

Letter to the Editor

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Preliminary study in specific activity of molecular components in allergy: implications for diagnostics and relationship with disease severity

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To the Editor,

In an ideal situation, specific IgE (sIgE) in allergy testing should correlate with patients' clinical history and additional testing would not be necessary. Unfortunately, there is a significant number of patients where biochemical testing does not correlate with their clinical history and the test outcomes are not a reliable predictor for severe reactions in this patient group [1]. This leads to further diagnostic testing such as skin prick testing (SPT), oral provocation testing and unnecessary dieting. The diagnostic value of the extracts and molecular components (MC) sIgE is questionable as the cut-off indicates only sensitization [2]. Furthermore, the information thus provided is incomplete, as patients may still report anaphylactic shock on the same sIgE values as those who fully tolerate food allergens. Progress has been made in biochemical

testing and the use of sIgE_{MC} allows more specific testing, but the fundamental problem of poor correlation between test results and clinical presentations remains [3–5]. Studies have shown that the ratio of food extract sIgE to total IgE (tIgE), the so called specific activity of extracts (SA_{EXT}) is a useful parameter in predicting clinical outcome as compared to the absolute value of sIgE of extracts [6–8].

The concept behind specific activity (SA) is that it relates sIgEs to the total pool of IgEs. Theoretically, the sum of all IgEs is reflected by tIgE. The IgE receptor does not appear to have a predisposition for any specific type of sIgE. These sIgEs rather compete for the same binding sites [7]. The SA of molecular components (SA_{MC}) might be the missing piece that we are looking for to provide a specific test that relates to disease severity.

sIgE for molecular components can be determined by different techniques, with the largest portfolio of components offered by Thermofisher Scientific Phadia. sIgE can be measured in quantitative way (ImmunoCAP Components) or semi-quantitative way (ImmunoCAP ISAC). On the ImmunoCAP, approximately a million times more allergen is coated on a cap, compared to ISAC [9]; therefore, when using ISAC, it is possible that sIgG [particularly specific IgG4 (sIgG4)] generated as a result of activation of the immune system in patients, might interfere with sIgE for the allergen binding site. The results of sIgE measured on the ImmunoCAP and ISAC may potentially diverge due to the sIgG (sIgG4) concentration [10].

The primary objective of this study was to evaluate the use of SA_{MC} as a clinical predictor for disease severity and to compare it to ISAC and ImmunoCAP sIgE measurements. In addition to this, we examined whether the discrepancy between ISAC and ImmunoCAP was explainable by sIgG4. The secondary objective was to investigate the relationship of SA_{MC} with disease severity for the peanut and egg components Ara h 2 and Gal d 1 in a larger group and estimate SA_{MC} cut-off values for Gal d 1 and Ara h 2.

The study was approved by Medical Ethical Committee of the Maasstad Hospital, Rotterdam. Group A patients

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were eligible for inclusion after referral for ImmunoCAP ISAC testing, which occurred between January 2013 and December 2013. Patients were included if they tested positive on ISAC for MC related to severe food allergy; 39 positive IgE_{MC} were obtained from 17 patients. The stored samples were measured by ImmunoCAP. The group A test population consisted of complex multisensitized patients [n = 17, six males, 11 females, with median (IQR age) of 13 (6–35) years] who were divided in groups based on prominent complaints as follows: five rhinoconjunctivitis/asthma, one acute urticarial/angioedema, two eczema, four anaphylactic reactions and finally five suffered from a combination of symptoms. Those with severe disease, had severe symptoms (anaphylaxis or angioedema) related solely to direct exposure to culprit food.

Group B was selected to investigate the secondary objective of this study (June 2014–December 2015). Patients with reported tIgE and who tested positive for Ara h 2 [n = 64, 42 males and 22 females with median (IQR) age of 5 (3–11) years] and/or Gal d 1 [n = 50, 36 males and 14 females with median (IQR age) of 3 (2–4) years] were included. Patients allergic to peanut or egg suffered most frequently from eczema and combination of allergic symptoms, in 86% and 78% of cases, respectively.

The disease severity was defined by an experienced allergy specialist as “severe” or “not severe” dependent on presence or absence of systemic reaction and reporting clinical symptoms after ingestion of a certain food source. The diagnosis was made based on clinical history, SPT and biochemical testing results. In several cases food allergy was confirmed with double-blind food provocation testing, however this was not performed in young children or patients with previously reported anaphylaxis. In cases where diagnostic testing was missing, the experienced allergist classified the disease severity (expert opinion).

Blood was collected by venipuncture. Sera from patients were primarily used for the ISAC analysis and remaining material was stored at –20 °C until further analysis. These analyses consisted of determining tIgE, sIgE of extracts and sIgE and sIgG4 of food molecular components. The ISAC analysis was performed in the University hospital of Erasmus Medical Centre Rotterdam (The Netherlands) by experienced technicians and authorized by a clinical immunologist blinded to all laboratory and clinical data. The skin prick testing as previously reported [11].

The agreement between ImmunoCAP and ISAC was reasonable ($r = 0.588$). No significant correlation between ImmunoCAP/ISAC sIgE and sIgG4/sIgE ratio was observed ($r = 0.0387$). The discrepancy between IgEs measured on

ImmunoCAP and ISAC, could not be explained in our study by the IgG4/IgE ratio, both expressed in mg/L.

There was a poor concordance of ImmunoCAP and ISAC in relation to disease severity, yielding many false positives (Table 1). The use of conventional sIgE cut-off values for both, the ImmunoCAP (0.35 kU_A/L) and ISAC (>0.3 ISU) gave 85% and 82%, respectively, abnormal results and even >70% abnormal results after increasing both thresholds tenfold. An improved correlation with disease severity of SA_{MC} was apparent without having yet established a potential cut-off value. In two patients the SA_{MC} obviously did not correlate with disease severity (pat #11 for Gal d 1 and pat #8). Pat #11 showed high tIgE and had many sensitizations (2109 kU/L). Pat #8 had severe (anaphylactic) reaction to peanut. Severe allergic reaction to hazelnut, walnut and peach was highly suspected. Based on positive skin prick testing, an elimination diet was prescribed. Because of severe reactions in the past and positivity for the four LTPs from the food sources described above, no oral provocation was performed. These discrepancies could be explained either due to multiple sensitizations (reflected by high tIgE as in pat #11), or due to cross-reactivity as in pat #8 (for instance between LTPs). This hypothesis of relationship of SA_{MC} with disease severity was studied in more detail in patients from Group B.

Figure 1 depicts the ROC analysis of different diagnostic markers in patients' groups allergic to peanut (Figure 1A) or egg (Figure 1B). In the patient group allergic to peanut, SA_{Ara h 2} had the highest combination of positive and negative predictive values among the tested markers at cut-off of $\geq 2.9\%$ to discriminate between severe and non-severe disease (Figure 1C). In analogy, for the patient group allergic to egg, a cut-off value of $\geq 3.6\%$ for SA_{Gal d 1} was determined.

There are some limitations of this study. Our first aim was to compare ISAC and ImmunoCAP results and explore whether measurement of sIgG4 may help to explain eventual discordance between ISAC and ImmunoCAP results. We chose the backward approach, as sIgE to severe food MC are occurring at a low frequency. Our “backward” approach enabled us to discover the “pattern” in the results and form the hypothesis that severity correlates better with SA of components than with the absolute sIgE results, which we further explored and confirmed with group B patients (mainly young patients with mono- or oligo-sensitizations). The found cut-off values for the SA_{MC} might however not be applicable to Group A patients due to mixed age and multiple sensitizations. In a prospective approach, it would take approximately 20 years to gather the data from our population. Additionally, food provocation testing was not performed in all patients and

Table 1: Clinical validation of molecular components (Group A).

Molecular allergen component	Patient No.	Age, year	Gender	SA,%	tlgE, kU/L	slgE, kU _A /L	slgE ^b , ISU	IgG4, mgA/L	SPT ^c	Severity ^a
Ara h 2	1	14	F	0.60%	5001	28.4	12.82	0.29	pos	0
	2	23	F	16.88%	266	44.9	26.83	0.01	*	1
	3	12	M	1.30%	3075	39.4	9.59	0.18	pos	0
	4	21	F	0.34%	1643	5.63	6.7	0.06	pos	0
	5	1	M	3.43%	507	17.4	0.95	1.17	pos	0
Ara h 9	5	1	M	0.01%	507	0.03	<0.3	0.04	pos	0
	6	5	M	1.71%	1762	30.2	2.74	<0.03	pos	0
	7	51	F	9.70%	264	25.5	4.93	0.59	neg	1
	8	47	F	13.08%	772	101	16.96	7.14	*	1
Gly m 5	1	14	F	0.20%	5001	10.9	6.68	0.09	pos	0
	2	23	F	1.55%	266	4.13	4.45	<0.03	*	0
	3	12	M	0.01%	3075	0.20	<0.3	20.3	neg	0
	4	21	F	0.01%	1643	0.13	<0.3	<0.03	pos	0
	22	84	M	0.01%	1884	0.14	<0.3	0.05	neg	0
Gly m 6	1	14	F	0.20%	5001	4.06	3.11	0.49	pos	0
	2	23	F	2.28%	266	6.07	5.28	<0.03	*	0
	3	12	M	0.02%	3075	0.59	<0.3	<0.03	neg	0
	4	21	F	0.02%	1643	0.29	<0.3	0.04	pos	0
	22	84	M	0.01%	1884	0.20	<0.3	0.21	neg	0
Gly m 4	14	13	M	0.55%	481	2.13	0.6	0.03	*	0
	15	11	M	5.18%	1652	85.5	38.4	<0.03	*	0
	17	10	F	1.69%	257	4.35	0.5	<0.03	*	0
	22	84	M	2.83%	1884	53.4	31	0.57	neg	0
	23	50	M	8.71%	481	41.9	1.2	0.09	*	1
Bos d 8	3	12	M	0.40%	3075	13.4	2.1	2.74	pos	0
	12	13	M	2.69%	3752	101	10.4	8.87	pos	0
Gal d 1	5	1	M	2.90%	507	14.7	0.84	3.19	pos	0
	9	3	M	0.26%	6470	17.0	6.57	0.13	pos	0
	10	4	M	0.08%	6645	5.17	8.60	0.05	pos	0
	11	7	M	1.69%	2109	35.7	13.2	0.30	pos	1
Cor a 8	6	5	M	1.01%	1762	17.8	2.40	0.03	neg	0
	7	51	F	1.40%	264	3.58	2.38	0.28	neg	0
	8	47	F	11.00%	772	84.9	6.42	3.18	*	1
Jug r 3	6	5	M	2.20%	1762	38.7	13.9	0.35	pos	0
	7	51	F	10.70%	264	28.3	16.5	1.21	pos	0
	8	47	F	13.08%	772	101	22.8	9.49	*	1
Pru p 3	6	5	M	3.83%	1762	64.7	14.3	0.04	*	0
	7	51	F	12.70%	264	33.6	14.1	0.90	*	0
	8	47	F	13.08%	772	101	27.2	10.0	*	1

^a1 = severe disease; 0 = non-severe disease. ^bISU < 0.3 is reported to compare to ImmunoCAP and skin prick results, but primary slgE positivity to another MC from the same allergic source was found on the ISAC and was reason for inclusion to group A. ^c* = not performed.

therefore expert opinion was used to classify patients in severe and non-severe categories.

In conclusion, by this preliminary study we showed in a small sample size that IgE antibodies to molecular food components, whether measured on ISAC or ImmunoCAP, were frequently abnormal and did not reflect disease severity sufficiently. This was reiterated when we increased the cut-off values tenfold and produced similar abnormal results. To the best of our knowledge, this is

the first study using SA_{MC} in relation to disease severity for important food molecular components. The previous studies relating SA to clinical outcomes were using extracts and therefore less accurate with more conflicting results between studies.

The necessity for using and establishing cut-off values for SA_{MC} in order to define a more reliable and patient dependent policy on treatment of allergic disease is evident and should be explored in further larger studies.

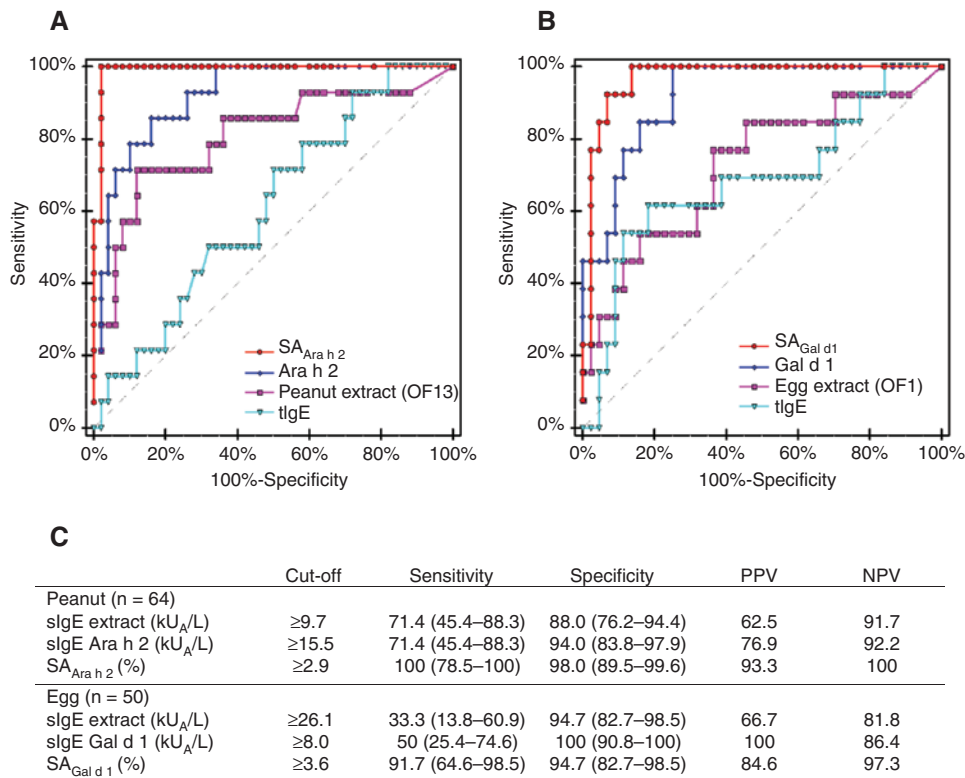


Figure 1: ROC analysis of peanut and egg diagnostic markers: disease severity was defined as presence of systemic disease meaning that at least two or more organs were affected.

(A) Patient group allergic to peanut, (B) patient group allergic to egg and (C) summary of ROC curve analysis in peanut and egg allergic groups of patients.

Nevertheless, as has been shown in previous studies, these cut-off values may not be extrapolated to different populations.

interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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