What is the limit? Anaphylaxis after whey sport supplement shake intake in a cow's milk tolerant patient

Villalobos-Vilda C[1]*, Lendínez MA[2]*, Lorente-Sorolla C[2], Rodrigo-Muñoz JM[2,3], Del Pozo V[2,3]

[1]Department of Allergy, Fundación Jiménez Díaz, Madrid, Spain
[2]Department of Immunology, Instituto de Investigación Sanitaria (IIS) Fundación Jiménez Díaz, Madrid, Spain
[3]CIBER de Enfermedades Respiratorias (CIBERES), Madrid, Spain

*Both authors contributed equally as first authors.

Corresponding author

Victoria del Pozo

Hospital Universitario Fundación Jiménez Díaz

Avenida de los Reyes Católicos 2

28040, Madrid

E-mail: VPozo@fjd.es

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.18176/jiaci.0836

Key words: Anaphylaxis. Cow's milk. Whey protein. Immunotherapy. Sport supplementation.

Palabras clave: Anafilaxia. Leche de vaca. Suero de leche. Inmunoterapia. Suplemento deportivo.

Cow's milk protein allergy (CMPA) is one of the most common food allergies worldwide. It is a common disease in childhood that usually appears in the first two or three months of breastfeeding supplemented with artificial formula. It is estimated that the prevalence ranges between 0.5 and 3% in children under one year of age [1], with the majority evolving towards spontaneous resolution. Cow's milk (*Bos domesticus*) is composed of lactose, simple lipids and proteins, of which 80% is casein and 20% whey proteins (β-lactoglobulin [BLG], α-lactalbumin [ALA], bovine serum albumin [BSA], among others) [2].

Here, we report a case of a 20-year-old man with a history of CMPA treated with oral immunotherapy (OIT) from 4 to 6 years old, currently tolerating daily doses between 200-300 mL (7-8 g) of cow's milk, who presented an episode of anaphylaxis after ingesting a whey-rich protein shake (composed of 30 g of whey protein supplement (WPS), creatine monohydrate and 200 ml of milk). The patient reported that, ten minutes after taking the mix, he presented epigastric pain, general urticarial lesions, facial angioedema and rhinoconjunctivitis, as well as dyspnoea that began after forty minutes. Due to the clinical manifestations presented, he required the administration of adrenaline (0.5 mg), methylprednisolone (60 mg) and dexchlorpheniramine (5 mg), resolving the symptoms in less than one hour. Since then, the patient continued taking milk dairy products at the doses stated above, avoiding the ingestion of the shake that caused the symptoms, without having new episodes.

He was sent to the Allergology Service, where he denied the influence of associated cofactors such as physical exercise or taking anti-inflammatories. He also commented that he had a meal twenty minutes before this episode based on a vegetable cream, gilthead bream, strawberries and nuts (all of which were previously tolerated).

Skin tests were performed with milk, the components of the protein shake (milk, WPS and creatine monohydrate), the previously mentioned groceries and the commercial anisakis extract. The results of the skin tests revealed a very positive prick-prick for commercial milk extract, casein, ALA, BLG and BSA and WPS, with the rest of the allergens testing negative.

Given the results obtained, we performed prick to prick tests with serial dilutions of milk and WPS, obtaining positive results from doses of 5 mg/mL to 0.1 mg/mL. Likewise, two *in vitro* studies were carried out: Western Blot and Basophil Activation Test (BAT).

We requested a test to measure specific IgE (sIgE) to milk components using ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden), considering positive values >0.35 kU/L. Total IgE (25.5 kU/L), cow's milk sIgE (0.47 kU/L) BSA (0 kU/L), ALA (0.38 kU/L), BLG (0.35 kU/L) and casein (0.04 kU/L) values were measured. The results were significantly lower compared to the previous registers (Supplementary Table 1).

doi: 10.18176/jiaci.0836

Western Blot revealed a positive response by the patient's serum to various proteins from both the supplement and milk (Figure 1A), which by molecular weight could correspond to lactoferrin [80 KDa], BSA [66 KDa], secretory component [≈ 60 KDa], caseins [21-27 KDa] and IgG [≈155KDa].

BAT was performed using the BasoFlowEx reagent kit (EXBIO, Vestec, Czechia), following the protocol described by the manufacturer. The basophil population was defined as $CD203c^{+}/SSC^{low}$ by flow cytometry. As shown in Figure 1B, the patient's basophils reached maximum activation after stimulation with 0.5 µg/µl of whey protein powder (24% activation) and with 0.005 µg/µl of milk (33% activation).

As mentioned above, skin-prick test with milk and WPS was positive at very low doses (0.1 mg/mL), which suggests a high degree of sensitization. Regarding immunoblotting, the possible proteins against which the patient's serum reacted were caseins, BSA, lactoferrin, secretory component and whey proteins, as described in the literature [3].

Furthermore, we observed a positive result for both WPS and whole milk in BAT. It is striking that the concentrations at which basophils are top activated are higher for milk (0.5 µg/µl) than WPS (0.005 µg/µl). Although the graphs of different studies that show the degree of activation of basophils in patients with CMPA display heterogeneous patterns as basophil reactivity differs from patient to patient [4,5], sensitized people's curves indeed grow as the concentration of the allergen increases. Our BAT is similar to the BAT of an allergic person but in a clinically asymptomatic patient (sensitized). There are no conclusive studies, so we hypothesize that it could be because our patient was treated with OIT, currently tolerating higher doses than the recommended by SEAIC's immunotherapy guideline [6], or due to inactivation of basophils because of exposure to high doses of allergens involved (inhibition by overactivation).

According to the results, it is demonstrated that our patient presents sensitivity to milk without clinical repercussion if the dose is 200-300 mL but present anaphylactic reaction when he take a milk protein overload like a sport supplement shake.

In conclusion, the patient may have presented the anaphylactic reaction because the ingested a dose of whey protein (24 grams per serving [serving = 31 g]) is much higher than the usual tolerated amounts of milk (200ml/day). The extensive laboratory evaluation supports the notion that OIT is a process of desensitization that does not predictably result in tolerance; it is not clear that patients who achieve negative skin prick tests, undetectable sIgE and non-reactive BAT are equivalent to those who acquire natural tolerance. However, our patient did achieve desensitization successfully, and even maintains its tolerance to CMP after the reported episode.

To our knowledge, this clinical case represents the first report of anaphylaxis without cofactors due to sports supplementation after CMPA overcome by immunotherapy, which is reflected in a small number of related articles [7]. Given that the anaphylactic reaction seems to correspond with the dose, it would be necessary to carry out a review that offers conclusive recommendations on the maximum tolerated dose of CMP suitable for those patients treated with OIT. It would be interesting to warn these patients of the possible risks of taking sports supplements based on hydrolysed whey protein.

Informed consent

The patient declared that he had read and understood the provided information and had the opportunity to ask questions. He understood that his participation is voluntary and is free to withdraw at any time, without giving a reason and without cost. He voluntarily agreed to take part in this study.

doi: 10.18176/jiaci.0836

Conflict of Interest

VdP reports having served as a consultant to Astra Zeneca and GSK; having been paid lecture fees by Astra Zeneca and GSK. Also, VdP is Associate Editor of JIACI. Other authors declare no conflicts of interest. Other authors declare no conflicts of interest

Clinical implications

We report one of the first described anaphylaxis to whey protein supplements in a patient allergic to cow's milk who went under oral immunotherapy. Our findings may be relevant for users of protein supplements with the same clinical background.

Financial sources statement

The authors declare that non funding was received for the present study.

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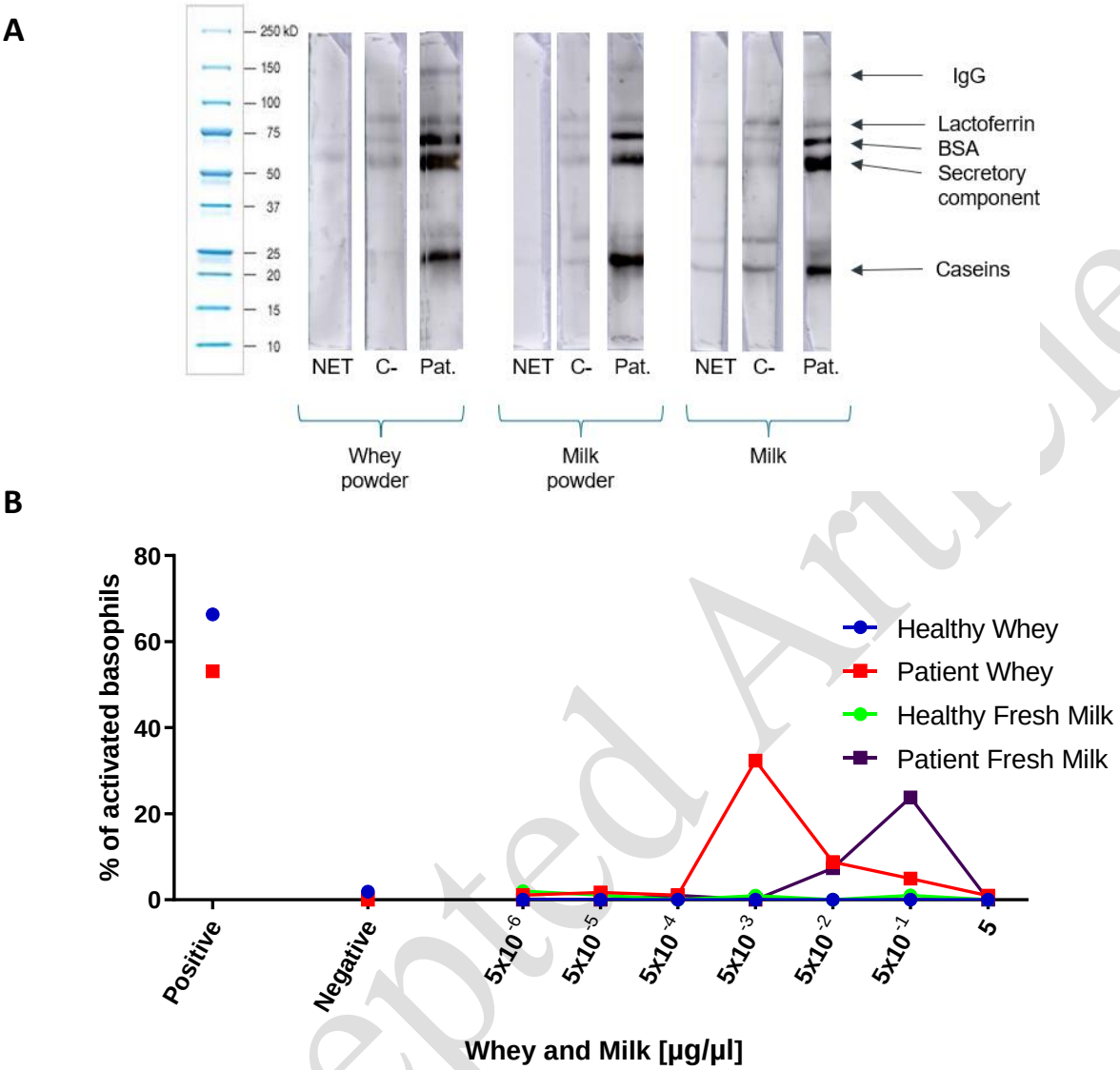

Figure 1. A, Western Blot results. Electrophoresis performed by SDS-PAGE under non-reducing conditions. Milk and supplement were dissolved in PBS1X. **NET:** Net 1X (negative technique control). **C-**: (Control serum). **Pat:** Patient serum. Binding of the immune complexes was performed using anti-IgE-HRP. **B,** BAT performed with WPS and fresh milk**.** The blood of the patient and a healthy control are confronted with decreasing concentrations of milk and whey protein powder. The result was considered positive with activation ≥15% (as established by the protocol). Results are expressed as the percentage of $CD63^{+}$ basophils (activated basophils).