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Regarding MS ALL-2016-00311:

Lange L et al. Ana o 3-specific IgE is a good predictor for clinically relevant cashew allergy in children

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Cashew Allergy Risks, 2S albumins, and Predictions based on IgE Antibody Levels

We read with great interest the report of Lange et al (1) that studied cashew allergic children and the potential predictive value of cashew 2S albumin storage protein (Ana o 3)-specific IgE measurements in determining the need for an oral cashew challenge to prove disease. They report that IgE antibody levels >2 kU_A/l to Ana o 3 improved their prediction (95% probability) of a positive oral food challenge after cashew consumption in comparison to IgE against the whole cashew extract. In other words, increased analytical specificity of molecular Ana o 3-specific IgE testing (2) reportedly translates into an improved diagnostic efficacy and a superior prediction for a positive oral cashew challenge.

Three issues are raised by the study: A) the report supports other studies that suggest a quantitative IgE measurement specific for a 2S albumin can serve as a biomarker of clinical response, B) there are implications regarding other potentially cross-reactive food allergens, and C) the study may only be narrowly valid for a selective population because there are additional factors that determine the relationship between a quantitative measurement of allergen-specific IgE and clinical presentation of allergic symptoms. Following paragraphs expand on these issues and discuss the advantages and limitations of the use of a quantitative IgE measurement as a biomarker of allergic disease.

A) We and others have reported that the general ability of molecular allergen-specific IgE antibody testing to potentially distinguish a clinically relevant from a silent IgE sensitization is one of molecular allergen based assays' notable strengths (2). Thus, this outcome in their report expands on the original conclusions of Sampson et al. (3) for other foods and it is not

a surprise. Seed storage proteins, in particular 2S albumins (4) in legumes, tree nuts, and oil seeds, are known to be highly abundant and stable. Their extraordinary thermal and digestive resistance allows them to effectively trigger severe anaphylactic reactions. Increased levels of IgE antibodies to a number of the 2S albumins (e.g. Ara h 2 (5, 6) and 6 (7, 8) from peanut, Gly m 8 (9, 10) from soy, Cor a 14 (6, 11) from hazelnut, Jug r 1 (12) from walnut, Ber e 1 (13) from brazil nut, Ses i 1 (14) from sesame, and others) seem to generally indicate an increased risk concerning severe reactions in a sensitized individual following exposure to the offending allergen source.

B) What's about other related foods in this situation? Does the presence of IgE antibody specific for cashew 2S albumin indicate an increased risk from exposure to other potentially cross-reactive 2S albumin-containing plant foods? Due to fairly limited cross-reactivity among these different storage proteins (4, 15), IgE-levels in any given patient are expected to vary considerably if different 2S albumins from these various sources are tested. Ideally, a clear negative result to a 2S albumin of one source could potentially rule out serological as well as clinical cross-reactivity. Thus, totally negative results using a singleplex IgE assay can be considered of value in ruling out sensitization! Moreover, the highest IgE antibody level against one of a panel of these different 2S albumins would likely point to the "primary sensitizer" which would also be helpful for counseling on avoidance. Clearly, a more accurate indication of the responsible allergen that initiated the 2S albumin-specific IgE antibody response detectable in a patient's serum can provide helpful information as a result from a diagnostic allergy work-up.

One might ask whether it is of value to test a patient's serum for IgE antibody to as many 2S albumins as are available in the routine clinical immunology laboratory. Could placing 2S albumin-specific IgE levels from a panel of different specificities (i.e. Ara h 2, Gly m 8, Cor a 14, Ana o 3, Jug r 1, Ber e 1 and Sesi 1) in a hierarchical order from highest to lowest aid in distinguishing clinically relevant from silent sensitizations and cross-reactivities? This question, unfortunately has not yet been systematically addressed and awaits a convincing answer. By utilizing a singleplex IgE antibody assay with an optimal analytical sensitivity, it will be possible to answer this better as more 2S albumins become available in the diagnostic allergy laboratory.

One general rule of thumb in molecular allergy diagnostics advocates for limited testing in the case of highly cross-reactive allergen families (i.e. profilins, polcalcins, non-specific lipid transfer proteins, tropomyosins, parvalbumins, lipocalins, PR10 like proteins and serum albumins) (2). Testing IgE antibody to more than one candidate of these broadly cross-reactive allergen families tends to duplicate a previously detected positive or negative serology to one member of the family and unlikely adds any useful additional information. In

contrast, limited cross-reactivity within one allergen (sub)family (i.e. 2S albumins) could represent a unique case where broad testing of many family members would be beneficial in identifying the potentially significant from the insignificant (negative) cross-sensitizations. But how does one interpret the case where specific IgE antibody levels are not entirely negative to the other 2S albumins, but rather positive to a subset of those allergens with shared allergenic epitopes (15) in comparison to the presumed primary sensitizer? Clinically valid information from the patient's medical history and/or a supervised oral challenge would thus be absolutely necessary to successfully work-up such a case and clarify the clinical significance of the patient's serological sensitization pattern.

C) One must also consider the complex set of variables that ultimately determine whether an allergen exposure will translate into an IgE-mediated hypersensitivity induced clinical reaction: A number of interrelated variables play a major role in translating a quantitative level of IgE antibody specific for an allergenic molecule into an end-organ immediate-type hypersensitivity response to that corresponding allergen source. Of the 10 highly variable parameters involved in this biological process noted below (highlighted in (16-18)), only few are actually measured by routine diagnostic testing (shown with *italic characters*). These parameters include:

- I) *quantitative allergen-specific IgE concentration in kU_A/L* distributed between the serum and tissue
- II) avidity spectrum of the allergen-specific IgE-antibody repertoire (19)
- III) *ratio of allergen-specific to total IgE*, termed specific activity (20)
- IV) total number of IgE-receptors on effector cells (mast cells, basophils) (21), as determined by constitutive production which depends on the (local) cytokine milieu (22) and internalization (recycling) vs. (passive) stabilization depending on total IgE levels (23). This results in a general, but loose log-linear relationship between total IgE and total number of Fc_εRI on effector cells (24)
- V) number of cross-linked IgE-receptors required for a significant (i.e. half-maximum) cellular response, termed intrinsic sensitivity (25)
- VI) status of cell activation (i.e. early Fc_εRI-transduced signals via Syk phosphorylation) (23)
- VII) number of effector cells within a certain tissue
- VIII) tissue response to instantly released, newly synthesized and prolonged produced mediators

IX) the end-organ immediate-type hypersensitivity response.

X) co-factors of immediate type reactions (i.e. exercise, alcohol consumption, NSAID intake).

In addition, the following assumptions should be kept in mind when considering the predictive value of an allergen-specific IgE antibody result:

1. A prediction on a clinical outcome based on a single parameter such as allergen-specific IgE levels will never be definitive, and at best, it can establish a rather loose and scattered association with clinical disease (2).
2. Any statistically-calculated threshold (i.e. allergen-specific IgE concentration for 95% probability of a positive or negative clinical response) has limited value and largely depends on the context relating to the patient, i.e. the geographical region (regional exposure to food and/or aeroallergens, dietary habits); cohort (age group, clinical phenotypes, inclusion criteria, control group); and procedures (comparators, oral challenge protocol, assay type)
3. Thresholds are clearly limited to the assay type which needs to be the same as the one that was used to generate the original predictive threshold results.
4. Threshold levels, also coined "decisions points" should not be broadly generalized; rather they should be cautiously interpreted while considering the clinical history context of each individual case.
5. Even if normalization of allergen-specific IgE (considering the specific IgE/total IgE ratio) does not immediately improve a prediction on clinical outcomes as in the cashew study or recently reported peanut studies (1, 26), the concept (20) carries significant value for individual interpretation of IgE levels, particularly if the total IgE levels are extremely low (i.e. <10 kU/l) or high (i.e. >1000 kU/l).
6. Interpretation of specific IgE/total IgE ratios requires comparable units ($\text{kU/l} = \text{kU}_\text{A}/\text{l}$) of the assays employed, which has only rarely been demonstrated with one method (27).
7. At best, one can assume qualitative concordance, but no absolute quantitative correlation should be expected by comparing allergen-specific IgE levels (even against allergenic molecules) with in vivo skin test or ex vivo cellular responses (28). This is due to the many underlying parameters highlighted above in I – X (2, 18).
8. A positive allergen-specific IgE serology, skin prick test or basophil activation test result indicates "sensitization". Their results are only clinically relevant in case of corresponding

allergic symptoms. They might therefore be considered as "sensitization tests", and not "allergy tests".

9. Only the person who knows the patient's clinical history is able to interpret their sensitization test results.

10. Clinical relevance has to be determined by the allergist or the responsible health care professional who knows the patient's clinical history and not by the test itself, even if single allergen molecules (2) are used in the analysis.

The difficulty in extrapolating from "sensitization" to "clinical allergy" is also highlighted by a recent study, showing that IgE antibody responses to common foods were detected in 26% of a representative cohort in South India. This contrasted with the (self reported) clinical food reactions that occurred in only 1.2% of these subjects (29). The authors did not provide total serum IgE levels, but mention the concern about high total IgE levels in the Indian cohort as one potential confounding factor for the disappointing low positive predictive value of allergen-specific IgE. The high total serum IgE levels in this population could result from wide-spread nematode parasitism that is common throughout South India.

Returning to the cashew study by Lange and colleagues (1), how would its predictive value turn out if IgE anti-Ana o 3 were measured in this South India region where high total serum IgE levels are common? Would one expect similar cut-off levels for Ana o 3 specific IgE? Importantly, more than one variable, not only allergen-specific IgE or the specific to total IgE ratio as in the case of the cashew study (1) should be considered to understand its predictive value in relation to the observed IgE-mediated hypersensitivity reaction. Total IgE is a rarely requested analyte that should be routinely integrated into diagnostic testing plans to adequately interpret the clinical significance of an allergen-specific IgE measurement (30).

In conclusion, predictions on allergic clinical outcomes using a single IgE antibody analysis involving components such as a 2S albumin would be nice to have, but it is not as straight forward as many studies such as cashew component investigation of Lange et al (1) suggest. A successful diagnostic allergy work-up still requires individually planned diagnostic steps and proper interpretation, while always taking into consideration the historical context of the patient on a case by case basis.

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