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Cardoon allergy

To the Editor:

The cardoon (*Cynara cardunculus*), a Mediterranean plant,[1] belongs to the Compositae family closely related to the artichoke. This vegetable is very popular in France, Italy and Spain and the fleshy root and the buds are eaten raw or cooked.

There is no published information regarding cardoon allergy.

A 12-year-old boy reported asthma and rhinoconjunctivitis in the spring seasons during the last 6 years. He referred, as well, pruritus around mouth after eating nuts, apple, melon, peach, oranges and tomatoes and in the last year, pruritus in mouth and ears and lip swelling after eating raw cardoon. He tolerated cooked cardoon and artichoke.

The patient was tested with a battery of aeroallergenes comprising pollens (grass, *Olea europaea*, *Artemisia vulgaris*, *Parietaria judaica, Platanus acerifolia* and *Chenopodium album*), house-dust mites, fungi, and animal dander. He was also prick tested with commercial food extracts, including nuts (hazelnut, almond, peanut, sunflower seed, pistachio and walnut), fruits (peach, apple, orange and melon), green vegetables (tomato and cardoon), profiline and prick-by-prick with cooked and raw cardoon. Histamine phosphate (10 mg/mL) and sterile 0.9% saline were used as positive and negative controls, respectively.

Specific IgE to extracts from cooked and raw cardoon and pollens from *L. perenne, O. europaea, A. vulgaris, P. judaica* and *P. acerifolia* was measured by an immune assay (Enzyme AllergoSorbent Test EAST})

SDS-PAGE Immunoblotting was performed according to the method of Laemmli.[2]

Separated protein bands were electrophoretically transferred to polyvinylidine difluoride (PVDF) essentially as described by Towbin et al.[3] and blocked during one hour at room temperature with 5% defatted dry milk in Tris buffered saline. Membranes were incubated overnight at 4° C with patient's serum followed by anti-human IgE-horseradish peroxidase conjugate. Detection was carried out by a chemioluminiscent (Amersham ECL Plus Western Blotting Detection System. GE Healhcare). When Western inhibition assay was performed, patient serum was preincubated with the inhibition phases during four hours at room temperature, and afterwards the serum samples were incubated with the PVDF membrane and IgE binding revealed as described herein.

Doubled-blind, placebo-controlled oral challenge tests with raw and cooked cardoon were made.

Skin prick tests with pollen of grass, *Olea europaea, Artemisia vulgaris, Parietaria judaica, Platanus acerifolia* and nuts (hazelnut, almond, peanut, sunflower seed, pistachio and walnut) were all positive. The prick test with profiline was also positive.

Prick-by-prick with peach, orange, melon, apple and tomato were positive.

Prick-by-prick with raw cardoon was positive, 11×10 mm (with negative controls) and negative with cooked cardoon.

Specific IgE by means of EAST assay was positive to extracts from raw cardoon (0.6 kU/L; class 1), cooked cardoon (0.4 kU/L; class 1) and pollens from *Lolium perenne* (>100 kU/L; class 5), *Olea europaea* (>100 kU/L; class 5), *Artemisia vulgaris* (10.3 kU/L; class 3), *Platanus acerifolia* (12.5 kU/L; class 3) and *Parietaria judaica* (1.9 kU/L; class 2).

The protein composition of the raw cardoon extract was studied by the SDS-PAGE and Coomassie staining with protein bands ranging from 66 to 14 kDa being observed. The electrophoresed and electrotransfered raw cardoon extract was incubated with patient's serum, revealing a broad and intense IgE binding area between 97 and 20 kDa.

Western blotting inhibition of raw cardoon extract with patient serum showed a total IgE-binding inhibition when

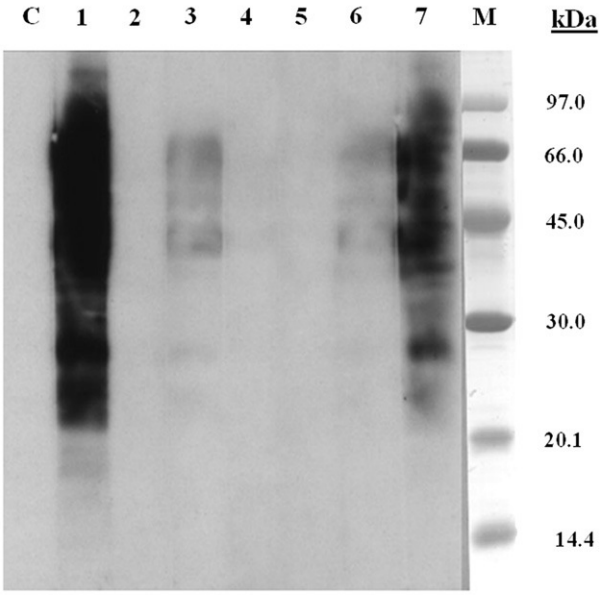

Figure 1 SDS-PAGE immunoblotting inhibition. **Lane C**: control serum, **Lane 1**: patient's serum, **Lane 2**: patient's serum preincubated with cardoon extract(1mg/ml)(Positive inhibition control), **Lane 3**: patient's serum preincubated with *Lolium perenne* pollen extract(1mg/ml), **Lane 4**: patient's serum preincubated with *Olea europaea* pollen extract(1mg/ml), **Lane 5**: patient's serum preincuabated with *Platanus acerifolia* pollen extract(1mg/ml) **Lane 6**: patient's serum preincubated with *Artemisia vulagaris* pollen extract(1mg/ml) **Lane 7**: patient's serum preincubated with lamb extract(1mg/ml) **M:** molecular weight marked proteins.

the serum sample was preincubated with pollen extracts from *Lolium perenne*, *Olea europaea*, *Artemisia vulgaris*, *Parietaria judaica* and *Platanus acerifolia*.(Figure 1).

Oral challenge test with raw cardoon produced pruritus in mouth and lips swelling 30 min after the ingestion whereas oral challenge with cooked cardoon did not produce any kind of symptomatology.

We report a case of cardoon allergy confirmed by positive results in in vivo and in vitro tests. Our patient had personal antecedents of rhinoconjunctivitis and asthma due to inhalation of various pollens: *Lolium perenne*, *Olea europaea*, *Artemisia vulgaris*, *Parietaria judaica* and *Platanus acerifolia*. The symptomatology due to cardoon ingestion had taken place several years after the pollen allergy status began. We demostrated the existence of serologic cross-reactivity among proteins from cardoon and some others from the pollens against which the patient was sensitised.

References

1. Sonnante G, Pignone D, Hammer. K. The Domestication of Artichoke and Cardoon: From Roman Times to the Genomic Age. Annals of Botany. 2007;100:1095–100.
2. Laemeli UK. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature. 1970;227:680–5.
3. Towbin H, Stachelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci.USA. 1979; 76:4350–6.

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Different clinical presentation of *Anisakis simplex* associated urticaria is dependent on the frequency of raw fish intake

To the Editor:

The fish nematode *Anisakis simplex(A.s.)* has widely been implicated in urticaria, angio-oedema and anaphylaxis, both as a hidden food allergen as well as in acute parasitism.[1,2] Gastro-allergic Anisakiasis (GAA) has been described as an acute allergic reaction like urticaria, angio-oedema or anaphylaxis accompanying the penetration of the live larvae of *A.s.* through the gastric mucosa.[3] This entity has been associated with fish-eating habits, where raw fish intake is a risk factor. Patients with these IgE-mediated symptoms tolerate well-cooked fish with non-viable *A.s.*, because excretory-secretory proteins are responsible for the allergic reaction, released only by the live larvae. It has been postulated that the urticarial reaction is accompanied by an inflammatory reaction in the gastric mucosa that helps the host to get rid of the larvae. Furthermore, all patients with GAA have detectable specific antibodies of all isotypes from the first day, thus confirming previous episodes of acute parasitism, mainly without clinically allergic symptoms.[4] The immunologic primary response corresponds thus to at least one previous episode of Anisakiasis with subclinical or only light symptoms.

Chronic urticaria has also been associated with *A.s.* sensitisation and possible dietary measures have been proposed.[5] In order to evaluate if dietary habits could be involved in the different clinical presentation (acute versus chronic urticaria) related to *A.s.* parasitism, we analysed eating habits in three clinical groups. We studied 32 patients with GAA and acute urticaria, and 75 chronic urticaria patients with detectable specific IgE $>$ 1.5 kU/l (CAP-PHADIA) against *A.s.*: CU+. The control group consisted of 25 patients with chronic urticaria without sensitisation against *A.s.* (CU−). Patients with GAA and CU+ displayed a positive skin prick test against *A.s.* and CU− patients had a negative skin prick test and no detectable serum specific IgE against *A.s.* Patients were interviewed about the overall frequency of fish intake per week and the frequency of raw fish intake per month.

No significant differences were stated for gender, age or overall frequency of fish consumption. Median specific IgE against *A.s.* was lower in CU+ (6.8; IQR 4.1–23 kU/l) than in GAA (40.2; IQR 19.7–80.1 kU/l; $p < 0.0001$), probably owing to the longer time interval from the last parasitic episode.[4] Whereas, as expected, the habit of consuming raw fish was associated with sensitisation against *A.s.* as shown by previous episodes of acute parasitism in both groups of *A.s.* sensitisation associated urticaria, GAA and CU+ showed an association with a different frequency of raw fish intake (Figure 1). This fact gains significance because of the further difference in the frequency of raw fish intake between both *A.s.* sensitisation associated urticaria groups and the control group: Patients with GAA eat raw fish with a higher frequency than patients with CU+, whereas the frequency of raw fish eating was higher in both groups compared with the group of CU−.

In our preliminary estimation, the frequency of raw fish intake is considered equivalent to the risk of acute parasitism. Whereas a high risk of parasitism leads, after an unknown number of previous subclinical parasitic episodes, finally to an acute urticaria in GAA, our results show evidence that an existing, but lower, frequency of raw