanese foods, and pasta sauces.² Both raw and cooked PR are edible. Raw PR contains 240 mg/g of protein, and cooked PR contains 283 mg/g. The amount of PR that one person typically eats is 10–20 g. Therefore, we selected 10–20 g of PR as the challenge level for the OFC.

Yanagida *et al.* have reported on OFCs with raw SR.⁶ In their report, 62 patients with suspected or confirmed SR allergy underwent OFCs with 10 g of raw SR. In that study, the median age of all patients was 6.7 years, 39% of all patients had a history of immediate reactions to SR, and the median SR-specific IgE value was 3.4 kUA/L. The positivity rate for the OFC was 54%, which was more than half of all patients. In contrast, in

Table 3

Comparison of patient characteristics by OFC response.

Characteristic	OFC-positive (n = 7)	OFC-negative (n = 44)	p-value
Male sex (%)	5 (71)	25 (57)	0.685
Median age at challenge (years)	7.1 (6.5–8.4)	6.4 (4.5–8.8)	0.427
History of immediate reactions to PR (%)	1 (14)	5 (11)	>0.999
History of anaphylaxis to PR (%)	0 (0)	1 (2)	>0.999
Avoiding SR (%)	6 (86)	39 (89)	>0.999
History of immediate reactions to SR (%)	3 (43)	15 (34)	0.686
History of immediate reactions to hens' eggs (%)	5 (71)	24 (55)	0.684
Atopic dermatitis, current (%)	3 (43)	19 (43)	>0.999
Bronchial asthma, current (%)	2 (29)	7 (16)	0.592
Allergic rhinitis, current (%)	1 (86)	5 (11)	>0.999
Total IgE (kIU/L)	1350 (1085–3290)	1495 (445–2402)	0.477
PR-specific IgE (kUA/L)	3.52 (2.45–14.25)	3.18 (1.10–5.81)	0.239
SR-specific IgE (kUA/L) [†]	5.94 (4.45–8.88)	13.55 (4.98–31.80)	0.219
PR-specific IgE/SR-specific IgE [‡]	1.08 (0.36–1.43)	0.25 (0.10–0.53)	0.009
Egg white-specific IgE (kUA/L) [‡]	44.40 (14.40–47.00)	10.30 (4.50–9.40)	0.198
Codfish-specific IgE (kUA/L) [§]	0.74 (0.44–5.67)	0.17 (0.05–0.48)	0.178

Data are presented as medians (25–75 percentiles) or n (%). Differences were evaluated using the Mann–Whitney test for continuous variables or the Fisher's exact test for categorical variables.

No significant differences were observed for age, sex, history of immediate reactions to PR and SR, complications of other allergic diseases, and values of specific IgE (PR, SR, hen's egg white, and codfish). OFC-positive patients had significantly higher PR-specific IgE/SR-specific IgE ratios than did negative patients ($p = 0.009$).

[†] n = 49.

[‡] n = 46.

[§] n = 32.

our study, the positivity rate for the OFC with 10–20 g of cooked PR was 14%, which is relatively low. In addition, induced symptoms were mild, and only one patient was treated with oral antihistamines.

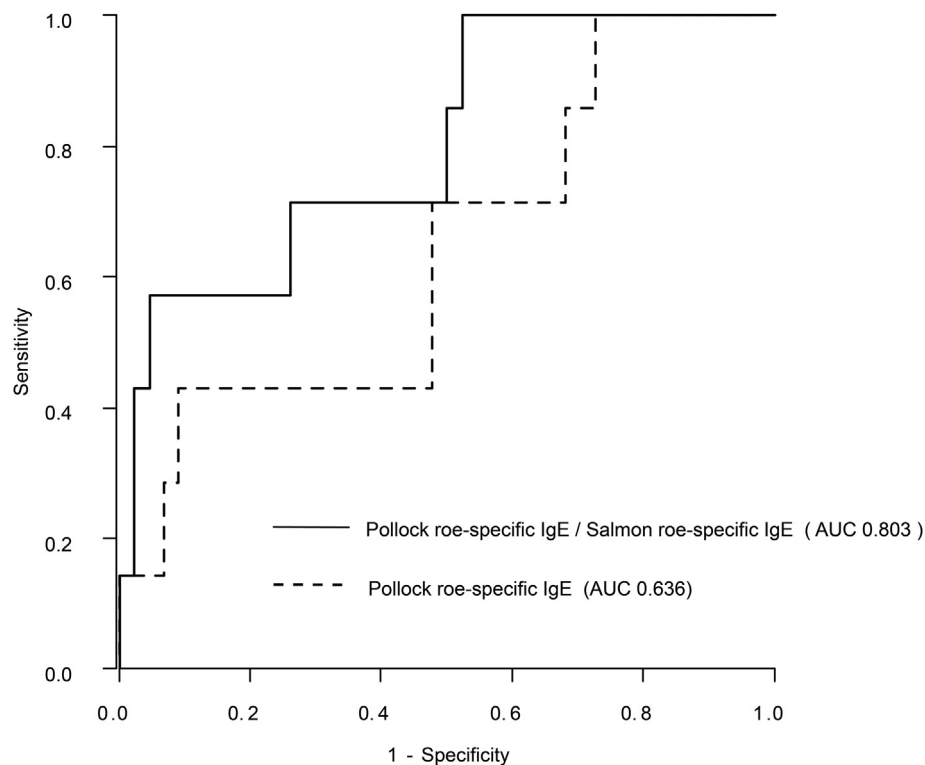


Fig. 3. ROC curves showing the performance of PR-specific IgE/SR-specific IgE and pollock roe-specific IgE.

Table 4
Logistic regression analysis.

	Odds ratio	<i>p</i> -value	Adjusted odds ratio [†]	<i>p</i> -value
PR-specific IgE (kUA/L) (10-fold increments)	3.55 (95% CI, 0.57–22.0)	0.173	20.20 (95% CI, 1.45–283.0)	0.0255

[†] Adjusted SR-specific IgE.

Yanagida *et al.* also reported that SR-specific IgE is useful to estimate positive OFCs with SR.⁶ However, in our comparisons between OFC-positive and OFC-negative patients, no significant differences were observed for PR-specific IgE. Nevertheless, PR-specific IgE levels adjusted for SR-specific IgE were considered to be significant risk factors for OFC positivity. Moreover, OFC-positive patients had significantly higher PR-specific IgE/SR-specific IgE ratios than did OFC-negative patients. In our ROC analysis, the PR-

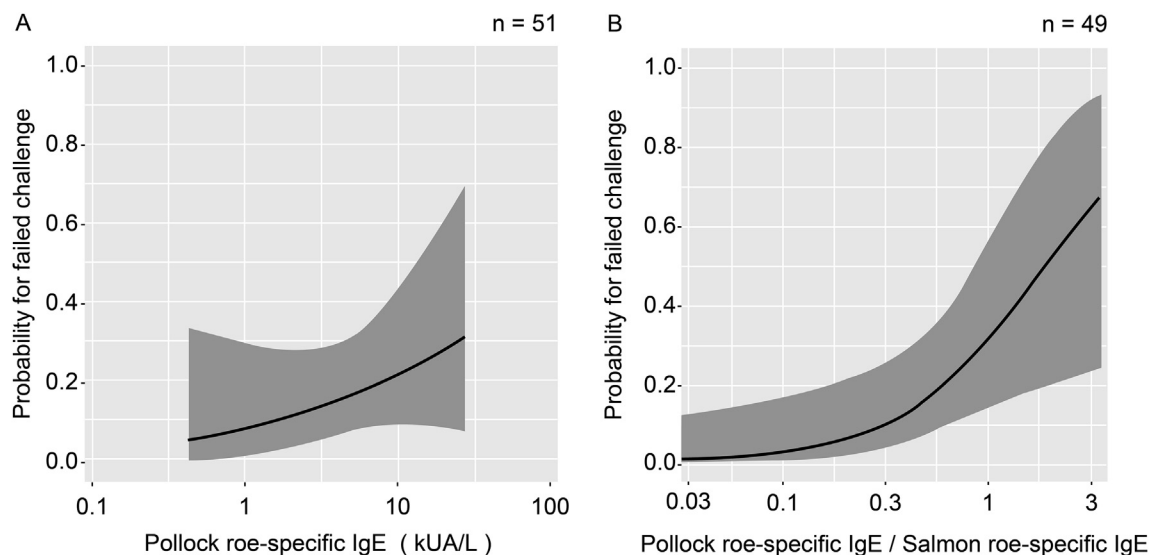


Fig. 4. Fitted predicted probability curves for the outcome of an oral food challenge at a given IgE level of (A) PR-specific IgE (kUA/L) and (B) PR-specific IgE/SR-specific IgE. Shaded areas indicate the range of the 95% CI. Probabilities for the outcomes of oral food challenges for PR-specific IgE levels and PR-specific IgE/SR-specific IgE ratios were calculated using logistic regression analysis.

specific IgE/SR-specific IgE ratio was more useful than the PR-specific IgE for predicting OFC-positive results.

In our study, the rate of positive results from the cooked PR OFC was low, and symptoms of positive patients were mild. Thus, this OFC is recommended for diagnosing PR allergies. Moreover, if a patient has a PR-specific IgE/SR-specific IgE ratio lower than 0.16 (5% PPV), eating cooked PR at home is acceptable. However, if the patient has a PR-specific IgE/SR-specific IgE ratio higher than 2 (approximately 50% PPV), the OFC should be conducted carefully.

Cross-reactivity between the beta-component of PR and Onc k 5, which is the main allergen of SR, has been identified.^{13,14} In our study, we observed moderate positive correlations between PR-specific IgE and SR-specific IgE. Moreover, we found no significant correlations between PR-specific IgE and hens' egg white-specific IgE or codfish-specific IgE. These results are consistent with those of previous studies.^{7,15}

If the beta-component of PR is the main allergen inducing PR allergy, patients who have positive reactions to PR on OFCs should have high values of both PR- and SR-specific IgE. However, on our OFCs, the proportion of patients who had higher levels of SR-specific IgE than PR-specific IgE was low. We suspect that the reason for this is that, in contrast to SR, the beta-component of PR is not the major allergen. However, the specific allergenic components inducing sensitization in our OFC-positive patients are unknown. Our study did not use western blotting to assay sera from patients with positive OFCs. Further analysis to identify the true allergenic components is therefore necessary.

One of the 44 negative OFC patients who underwent an OFC with raw PR after the OFC with cooked PR had positive OFC results with raw PR. Thus, it is possible that the antigenicity of PR decreases when PR is cooked, as is the case with hens' eggs.¹⁶ To confirm true tolerance, an OFC using raw PR is needed following an OFC with cooked PR. However, most of our patients were satisfied that they could safely eat cooked PR, and, therefore, they felt that their quality of life was improved.

Of the six patients with a history of immediate reaction to PR, five had no reaction to cooked PR on the OFC (Table 3). There are two possible reasons for this: First, the patients may have developed tolerance as they grew older; second, the patients may have experienced an immediate reaction after eating undercooked PR. We do not have detailed information about the methods used to cook the PR eaten by the patients with immediate reactions.

Limitations

Our study has certain limitations. First, the percentage of patients who had a history of immediate reactions to PR was low. Generally, as the percentage of patients with a history of immediate reactions to an allergen becomes higher, the probability of failed OFCs increases. In our study, 11.8% of subjects had a history of immediate reactions to PR, which is lower than that found in a previous study of OFCs to SR.⁶ Most subjects in our study underwent this OFC because of sensitization to PR. If the majority of subjects had had a history of immediate reactions to PR, there would have been a possibility that the percentage of positive OFC results and severity of symptoms would have increased. Therefore, further studies using the same OFC but with different study populations are required.

Second, the influence of SR allergy complications was unknown. In our study, most subjects avoided SR because of sensitization to it. However, the percentage of patients with a true SR allergy was unknown because most patients had not undergone an SR OFC. Our study suggests that highly SR-specific IgE, which may cross-react with PR seems to have little influence on PR OFC positivity. Further studies on the relationship between SR allergy

complications and PR OFC outcomes are needed. Third, this OFC was an open OFC using cooked PR. A double-blind placebo-controlled food challenge is the gold standard in food allergy diagnosis; however, this would not be realistic for PR because of its very strong taste.

Conclusion

Although all patients had sensitization to PR and some patients had a history of immediate reaction to PR, the positivity rate for the OFC with cooked PR was low. In addition, the symptoms of OFC-positive patients were mild. Thus, this OFC can be recommended to exclude PR allergy. Furthermore, an increased PR-specific IgE/SR-specific IgE ratio or increased PR-specific IgE levels adjusted for SR-specific IgE levels can be considered risk factors for OFC positivity. Therefore, if a patient has a high PR-specific IgE/SR-specific IgE ratio, this OFC should be conducted carefully.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.alit.2017.11.005>.

Conflict of interest

The authors have no conflicts of interest to declare.

Authors' contributions

EM wrote the manuscript. EM, NY, SS, TA, and ME contributed to data collection and analysis. ME organized the study. All authors read and approved the final manuscript.

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Supplementary Table 1. Grading of symptoms.

	1 (mild)	2 (moderate)	3 (severe)
Skin	Localized urticaria, exanthema, wheal,	Generalized urticaria, exanthema, wheal,	-
	pruritus	pruritus	
	Swollen eyelid or lip	Swollen face	-
Gastrointestinal tract	Pruritus of the throat or oral cavity	Throat pain	-
	Mild abdominal pain	Moderate abdominal pain	Cramps
	Nausea, emesis, diarrhea	Recurrent emesis, diarrhea	Continuous emesis, loss of bowel control
Respiratory tract	Intermittent cough, nasal congestion,	Repetitive cough	Persistent cough, hoarseness,
	sneezing, rhinorrhea		“barking” cough
	-	Chest tightness,	Audible wheezing, dyspnea,
		Wheezing detectable via auscultation	cyanosis, saturation <92%, swallowing or speaking

			difficulties, throat tightness, respiratory arrest
Cardiovascular	-	Pale face, mild hypotension, tachycardia (increase >15 beats/min)	Hypotension, dysrhythmia, severe bradycardia, cardiac arrest
Neurological	Change in activity level, tiredness	Light-headedness, feeling of “pending doom”, somnolence, headache	Confusion, loss of consciousness, incontinence

The severity score was based on the organ system that was most affected by the symptoms.

Hypotension was defined as systolic blood pressure of <70 mmHg (ages: 1 month to 1 year), <70 mmHg + [2 × age] (ages: 1–10 years), and <90 mmHg (>11 years). Mild hypotension was defined as systolic blood pressure of <80 mmHg (ages: 1 month to 1 year), <80 mmHg + [2 × age] (ages: 1–10 years), and <100 mmHg (>11 years). Wheezing detectable via auscultation was defined as mild wheezing that was audible only through a stethoscope. Audible wheezing was defined as wheezing audible without a stethoscope. This definition was modified using the anaphylactic symptom grading of the European Academy of Allergology and Clinical Immunology Guidelines.

Supplementary Table 2. Induced symptoms, severity and treatments in the patients with positive test results.

No	Age (Year)	Sex	Eliciting dose (g)		Symptoms	Grade [†]	Treatment
			Pollock roe	Protein			
1	6	F	20	5.66	Swollen lip, Pruritus of the oral cavity, Intermittent cough	1	—
2	6	M	10	2.83	Localized urticaria, Pruritus of the throat, Mild abdominal pain	1	—
3	9	M	20	5.66	Localized urticaria, Pruritus of the oral cavity	1	—
4	7	F	15	4.25	Pruritus of the throat and lip	1	—

5	8	M	5	1.42	Pruritus of the throat, Mild abdominal pain	1	Antihistamine
6	6	M	20	2.83	Mild abdominal pain, Emesis ‡	1	—
7	7	M	5	1.42	Localized urticaria, Pruritus of the throat	1	—

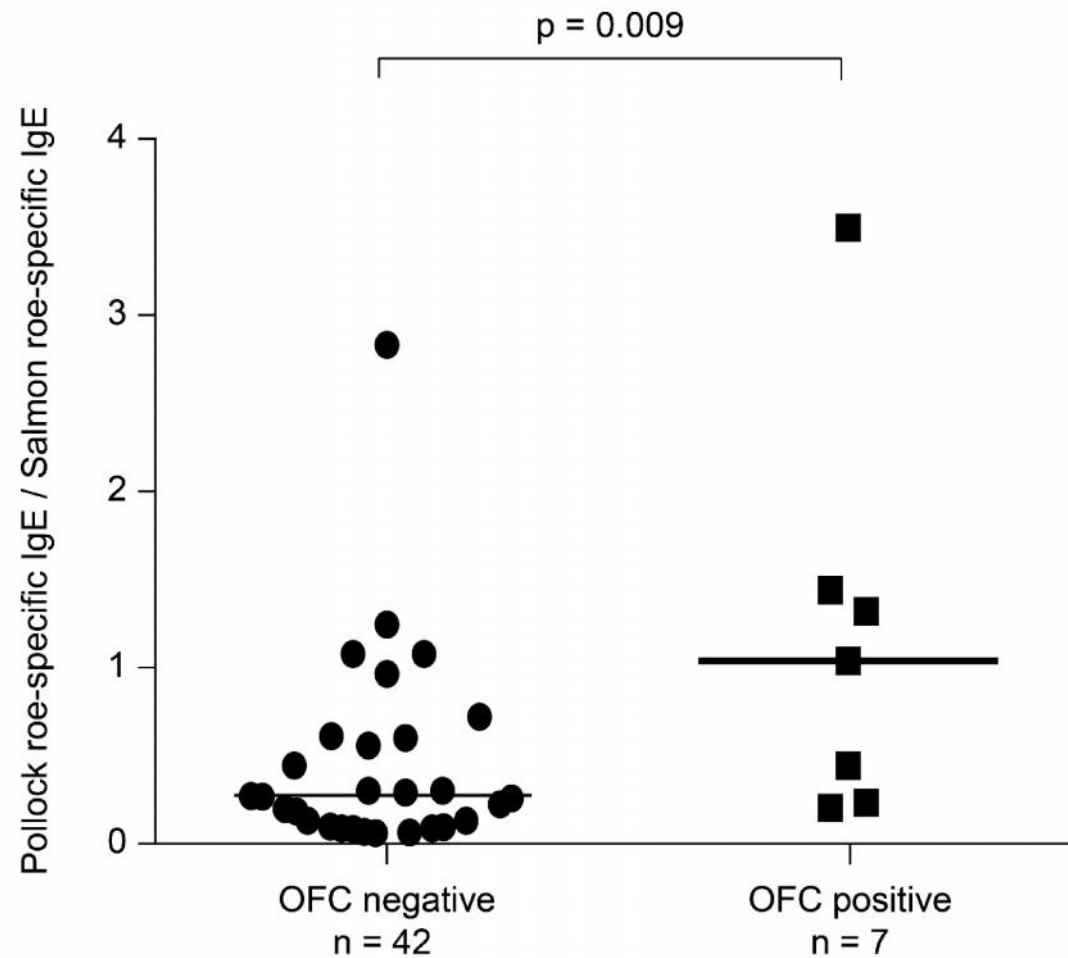
† The definitions of severity grade was based on anaphylaxis guidelines.

‡ Although the patient had no symptoms in hospital, he had symptoms on consuming heated PR at home.

Supplementary Table 3. Specificity, sensitivity, positive predictive values, and negative predictive values.

Cut off (kUA/L)	Specificity (%)	Sensitivity (%)	PPV (%)	NPV (%)
0.2	45.2	100	23.3	100
0.47	73.8	71.4	31.2	93.9
1.3	97.6	42.9	75.0	91.1

PPV, positive predictive value; NPV, negative predictive value.



Supplementary Figure 1. Comparison of pollock roe-specific IgE/salmon roe-specific IgE ratio in the positive and negative response groups. *P*-values for the comparison between groups were calculated using the Mann-Whitney U test.