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# IgE cross-reactivity measurement of cashew nut, hazelnut and peanut using a novel IMMULITE inhibition method

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**Abstract**

**Background:** Tree nut-allergic individuals are often sensitised towards multiple nuts and seeds. The underlying cause behind a multi-sensitisation for cashew nut, hazelnut, peanut and birch pollen is not always clear. We investigated whether immunoglobulin E antibody (IgE) cross-reactivity between cashew nut, hazelnut and peanut proteins exists in children who are multi-allergic to these foods using a novel IMMULITE®-based inhibition methodology, and investigated which allergens might be responsible. In addition, we explored if an allergy to birch pollen might play a role in this co-sensitisation for cashew nut, hazelnut and peanut.

**Methods:** Serum of five children with a confirmed cashew nut allergy and suffering from allergic symptoms after eating peanut and hazelnut were subjected to inhibition immunoassays using the IMMULITE® 2000 XPi. Serum-specific IgE (sIgE) to seed storage allergens and pathogenesis-related protein 10 (PR10) allergens were determined and used for molecular multicomponent allergen correlation analyses with observed clinical symptoms and obtained inhibition data.

**Results:** IgE cross-reactivity was observed in all patients. Hazelnut extract was a strong inhibitor of cashew nut sIgE (46.8%), while cashew nut extract was less able to inhibit hazelnut extract (22.8%). Peanut extract showed the least inhibition potency. Moreover, there are strong indications that a birch pollen sensitisation to Bet v 1 might play a role in the observed symptoms provoked upon ingestion of cashew nut and hazelnut.

**Conclusions:** By applying an adjusted working protocol, the IMMULITE® technology can be used to perform inhibition assays to determine the risk of sIgE cross-reactivity between very different food components.

**Keywords:** allergy diagnostics; cashew nut; hazelnut; IgE cross-reactivity; IMMULITE® technology; peanut.

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## Introduction

Among food allergies, an allergy to tree nuts is relatively common affecting ~0.05–7.3% of the population and its prevalence seems to be increasing, especially in children [1–3]. The majority of severe food allergy reactions such as anaphylaxis are related to tree nut ingestions [4], and tree nut-allergic individuals are often sensitised to multiple nuts and seeds [5]. Indeed, in the multi-centre Improvement of Diagnostic mEthods for ALlergy assessment (IDEAL) study by van der Valk et al. [6], co-sensitisation towards peanut and hazelnut was observed in more than 60% of Dutch cashew nut-allergic (multi-sensitised) children, of which 13% (n = 14) indicated to also suffer from clinical symptoms upon ingestion of all three seeds/nuts (cashew nut, hazelnut and peanut). Although cross-sensitisation seems less

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likely due to low level of botanical relations [7], structural identity between certain proteins like 2S albumins might be possible, and consequently may result in cross-reactive clinical symptoms. Cashew nut allergies cause predominantly severe reactions at very small exposure levels [6]. However, all except one child suffered from oral allergy syndrome (OAS)-related symptoms next to gastrointestinal complaints upon cashew nut ingestion and are immunoglobulin E antibody (IgE)-sensitised to birch pollen. Five of the 14 multi-allergic children in the IDEAL cohort could be selected for further research on co- and/or cross-sensitisation patterns to specific allergen components.

Reported co-allergy and IgE cross-reactivity between major and minor allergens in hazelnut, peanut and birch pollen has been reviewed extensively [3, 8–10]. However, an underlying cause that explains a multi-sensitisation to cashew nut, hazelnut, peanut and birch pollen has not been studied in detail. Thus, our aim in this study was to investigate whether IgE cross-reactivity between cashew nut, hazelnut and peanut proteins exists in children who are multi-allergic to these foods using a novel IMMULITE®-based inhibition methodology, and which allergens might be responsible for the observed IgE cross-reactivity. In addition, we explored if an allergy to birch pollen might play a role in this co-sensitisation for cashew nut, hazelnut and peanut.

# Materials and methods

## Study design and subjects

Case histories including clinical symptoms after eating hazelnut and peanut were collected from the registered electronic patient files and questionnaires in the IDEAL study (trial number NTR3572 [11]), as well as the result of the double-blind placebo-controlled food challenge (DBPCFC) with cashew nut, skin prick test (SPT) and IgE data specific for whole cashew nut (f220), hazelnut (f17), peanut (f13) and birch pollen (t3) [6].

## SPT measurements

SPTs against whole nut extracts were performed with cashew nut, hazelnut and peanut, a positive control (histamine 10 mg/mL; ALK-Abello, Nieuwegein, The Netherlands) in duplicate and PBS as a negative control. The histamine equivalent prick (HEP) index area was measured as described previously [11].

Protein extracts for SPTs were obtained from unsalted roasted cashew nut and unsalted fresh hazelnut and peanuts (not roasted). Seeds were mechanically homogenised using a mortar and pestle, defatted by ether extraction and air-dried. A 10% (w/v) extract in PBS was centrifuged for 10 min at 2000 *g*, and the supernatant was passed through a 0.22-m filter. All extracts were stored in appropriate aliquots at –20 °C until use in the skin test. Before the skin tests, the extracts were defrosted and mixed [12].

## sIgE-inhibition study

For the IgE-based inhibition tests with cashew nut, hazelnut, peanut and birch pollen, we developed a methodology for specific IgE (sIgE)-inhibition testing on the fully automated IMMULITE® 200 XPi (see a visual overview in Figure 1). This method is purely experimental without extensive validation and was not performed before. For standard routine sIgE quantification, IMMULITE® makes use of an enzyme-enhanced chemiluminescent enzyme immunoassay. In short, a streptavidin-coated bead, a biotinylated liquid allergen and a patient serum sample were mixed and incubated for 30 min. After a spin wash, an alkaline phosphatase-conjugated monoclonal antibody specific for human IgE (AP-IgE) was added and incubated for 30 min. After another spin wash, the presence of the AP conjugate was measured by adding an AP-specific chemiluminescent substrate (phosphate ester of adamantyl dioxetane) which is converted to light. The intensity of the light produced is proportional to the amount of IgE present in the adjustor.

Allergens for the inhibition steps were prepared from a stock solution of nut/seed extract (5 mg/mL) that was provided by Siemens Healthcare Diagnostics (Los Angeles, CA, USA). For the whole food-inhibition experiments, a 2% dilution in PBS (100 µg/mL) of the allergen stock of choice was used (cashew nut [f202], hazelnut [f17], peanut [f13]), while for the Bet v 1-specific inhibitions, a concentration of 1.6 mg/mL (purified as described in [13]) in PBS was used. The nut/seed extracts were produced according to the same procedure as the extracts used in the normal IMMULITE® XPi sIgE tests.

Inhibition experiments were performed singly by pre-incubating sera with inhibitory allergen preparations mixed 1:1 for 1 h at room temperature before proceeding with the normal IMMULITE® XPi sIGE testing. Pre-incubations with PBS served as negative controls. The percentage of inhibition was calculated using the following formula:

$$\%\ \text{Inhibition} = (\text{serum pre-incubated with PBS} - \text{serum pre-incubated with inhibitor}) / \text{serum pre-incubated with PBS}) \times 100\%$$

## Allergen sIgE measurements

Serum samples were analysed for sIgE antibodies against cashew nut-specific allergens (Ana o 1,2,3) using the Siemens IMMULITE 2000 XPi® Immunoassay System (Siemens AG, Munich, Germany) [14]. Additional sIgE antibodies specific for nCor a 9 and rCor a 14 were determined using the ImmunoCAP 250 systems. Other sIgE measurements for hazelnut (rCor a 1), birch pollen (rBet v 1) and peanut (rAra h 1, rAra h 2, rAra h 3 and rAra h 8) were measured using the ImmunoCAP ISAC kit (Thermo Fisher Scientific, Waltham, MA, USA). An assay for Cor a 11 was not commercially available. Antibody levels above 0.35 kU/L as obtained by IMMULITE® and ImmunoCAP 250 were considered positive.

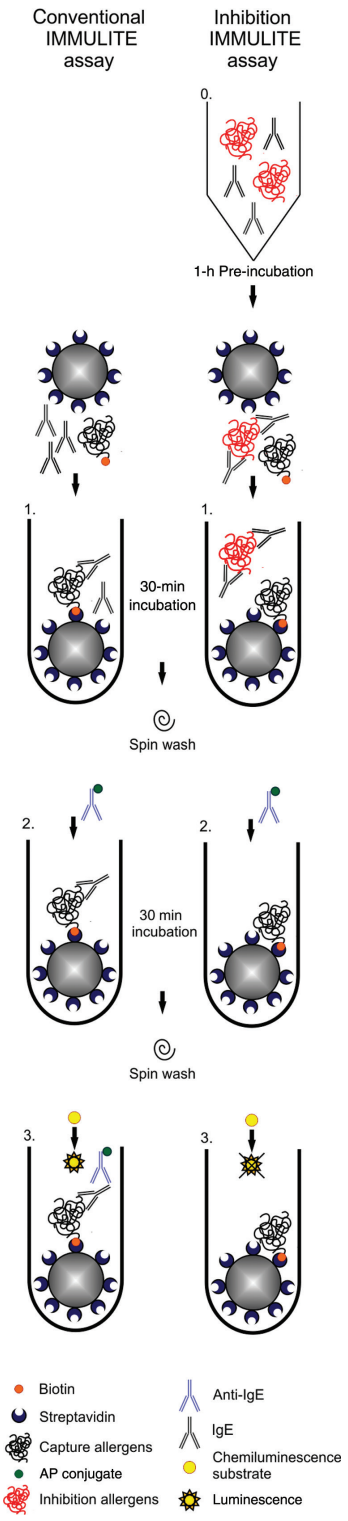


**Figure 1:** IMMULITE® inhibition methodology.
0. Serum sIgE is pre-incubated with or without an inhibition protein extract; 1. Serum and biotinylated capture allergens are incubated with streptavidin-coated beads; 2. AP-conjugated anti-IgE antibodies are added to the reaction mix; 3. Addition of AP-specific substrate results in luminescence that can be quantified. AP, alkaline phosphatase.

# Results

## Clinical history

Of the 179 children included in the IDEAL study [6], five children with a confirmed DBPCFC test against cashew nut plus a positive history of allergic symptoms after hazelnut and peanut ingestion were selected for this small follow-up study to investigate possible IgE cross- and/or co-reactivity between cashew nut, hazelnut and peanut allergens. In addition to a clinically relevant food allergy, all children suffered from a birch pollen-related inhalation allergy. Baseline characteristics including SPT, whole food/pollen-sIgE and case history for cashew nut, hazelnut, peanut and birch pollen in the five selected patients from the IDEAL study can be found in Table 1.

## Inhibition assays

To characterise possible cross-reactive allergens in the cashew nut-allergic children, each serum sample was exposed to six inhibition tests using biotinylated cashew nut, hazelnut and peanut extracts as detection allergens and non-biotinylated extracts as inhibitors. First, the inhibition of IgE that would be captured by cashew nut was investigated. As expected, inhibition of cashew nut-sIgE with cashew nut protein extract (=positive control) reached 90%–99% (Figure 2). Hazelnut, on the other hand, was able to inhibit cashew nut-sIgE detection in four of the five patients with a mean inhibition rate of 46.7%. Lowest mean inhibition of cashew nut-sIgE was seen for peanut extract (2.6%).

Next, we attempted to inhibit hazelnut-sIgE binding. Cashew nut protein extract was able to inhibit hazelnut-sIgE detection in four of the five patients with a mean of 24.2%, while peanut was able to inhibit hazelnut-sIgE only in patients #1110015 and #3330002 (mean inhibition rate 5.0%). The positive control extract (hazelnut) was again able to inhibit up to 99% of the hazelnut-sIgE.

Peanut-sIgE was inhibited more efficiently by a hazelnut than by a cashew nut extract, especially in patient #1110063. These results indicate that IgE cross-reactivity between cashew nut and hazelnut clearly exists, but the role of peanut seems to be negligible.

## Allergen-sIgE diagnosis

Hazelnut protein showed to be a strong inhibitor of IgE that also specifically binds to cashew nut protein,

**Table 1:** Baseline characteristics including SPT, sIgE and case history for cashew nut, hazelnut, peanut and birch pollen in the five selected patients.

| Patient | Cashew nut | | | Hazelnut | | | Peanut | | | Birch pollen | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Symptoms | SPT (HEP) | sIgE, kU/L | Symptoms | SPT (HEP) | sIgE, kU/L | Symptoms | SPT (HEP) | sIgE, kU/L | Symptoms | SPT (HEP) | sIgE, kU/L |
| #1110015 | Oral allergy, upper airway symptoms, nausea/stomach ache and vomiting | 2.1 | 38.6 | Oral allergy, redness in the mouth | 4.4 | 26.7 | Nausea/stomach ache/ vomiting | 5.9 | 2.8 | Seasonal rhinitis, runny nose, sneezing, stuffy nose, asthma | 1.3 | 88.9 |
| #1110063 | Oral allergy, nausea/ stomach ache and vomiting | 2.4 | 2.6 | Oral allergy, itching and irritation of the mouth/ throat/lips | 2.1 | 45.5 | Redness and coughing | 4.6 | 46.3 | Asthma | 0.9 | >100 |
| #2220011 | Oral allergy, nausea/ stomach ache and vomiting | 4.8 | 1.1 | Nausea/stomach ache/ vomiting | 2.1 | 45.5 | Nausea/stomach ache/ vomiting | 3.7 | 5.6 | Seasonal rhinitis, runny nose, sneezing, stuffy nose | 1.0 | >100 |
| #2220029 | Nausea/stomach ache and vomiting | 3.3 | 9.3 | Worsening of eczema | Neg | 7.2 | Upper airway symptoms | 4.7 | 1.9 | Seasonal rhinitis, runny nose, sneezing, stuffy nose | 1.4 | 38.6 |
| #3330002 | Oral allergy | 2.9 | 3.5 | Oral allergy, itching and irritation of the mouth/ throat/lips | 0.6 | Neg | Upper airway symptoms | 6.1 | 2.4 | Seasonal rhinitis, stuffy nose | 1.1 | 20.7 |

Neg, negative.

especially for patients #1110015 and #2220029. Allergen cross-reactivity between nuts might be predominantly based on storage proteins [15]. In order to determine for each patient whether the albumin- (2S) or globulin-type (7S/11S) seed storage allergens might be involved in the observed whole food-sIgE-inhibition activity, allergen-sIgE levels for cashew nut (Ana o 1, Ana o 2 and Ana o 3), hazelnut (Cor a 9 and Cor a 14) and peanut (Ara h 1, Ara h 2 and Ara h 3) were evaluated (Table 2). As all children suffered from a birch pollen inhalation allergy, also sIgE levels against the major birch pollen allergen Bet v 1 and their equivalents in hazelnut (Cor a 1) and peanut (Ara h 8) were measured.

We hypothesise that the relatively strong cashew nut/hazelnut inhibition observed in patients #1110015 and #2220029 might be primarily caused by cross-reactivity between globulin allergens Ana o 2 and Cor a 9 rather than between 2S albumin allergens. Even though a mean inhibition rate of 12.8% was observed of cashew nut-sIgE by peanut extract, a peanut-related globulin sensitisation seems not to play a role in these two patients, as Ara h 1- and Ara h 3-sIgE were both negative. Possibly, a cross-reactivity between the albumin allergens Ana o 3/Ara h 2/Cor a 14 may explain the observed peanut-inhibition activity.

Patient #1110063 hardly showed inhibition of cashew nut-sIgE with hazelnut and no inhibition of hazelnut-sIgE with cashew nut protein extract, even though the serum contains sIgE against the 2S and 11S storage protein allergens. On the other hand, peanut-sIgE in this serum was strongly inhibited by hazelnut protein extract. Also, this serum shows high sIgE levels for the Bet v 1-like allergens Cor a 1 and Ara h 8. This suggests that a pathogenesis-related protein 10 (PR10)-related hazelnut/peanut cross-reactivity might be a possible cause for the observed inhibition (although maybe not clinically relevant as no OAS is observed upon peanut ingestion).

The absence of cashew nut-sIgE inhibition by hazelnut or peanut was also observed for patient #3330002, indicating that cross-reactivity between the 2S albumins Ara h 2 and Ana o 3 is unlikely. Also for this patient, a PR10-related hazelnut/peanut cross-reactivity might possibly explain the observed inhibition of hazelnut-sIgE by cashew nut (41.2%) and peanut (31.4%) extracts.

Although the positive 2S albumin sensitisation to cashew nut (Ana o 3), hazelnut (Cor a 14) and peanut (Ara h 2) indicates possible cross-reactivity, neither hazelnut- nor cashew nut-sIgE inhibition with peanut extract was observed for patient #2220011. This suggests that co-recognition of allergens in cashew nut and hazelnut by peanut 2S albumin-sIgE is unlikely. The observed cashew nut/hazelnut inhibition in this patient (72.2% for cashew

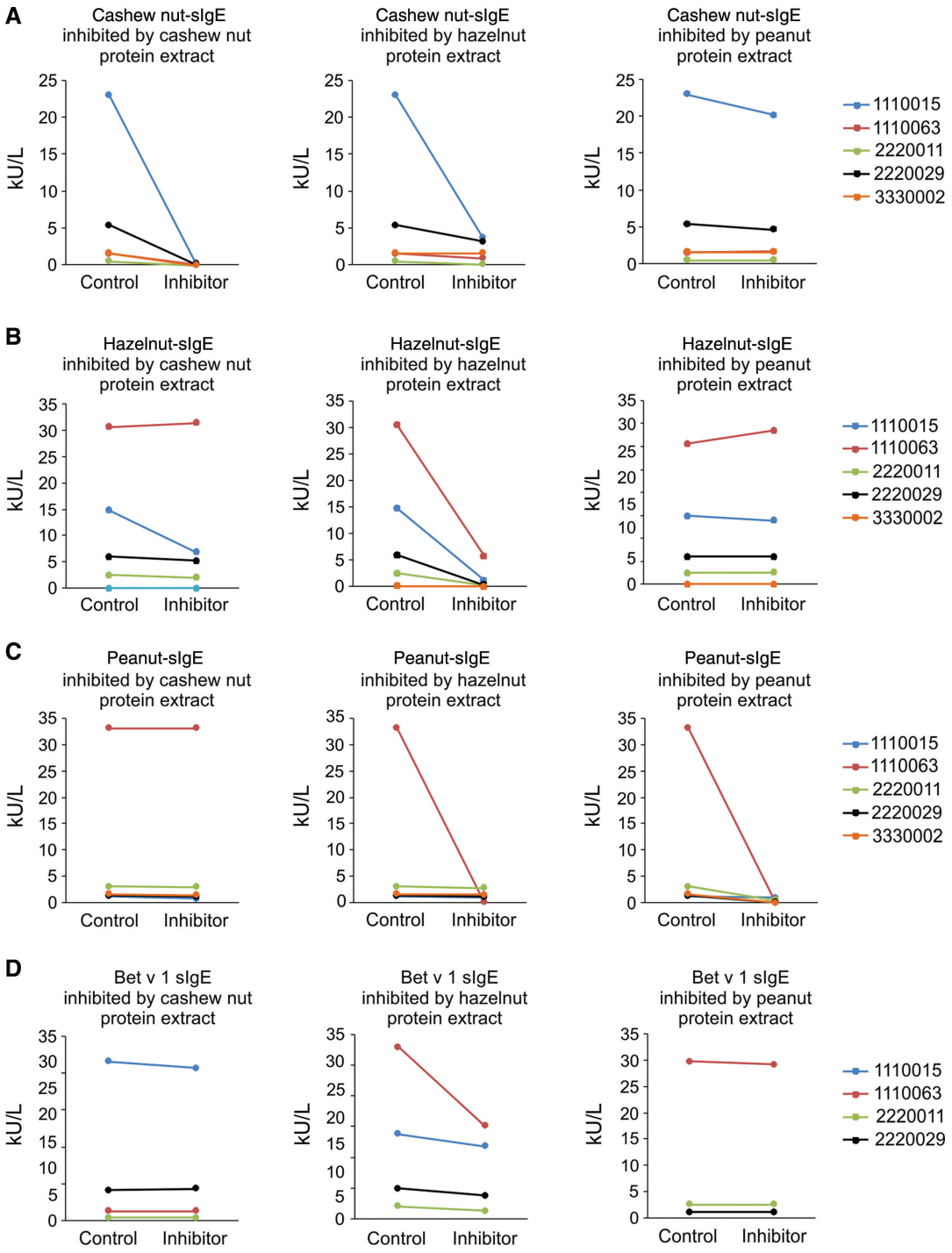


**Figure 2:** IMMULITE sIgE inhibitions by a total cashew nut, hazelnut or peanut protein extract.
(A) Inhibition of cashew nut-sIgE (f202); (B) inhibition of hazelnut-sIgE (f17); (C) inhibition of peanut-sIgE (f13); (D) inhibition of Bet v 1-sIgE (a89).

nut-sIgE and 16.7% for hazelnut-sIgE) could also be explained by the 11S globulin type of allergens.

Overall, the observed allergen component analysis cannot fully explain all cashew nut/hazelnut/peanut-sIgE cross-reactivity patterns in the individual patient sera, suggesting the involvement of additional allergens in the inhibition reactions.

## Bet v 1-specific IMMULITE® inhibitions

It was noticed that most patients, except #2220029, displayed mild OAS symptoms after consumption of cashew nut and hazelnut, next to the more severe gastrointestinal complaints. As all children are birch pollen-sensitised, we speculated that the observed clinical symptoms as

**Table 2:** sIgE (kU/L) levels of cashew nut, hazelnut, peanut and PR10 birch pollen allergens in the five selected sera, measured by ImmunoCAP (CAP), ImmunoCAP ISAC (ISAC) or IMMULITE® (IMM) methodology.

| Patient | Cashew nut-sIgE | | | Hazelnut-sIgE | | | Peanut-sIgE | | | | Birch pollen-sIgE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | nAna o 1 (7S) | nAna o 2 (11S) | nAna o 3 (2S) | rCor a 1 (PR10) | nCor a 9 (11S) | rCor a 14 (2S) | rAra h 1 (7S) | rAra h 2 (2S) | rAra h 3 (11S) | rAra h 8 (PR10) | rBet v 1 (PR10) |
| #1110015 | 2.4 | 34.0 | 10.5 | 16.8 | 43.7 | 0.8 | Neg | 0.9 | Neg | 7.8 | 63.2 |
| #1110063 | 0.7 | 2.4 | 3.9 | 77.4 | 0.9 | 0.4 | 8.5 | 6.1 | Neg | 15.6 | 119.2 |
| #2220011 | Neg | 0,9 | 0.9 | 8.0 | 0.5 | 13.0 | Neg | 5.7 | Neg | 15.6 | 61.3 |
| #2220029 | 1.9 | 10.9 | 16.0 | 6.8 | 2.1 | Neg | Neg | 3.0 | Neg | 2.47 | 15.8 |
| #3330002 | 1.3 | 5.0 | 9.2 | 4.2 | Neg | Neg | Neg | 3.9 | Neg | 0.8 | 14.9 |
| Analysed by | IMM | IMM | IMM | ISAC | CAP | CAP | ISAC | ISAC | ISAC | ISAC | ISAC |

2S, 2S albumin; 7S, 7S vicilin; 11S, 11S globulin; PR10, pathogenesis-related protein 10; Neg, negative.

well as the measured IMMULITE® sIgE inhibitions in some patients might be explained by a secondary (cross-reactive) reaction on Bet v 1-homologues in cashew nut, hazelnut and peanut. Therefore, an inhibition assay with nBet v 1 protein was performed on four of the five patients (for #3330002 not enough serum was left), as visualised in Figure 2D.

Hazelnut-sIgE detection was inhibited in all patients with an average of 28.9%, while cashew nut-sIgE was only reduced 4.17% in two of the four patients (#1110015 and #2220011). nBet v 1 hardly captured any peanut-sIgE, except in patient #1110063 (2.0%), which might be consistent with the lack of OAS symptoms in these patients upon peanut consumption. The Bet v 1-inhibition controls in each patient reached over 99% (data not shown). A summary of the mean inhibition rates in percentages is presented in Figure 3.

## Discussion

IgE cross-reactivity generally only occurs between proteins belonging to the same allergen family, mostly because of structural and sequential similarity [16, 17]. In the studied population, only in patients #1110015 and #2220029, a strong sIgE cross-reactivity was observed between hazelnut and cashew nut protein extracts, which might have possibly been caused by a specific 11S globulin sensitisation. IgE cross-reactivity between the globulin proteins Ana o 2 and Cor a 9 has been previously reported by Wallowitz et al. [18]. Also, *in vitro* cross-reactivity of cashew nut, hazelnut and peanut extracts with the walnut 11S globulin Jug r 4 has been observed [19].

For patient #2220011, a specific cashew nut/hazelnut globulin or albumin cross-reactivity could not be distinguished. For a cashew nut and hazelnut allergy,

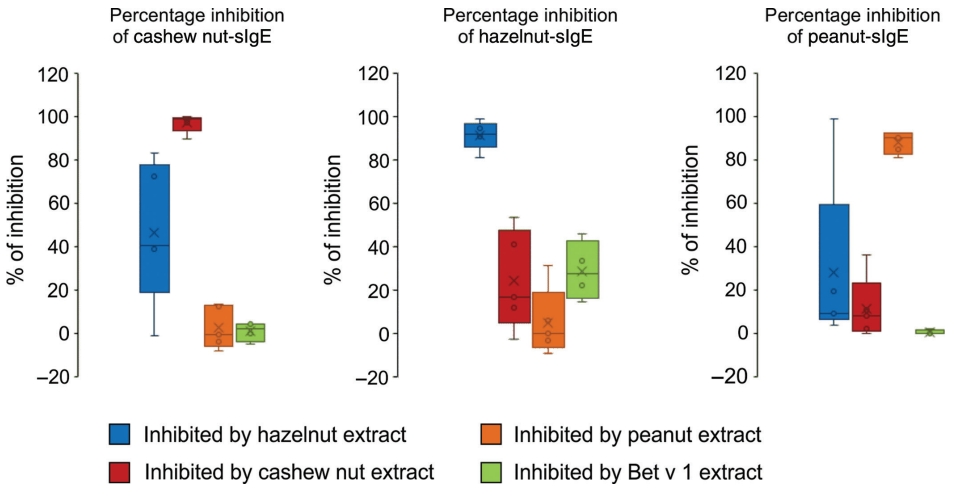


**Figure 3:** Overview of the mean inhibition rates in percentages.

sensitisation towards the 2S albumins, Ana o 3 and Cor a 14, respectively, is considered a prediction marker for clinical allergy [14, 20, 21]. However, cross-reactivity between these albumins sharing only 43% amino acid identity is considered rare [16], although this requires further verification.

Peanut displayed the lowest inhibition potency in this study. Only one patient (#1110063) was positive for Ara h 1-sIgE, while none of the patients studied were sensitised for the 11S-type globulins, although this could have been biased by the low sensitivity of the diagnostics method used (ISAC). A predominant 2S albumin sensitisation to peanut was detected, as well as a strong sensitisation to the birch pollen allergen Bet v 1 and its homologue Ara h 8. As none of the patients indicated OAS symptoms upon peanut ingestion, the Ara h 8 sensitisation in these patients seems to be clinically irrelevant, as also evident from the absence of a Bet v 1/peanut-inhibition activity in four of the five patients. Perhaps, the Ara h 8-sIgE in these patients recognise predominantly conformational epitopes that are destroyed upon heating of peanut. Although PR10 proteins are heat sensitive, Ara h 8 has been suggested as a major allergen in patients with a combined birch pollen and peanut allergy [22, 23]. Unfortunately, a Bet v 1-inhibition test could not be performed for patient #3330002 due to serum limitations, while in this patient peanut extract was a particular strong inhibitor of hazelnut-sIgE.

A 2S albumin sensitisation for peanut is commonly associated with severe systemic reactions [24], while from the clinical history only mild upper airway symptoms are described for three of the five patients. In general, cross-reactivity between 2S albumins seems to be uncommon due to their high amino acid sequence variability [16, 25], and IgE cross-reactivity of peanut-specific albumins occurs primarily between its isotypes rather than with tree nut 2S albumins [24, 26]. For instance, peanut did not display cross-reactivity with the 2S albumin Jug r 1 from walnut [27] nor with 2S albumins from Brazil nut [28], which could explain the low peanut-inhibition activity for these patients.

On the other hand, peanut-sIgE was inhibited on average 12.3 and 34.3% when pre-incubated with cashew nut or hazelnut extract, respectively. This contrasts a study by de Leon et al. [29], in which no inhibition of peanut-sIgE by cashew nut was observed, although cross-reactive allergen reactivity existed between hazelnut and peanut. de Leon et al. [29] applied immobilised peanut extract in their inhibition enzyme-linked immunosorbent assays (ELISAs) while in the IMMULITE® technique protein conformation during inhibition is preserved which possibly explains the contrasts observed in inhibition efficiency. Why peanut-sIgE can be captured by hazelnut and cashew nut while peanut extract displays only weak inhibition potency cannot be explained from the allergen multicomponent analysis performed. Possibly, differences in the extract's relative allergen concentrations and/or measurement methods may have interfered in the observed varying degrees of inhibitory potency.

Hazelnut and cashew nut extracts were able to inhibit the detection of Bet v 1-sIgE in some of the patients (#1110015 and #2220011), suggesting that the OAS-related symptoms upon ingestion of hazelnut and cashew nut in these children could very well be caused by Bet v 1-related homologues in both tree nut extracts. A birch pollen/hazelnut cross-sensitisation is well known as reviewed by Costa et al. [30] and Flinterman et al. [31]; however, evidence for a clinically relevant Bet v 1-related cross-reactivity with cashew nut is still lacking. Putative IgE-binding homologues of Bet v 1 (PR10) have been identified in cashew nut by our group (unpublished results), but whether these allergens have cross-reactive potency manifesting in clinical reactions needs further investigation.

The symptoms upon cashew nut or hazelnut ingestion could also be caused by a non-PR10-related allergen sensitisation. Allergic reactions towards profilin or nsLTP proteins can also result in OAS symptoms [32, 33]. However, as none of the patients showed an nsLTP or profilin sensitisation on the ISAC (results not shown), these allergens are most likely not involved in the clinical reactions of our five patients.

A limitation in our current study is the use of two different specific IgE measurement methods, the ImmunoCAP and the ISAC, due to low serum availability. Both methods were compared earlier [34, 35] and the detection rates for ISAC and ImmunoCAP were comparable: 65% and 71%, respectively, in patients with nut allergy. Although the detection rates apparently only slightly differ, we cannot rule out that this has influence on our results.

In this study, we have successfully demonstrated that the IMMULITE® technique can be used to perform IgE-inhibition assays, as previously also shown for the ImmunoCAP technique [36]. Although the reproducibility of the new method was not tested, the specificity of the inhibition data measured using this method was demonstrated by the strong inhibition obtained by the positive controls. The advantage of this technique over the ImmunoCAP inhibition technique [36] or the commonly applied immunoblot or ELISA-inhibition tests is that inhibition of biotinylated allergens and detection is conducted in the liquid form, before conjugation to streptavidin-coated beads takes place, meaning that the conformational properties

of proteins are conserved, increasing physiological relevance. However, using this method, the minimal amount of serum needed per inhibition assay is still substantial (90 μL), meaning that no inhibition concentration curves could be performed because of serum availability limitations. This prevented us to acquire EC50 values (amount of protein extract needed to inhibit 50% of sIgE binding), implying that the strength of inhibition or cross-reactive potency per protein extract could not be evaluated in this study. In addition, available serum levels limited the amount of specific allergen inhibitions that could be performed. Globulin-specific inhibitions with Ana o 2 and Cor a 9 in particular could have contributed significantly to the understanding of sensitisation factors in our study population.

From the inhibition data, we could not conclude which patients are primarily sensitised to cashew nut and secondary to hazelnut or vice versa. As only a small sub-population was tested, the patients might be just co-sensitised and have a primary food allergy for cashew nut, hazelnut and birch pollen, and display no secondary food allergy. In addition, we are not sure if the possible cross-reactivity observed in this study is caused by the major seed storage allergens, or minor allergens not yet identified in cashew nut.

Thus, future validation experiments should be performed using larger patient cohorts to compare results obtained using the IMMULITE® inhibition technology with those obtained using the currently applied inhibition ELISA or inhibition ImmunoCAP technologies as well as to further validate its reproducibility and applicability in allergy diagnostics.

## Conclusions

Molecular diagnostic testing by measuring sIgE against individual allergen molecules or components using purified or recombinant allergens (CRD) provides detailed information on sensitisation patterns to allergologists and enables a more accurate interpretation of allergic symptoms by distinguishing clinically relevant food protein sensitisation from non-relevant sensitisation that does not cause systemic reactions [37]. Moreover, such a CRD analysis can broaden our understanding of which IgE cross-reactivity reactions between foods are to be expected in a patient group, which may guide dietary advice [3]. We have demonstrated that the IMMULITE® technique can indeed be applied to evaluate IgE cross-reactivity between protein extracts and between specific allergens.

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**Author contributions:** SB, NS, HJW and NWdJ gave shape to the study design and interpretation of results. MRB performed the IMMULITE® sIgE measurements as well as the inhibition tests. ImmunoCap and ISAC measurements were performed by MWJS. Patient clinical details were summarised by JPMvdV. RGvW, HFJS, HJW and NWdJ acquired the necessary funding and all authors contributed to drafting and revising the article and approved the final version for publication.

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