Contribution of recombinant Parvalbumin Gad c 1 in the diagnosis and prognosis of fish allergy

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Abstract

Background: The prevalence of fish allergy increased in recent years. Gadc1 is a parvalbumin and a major cod allergen, used as a follow-up marker in patients with fish allergy.

Objective: Clinical and laboratory characterizationof a population of patients with fish allergy. Analyze the contribution of thespecific IgE(sIgE) of recombinant parvalbumin Gadc1(rGadc1) and skin prick tests(SPTs) to confirm the acquisition of tolerance to fish.

Methods: Retrospective study of patientswith fish allergy, from July 1st, 2005 to December 31st, 2016. The population was characterized according to demographic data, fish associated with allergic reactions and symptoms. The wheal diameter of the SPT and sIgEfor the fish and rGad c 1 were evaluatedbefore(T0) and after tolerance acquisition(T1).

Results: 81 patients(68% male).Hake(51%), mackerel(30%) and cod(26%) triggered the majority of reactions. The most frequent manifestations were urticaria/angioedema(72%), gastrointestinal symptoms(35%) and eczema(33%);42% of all patients had anaphylaxis. In (T0), the average sIgE values were:cod(32.2kUA/l), sardine(18.4kUA/l), hake(17.5kUA/l), salmon(13.9kUA/l), tuna(4.5kUA/l), and rGadc1(22.9kUA/l). In patients who acquired toleranceto at least one fish species(n=60; 74%), the mean value of rGadc1 in T1(5.1kUA/l) was significantly lower thanT0 (16.8kUA/l)(p=0.001).The values for the average diameter of SPT, evaluations in T0 and T1 for hake(9.42mm/3.79mm) and salmon(7.8mm/2.8mm)were also significant p=0.002 and p=0.026, respectively.

Conclusion: The decrease of sIgE to rGad c 1 and the mean wheal diameter of SPT forhake and salmon can be used as markers of prognosis in the acquisition of tolerance in fish allergy.

Key words: food allergy, fish allergy, parvalbumin, cross reactivity, recombinant allergen, rGad c 1.

Resumen

Antecedentes: La prevalencia de alergia al pescado ha aumentado en los últimos años. Gad c 1 es una parvalbúmina y un importante alérgeno del bacalao, utilizado como marcador de seguimiento en pacientes con alergia a pescado.

Objetivos: Caracterización clínica y de laboratorio de una población de pacientes con alergia a pescados. Analizar la contribución de la IgE específica (sIgE) a parvalbúmina recombinante (rGad c 1) y las pruebas cutáneas (SPT) para confirmar la aparición de tolerancia al pescado.

Métodos: estudio retrospectivo de pacientes con alergia a pescados, desde julio de 2005 hasta diciembre de 2016. Se recogieron datos demográficos, reacciones alérgicas y síntomas con los pescados; el diámetro total de la SPT y el valor de la IgE a rGad c 1 antes (T0) y después de la adquisición de tolerancia (T1).

Resultados: Se evaluaron 81 pacientes (68% hombres). La merluza (51%), caballa (30%) y bacalao (26%) desencadenaron la mayoría de las reacciones. Las manifestaciones más frecuentes fueron urticaria /angioedema (72%), síntomas gastrointestinales (35%) eccema (33%) y el 42% de los pacientes tuvieron anafilaxia. En (T0), los valores medios de sIgE fueron: bacalao (32.2kUA / l), sardina (18.4kUA / l), merluza (17.5kUA / l), salmón (13.9kUA / l), atún (4.5kUA / l) y rGad c 1 (22.9kUA / l). En pacientes que adquirieron tolerancia a al menos una especie de pescado (n = 60; 74%), el valor medio de rGad c 1 en T1 (5.1kUA / l) fue significativamente más bajo que T0 (16.8kUA / l) (p = 0.001). Los valores del diámetro medio de la SPT en T0 y T1 para merluza (9.42mm / 3.79mm) y salmón (7.8mm / 2.8mm) también fueron significativos p = 0.002 y p = 0.026, respectivamente.

Conclusión: la disminución de la sIgE a rGad c 1 y el diámetro medio de la SPT para merluza y salmón se pueden utilizar como marcadores de pronóstico en la adquisición de tolerancia de alergia a pescados.

Palabras clave: alergia a alimentos, alergia a pescado, parvalbúmina, reactividad cruzada, rGad c 1.

Background

Allergic diseases in most industrialized countries have been rising and the World Allergy Organization reported that 20 to 30% of the world population is affected by some allergy[1].

In Europe the highest fish consumption is found in the countries of Scandinavia, Spain and Portugal[2].This increase in fish consumption worldwide, due to its high nutritional valueand its association with a healthy diet, justifies the increase of reported cases of reactions to fish, particularly of allergic nature[3–7].

The first reported cases of fish allergy date backto 1930 when De Besqueand colleagues reported cases of allergic reactions with ingestion, contact and inhalation of fish allergens[1,2,8] but the most common sensitization route is through the gastrointestinal tract [1,2,9].

Fish allergy is the third most common food allergy in Europe, after milk and egg allergy[2]. Prevalence rates of fish allergy by self-reporting range from 0,2 to 2,29% in the general population but can be more than 8% in specific populations, such as fish processing workers[1].

Fish allergy usually develops in early childhood, when fish is first introduced to the diet, but unlike milk or egg allergy, it persists in about 80% of patients, even 10 years after the initial diagnosis[2,3]. The development of allergy to fish only in adulthood can also occur[5].

Mostallergic reactions to fish areIgE-mediated[2,4,9] but there are also reported cases of non-IgE mediated fish allergy likefood protein-induced enterocolitis syndrome (FPIES) cases [2].According to a Spanish study of 355 children with fish IgE-mediated allergy, this type of allergy appears before the secondyear of life[2].

Fish allergy is a multiorgan disease and patients may display symptoms compatiblewith oral allergy syndrome, rhinitis, urticaria, angioedema, bronchospasm, atopic dermatitis exacerbations and gastrointestinal symptoms such as abdominal pain, nausea, vomiting and diarrheaand, in more severe cases, anaphylaxis may even occur[1,2,4,5,8,9].

Parvalbumins were recognized as panallergens of fish in the 1970s [1,2,10], is a low molecular weight protein (10-12kDa) present in fish muscle fibers [5,9,10].

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Besidesparvalbumin, the major allergen associated with fish allergy, with most of patients (70% to 100%)presenting parvalbumin-specific IgE[5,10], there are a number of other allergens, such as enolase, aldolase and collagen[5],that are less concentrated in fish, less studiedand its relevance as food allergens is not yet fully understood[1,9,10].

Fish muscles are composed of two types of fibers: white/lightor red/dark,depending on their function and myoglobin content. Parvalbumin is in higher concentration in white fibers [3,8,9].Fish such as tuna, skipjack, or swordfish have a higher proportion of dark fibers when compared to fish such as cod, flounder or hake, making them hypothetically more allergenic species [3]. Parvalbumin is particularly stable (resistant to heat and chemical desaturation, as well as to the action of proteolytic enzymes)[1,3,5,10], which means that even cooked fish maintains its allergenicity[2].

Cross-reactivity between different fish species can be explained by the extensive homology between parvalbumins of different species (60-80%)[1,3,4,6,9–11].Patients allergic to fish most frequently (67%) will not tolerate several fish species, probably due to parvalbumin cross-reactivity[1,6,10]. More recently, however, cases of monosensitization have been reported and, some patients tolerate only specific fish[1,5,6,8,9,12,13].

Allergy to cod has been extensively studied and most patients allergic to fish have symptoms with cod.Furthermore,IgE has a higher affinity for cod parvalbumin (Gad c 1) than for other fish parvalbumins, making it one of the best studied parvalbumins[1,4,8,10].

The gold standard diagnostic method infish allergyis oral provocation test[1].The provocation can be preceded by skin prick tests (SPT, with commercial extracts and the food itself) whichare a quick and safe way to test patients with a suggestive history of fish allergy. The initial study of these patients is completed by performing IgE assaysfor parvalbumin and for each fish species[5]. It is accepted thata lower level of IgE for a particular fish is associated with less probability of reaction and less severe reactions[1].Studies have been carried out with the aim of determining serum specific IgE(sIgE)*cut-offs* for the diagnosis of fish allergy and it has been ascertained that sIgE>20kU/L for Gad c 1 is diagnostic of fish allergy(95% confidence)[9].

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The only recommended treatment is fish avoidance, but this is not always possible to follow[1,3]. Even though allergy to fish is considered a lasting allergy, there have been cases of patients who become tolerant to fish after an avoidance diet [1,9]. On the other hand, allergen immunotherapy is becoming an increasingly viable possibility, considering studies carried out with hypoallergenic parvalbumin[7,9,10,14].

The aim of this study was the clinical and laboratorial characterization of patients diagnosed with fish allergy followed in our department, by evaluatingtheir sensitization profile to different fish.We also intended to analyze the contribution of sIgEfor recombinant parvalbumin Gad c 1 (rGad c 1) and of SPTsin the diagnosis and prognosis in patients allergic to fish.

Methods

Study Population

The study consisted of a retrospective analysis of 81 patients followed in the Imunoallergology department, diagnosed with fish allergy. The diagnosis was confirmed by clinical history, positive skin prick-tests, and specific IgE determinations,from July 1st 2005 to December 31st 2016.

The population was characterized according to demographic data, type of fish associated to the reaction and symptoms.

The results of SPTswith commercial extracts (BialAristegui®) and sIgE to several fish, as well asrGad c 1 (lmmunoCAP®, Thermo-Fisher),at time of diagnosis,were analyzed in all patients (T0).

In the group that achieved tolerance to at least one fish species, confirmed by oral challenge or spontaneous ingestion at home, SPTs and sIgE to rGad c 1 and other fish species after the acquisition tolerance (T1) were evaluated and compared.

Statistical Analysis

Wilcoxon signed-rank test was used to compare levels of sIgEfor rGad c 1 and several other fish, before and after acquisition of tolerance to at least one fish species, as well to

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compare wheal diameter from SPTsto those fish.A *p* value <0,05was considered statistically significant.

The statistical analysiswas performed with SPSS (v23) and Excel (Microsoft Office 2016).

Results

Characterization of population

Eighty-one(81) patients (68% male, 32% female). Seventy-five(75, 93%) were atopic patients: 63 (78%) had rhinitis, 54 (67%) eczema and 35 (43%) asthma. Seventy seven (77,95%) patients developed symptoms under the age of 18 years old (average age 24.2±31.1 months) and 4 (5%) after the age of 18 (average age 37.8±13.0 years).(Table 1)

The five most common fish species associated with allergic reactions were hake (41, 51%), mackerel (24, 30%), codfish (21, 26%), Gilt-head bream (17, 21%) and sardine (16, 21%). (Table 2)

Average age at first contact with fish was 9 months. In 50% of patients, symptoms compatible with allergic reaction were presented during the first ingestion of fish.

Four (4, 5%) patients had an allergic reaction with the ingestion of all fish species but, only one, presented monosensitization to redfish. The remaining patients did not tolerate, on average, 2.9±3.8 fish species.

Patients did not present symptoms only with fish intake (78, 96%), but also, to a lesser extent, with skin contact (22, 27%) and inhalation of cooking vapors (16, 20%).Anaphylaxis manifested in 34 (42%)of all patientsincluded in this study.Considering only clinical manifestations, the frequency of symptoms associated to fish contact were urticaria/angioedema (58, 72%), gastrointestinal symptoms (28, 35%) and eczema (27, 33%). However, some of these patients, also presentedrespiratory symptoms (19, 23%), oral allergy syndrome (10, 12%) and cardiovascular symptoms (2, 2.5%).(Figure 1)

Skin Prick Tests and specific IgE

In all patients,SPTs were performedat first evaluation with commercial extractsof different fish and were positive for at least one fish species. (Figure 2)

The average wheal diameterof SPT at this initial evaluation (T0) was 9.4 mm to hake, 9.3mm to cod, 9.1 mm to sardine, 7.8 mm to salmon and 4.8 mm to tuna.

The average values of sIgE at first evaluation (T0)were: cod (32.2±40.8 kUA/L), sardine(18.4±32.0kUA/L), hake (17.5±31.4 kUA/L), salmon (13.9±25.5kUA/L), halibut (9.1±21.6kUA/L), tuna (4.5±6.9kUA/L) and rGad c 1 (22.9±34.9kUA/L). (Table 3)

ToleranceAcquisition

Of 81 patients, 60 (74%) acquired tolerance to at least one fish species.

The most and the earliest tolerated fish was tuna with 51 (63%) patients, followed by cod(20, 25%), salmon (20, 25%), hake (18, 22%), Gilt-head bream (14, 17%), mackerel (13, 16%), swordfish (13, 16%), sardine (12, 15%), halibut (11, 14%), perch (9, 11%), sea bass (8, 10%), whiting (7, 9%), white grouper (7, 9%), croaker (6, 7%), flatfish (6, 7%), ling (6, 7%) and redfish (5, 6%).

The average age for tolerance acquisition was 10.5±10.2 years. Five patients (8%) acquired tolerance to all fish, which was confirmed by oral food challenge.

For the patients who acquired tolerance for at least one species of fish, the average at (T0) of rGad c 1sIgE was 16.9kUA/L and was significantly lowerthan(T1), with an average value of 5.1kUA/L (*p*=0.001).

In addition, average values of sIgEwere analyzed for several fish (cod, hake, salmon, sardine and tuna) at T0 and T1 for patients who acquired tolerance to at least one species of fish. The average values of IgE were significantly lower in T1 for cod (10.2kUA/l), sardine (4.7kUA/l), hake (5.23kUA/l) and salmon (3.9kUA/l) when compared to T0: 16.8kUA/l (p=0.001), 18.6kUA/l (p=0.0007), 5.3kUA/l (p=0.004), 5.56kUA/l (p=0.01) and 16.15kUA/l (p=0.001), respectively. The values were not statistically significant for tuna. (Table 4)

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The average wheal diameter of SPTwaslarger before patients acquired tolerance,for all tested fish: hake (9.42mm vs 3.79mm), codfish (9.3mm vs 3.4mm), sardine (9.0mm vs 2.5mm) and salmon (7.8mm vs 2.8mm).Despite the fact that all the values were lower after acquiring tolerance, this difference only reached statistical significance for hake(p=0,002) and salmon (p=0,026). (Table 4)

Discussion

Despite the fact that females are believed to be overall more likely to become sensitized to fish[1], in the pool of 81 patientsincludedin this study the majority of these were male (68%, n=55).

As expected,most children and adults included in this study were atopic (75,92%);78% with rhinitis, 67% with eczema, and 43% with asthma).

Allergy to fish varies geographically according to the type of diet in each area of the planet with salmon, tuna, catfish, cod, flatfish, halibut, trout and sea bass being the most frequent culprits[1]. In our population the most common species associated with allergic reactions mirrored the most frequently consumed fish in our country:hake (41, 51%), mackerel (24, 30%) and codfish (21, 26%).

As expected, these patients did not present symptoms only with fish intake (78, 96%), but also with skin contact (22, 27%) and inhalation of fish cooking vapors (16, 20%). Although symptoms associated with the inhalation of vapors were less frequent in our group of patients, they were reported more often than when compared to a Spanish group of 197 children (11%)[1]. In those patients,symptoms after inhalation may comprise dyspnea, wheezing, angioedema of the upper airways or urticaria, among other complaints [1]. In our study, regardless of the kind of contact with fish,the most frequent symptoms werecutaneous, as expected and previously reported. Thesewere urticaria/angioedema (58, 71%), eczema exacerbation (27, 33%), but also gastrointestinal symptoms (28, 35%). In 34 (42%)of patients, symptoms presented as anaphylactic reaction.

In most cases, allergic symptoms are reported afterfirst contact with fish[1]. In Spain, diagnosis is most frequentbetween 6 and 12 months of age, which coincides with the age of introduction of fish into the diet [2].In another Portuguese study, the average age

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of first symptoms was between 4 and 12 months[11]. In this study, the average age of first contact with fishwas 9 months and half of these children presented symptoms compatible with allergic reaction inthe first ingestion. It would be expected that the first symptoms had also occurred around this age, however, according to information provided by the parents, on average, the first symptoms occurred only at 24.2 months (average age for 1st symptoms < 18 years).

Sensitization to individual fish species has been reported [6,12,13] and could be caused by allergens other than parvalbumin. In this study, only one patient was monosensitized, with redfish, probably due to the expression of another protein than parvalbumin. This case is currently under study.

From these 81 patients, 60 acquired tolerance to at least one fish species,most frequently tuna (51, 63%), likely associated with its lower concentration of parvalbumin.

On average, sIgEr Gad c 1, after patients acquired tolerance to at least one fish species, wassignificantly lower (p=0.001) at (T1) with a value of 5.1kUA/L when comparing with average value at (T0)16.9kUA/L. For all tested fish, the average wheal diameter in SPT was lower whencomparing T0-T1,but only reached significative lower values to hake: 9.42mm-3.79mm(p=0.002); and to salmon: 7.8mm-2.8mm (p=0.026).As expected, the same is also true for average sIgE values of all fish species tested at T0 and T1 and all reached significantly lower values except for tuna, possibly due to its low allergenicity due to the low levels of parvalbumin.

These data show thats IgE parvalbumin levels and SPT, probably, can be considered relevant in the follow-up of these patients.These evaluations may help the clinician to decide when to safely perform oral challenge teststo confirmacquisition of tolerance and introduce new fish species inthe patients' diet.We hypothesize that the SPT wheal diametersfor the remaining fish did not reach statistical significance because of the low number of patients in the study.

A limitation of our study was the low number of included patients. Therefore, we believe that prospective studies including a largernumber of patients will be required to support the presented data.

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Conclusions

Patients allergic to fish often don’t tolerate any species of fish. In this study,reported symptoms were most frequently associated to hake, due to its high consumption in the Mediterranean diet.

In patients with fish allergy, the decreasing of sIgE serum levels for rGad c 1 and the wheal diameter ofSPT withhakeand salmon can probably be used as markers of prognosis in the acquisition of tolerance to fish, and may justify future prospective studies using a control group, with patients without acquisition of tolerance to any species of fish.Overall, tuna was the most and the first tolerated fish.

We observed that half of the patients in this study had symptoms on the first contact with fish, which lead us to the possible assumption of an *inutero*or breast milk-associatedsensitization [15,16]. However, more studies are warranted to confirm this hypothesis.

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Previous Presentations

This study was presented in part as an abstract entitled "Contribution of Recombinant Parvalbumin Gad c 1 in diagnosis and prognosis of fish allergy" at 2017 annual meeting of the European Academy of Allergy and Clinical Immunology (EAACI) in Helsinki, Finland.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Table Legends

Table 1. Population characterization

		n	%
	Total Patients	81	
Gender	Male	55	67,90
	Female	26	32,10
	Atopic Background	75	92,60
	Rhinitis	63	77,78
	Eczema	54	66,67
	Asthma	35	43,21
	1ST Symptoms < 18 years Average age (24,2±31.1 months)	77	95,06
	1ST Symptoms > 18 years Average age (37.8±13.0 years)	4	4,94

Table 2. Species of fish associated with allergic reactions

	n	%
Hake	41	50,62
Mackerel	24	29,63
Codfish	21	25,93
Gilt-head bream	17	20,99
Sardine	16	19,75
Halibut	15	18,52
Salmon	14	17,28
Fresh Tuna	12	14,81
Redfish	11	13,58
Seabass	11	13,58
Swordfish	9	11,11
Perch	7	8,64
Whitegrouper	7	8,64
Croaker	6	7,41
Whiting	6	7,41
Canned Tuna	5	6,17
Flatfish	5	6,17
Ling	5	6,17
Croup	1	1,23

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Table 3. Average values of sIgE at first evaluation for several species of fish

(average values, kUA/l)	**1ST sIgE EVALUATION**
Cod	**32,2**±40,82
Sardine	**18,4**±32,03
Hake	**17,5**±31,44
Salmon	**13,9**±25,46
Tuna	**4,5**±6,90
Halibut	**9,1**±21,55
rGad c 1	**22,9**±34,99

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Table 4. Average values of wheal diameters in skin prick tests and average values of sIgE to rGad c 1 and several fish before (T0) and after acquisition of tolerance (T1) to at least one fish species

	BEFORE TOLERANCE ACQUISITION (T0)	AFTER TOLERANCE ACQUISITION (T1)	*P-VALUE*
Average wheal diameter (mm)			
COD	9,33	3,43	*0,066*
TUNA	4,82	3,40	*0,180*
HAKE	**9,42**	**3,79**	***0,002***
SALMON	**7,78**	**2,75**	***0,026***
SARDINE	9,07	2,50	*0,180*
Average values of sIgE (kUA/l)			
RGAD C 1	**16,85**	**5,07**	***0,001***
COD	**18,60**	**10,21**	***0,0007***
TUNA	2,63	2,61	*0,101*
HAKE	**5,56**	**5,23**	***0,016***
SALMON	**16.15**	**3,87**	***0,001***
SARDINE	**5,29**	**4,67**	***0,004***

Figure 1. Clinical manifestations associated with contact with fish

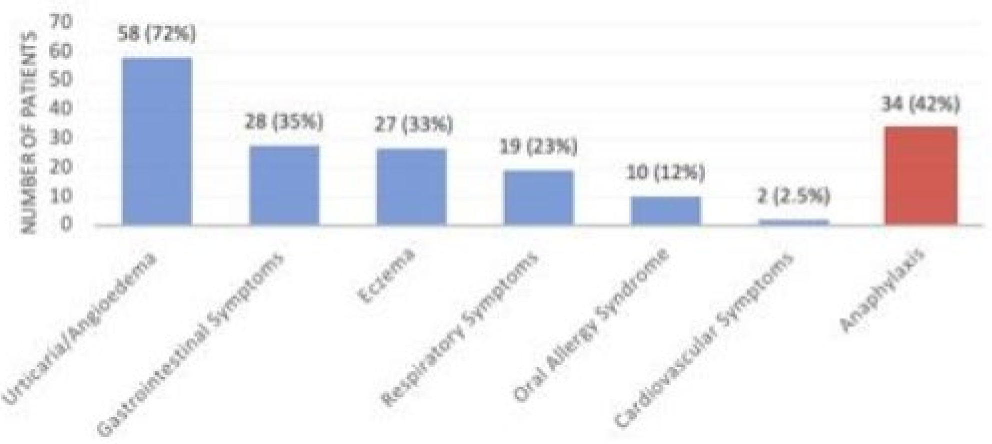
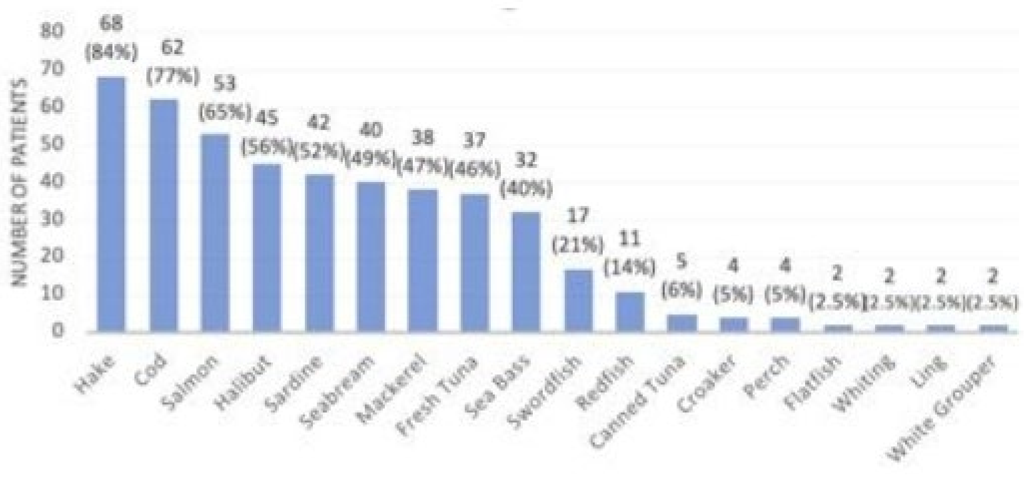


Figure 2. Positive results for skin prick tests performed on several fish



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