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Original Article

Diagnostic usefulness of trait specific IgE and multiple immunoglobulin production in allergic diseases

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Abstract: Purpose: This article is to evaluate the proportion of mono-sensitization and poly-sensitization based on sIgE composition and to develop a novel diagnostic tool and study the production of different immunoglobulins (Ig) in allergic diseases. Methods: The serum sIgE composition of 2409 patients diagnosed with allergic disease was analyzed using the sIgE immunoblot method. A specific formula was developed to calculate the theoretical proportion of mono-sensitization (TPM) in different gender, age, season, disease and pathway groups. A second formula also established a theoretical value for the proportion of compound sensitization (TPC) in the different pathways. IgG antibodies to hepatitis C virus (HCV), Treponema pallidum (TP), human immunodeficiency virus (HIV) and IgM antibodies to *Toxoplasma gondii (Toxoplasma*), Cytomegalovirus (CMV), Rubella virus (RV) were detected. Results: The observed proportions of mono-sensitization (OPM) were all lower than the predicted TPM. Poly-sensitization was predominant in allergic diseases. There were statistical differences in OPM and TPM in different groups ($P<0.001$). The observed proportion of compound sensitization (OPC) was higher than TPC. IgM antibodies to CMV and IgG antibodies to TP were significantly different in allergic diseases. Receiver operating characteristic curve (ROC curve) analysis of trait sIgE indicated an area-under-the-curve of 0.749. Conclusions: The co-sensitization instead of cross-reactivity was the major mechanism of poly-sensitization. Except for sIgE abnormality, changes in anti-CMV IgM and anti-TP IgG suggested an abnormal state of plasma cells. Trait sIgE may be used as a good indicator for diagnosis in allergic diseases.

Keywords: Allergic diseases, specific IgE, mono-sensitization, poly-sensitization, trait sIgE, immunoglobulin

Introduction

Allergic diseases are a critical public health problem regardless age, racial and gender groups. Examples of Allergic diseases are atopic dermatitis (AD), asthma, urticaria, allergic rhinitis (AR). In the past few decades, the prevalence of allergic disease has increased globally [1-3]. The pathological mechanism of allergic diseases is varied and complicated. Most allergic diseases are considered multifactorial. They can be influenced by genetic, epigenetic, developmental and environmental factors. Immunoglobulin E (IgE) associated allergic diseases belong to a broader family of inflammatory conditions. Their clinical manifestations range from mild to life-threatening.

It has become increasingly evident that IgE mediates type I hyper-sensitive reactions plays an essential role in the pathophysiology of allergic diseases. IgE is also an attractive target for pharmacologic intervention and blockade in allergic diseases [4]. SIgE is also the most important predictor of allergen-related symptoms [5]. IgE is predominantly produced by plasma cells after individuals are exposed to a variable number of allergenic sources. Helper T cell 2 (Th2) cells, Type 2 mediators and cytokines (IL-4, IL-5, and IL-13) have been implicated in the pathogenesis of allergic diseases [6]. Synthesis of IgE results from collaboration between Th2 and B cells when IL-4 and IL-13 interact with receptors on the surface of B cells to initiate a signaling cascade mediated by Janus kinase 3 and signal transducer and activator of transcription 6. Another signal is needed for class switch to IgE involving CD40 on the B cell interacting with CD40 ligand on the T-cell. Once IgE is produced by allergen-specific B cells, it is released into the circulation [7-10]. In spite of a half-life of only 2.8 days, there is evidence that the IgE response may last for several years even without allergen stimulation. This

is likely caused by long-lived IgE-producing plasma cells. The existence of long-lived IgE-producing plasma cells may explain finding of IgE with specificities to allergens that a patient has not been exposed for years. Such cells are located in the bone marrow. They are not easily targeted by therapeutics, but have some utility as a diagnostic marker [11].

In addition, the specific IgE (sIgE) level tends to be more meaningful than total IgE in the serum. The prevalence of sIgE may differ across gender or age groups, geographic areas as well as different diseases. The ability to identify and quantify the proportion of sIgE could provide more information which may be useful in diagnosis and treatment. These sIgE may provide guidance for allergen avoidance and insight into the morbidity and mortality of various allergic diseases. A study undertaken by the European Community Respiratory Health Survey of 11,355 persons (median age, 34 years) found 57.0% to 67.8% of European populations were not sensitized to any of the test allergens, 16.2% to 19.6% were mono-sensitized, and 12.8% to 25.3% were poly-sensitized to specific groups of allergens [12]. The third National Health and Nutrition Examination Surveys, 10,863 patients participated in skin testing: 45.7% were not sensitized to any of the test allergens, 15.5% were mono-sensitized, and 38.8% were poly-sensitized [13].

There is a debate around the mechanisms of poly-sensitization. Cross-reactive and co-sensitization are two hypotheses. In general, terminology describing cross-reactivity should be limited to defined clinical manifestations showing reactivity to a single allergic source without prior exposure. Cross-reactivity requires more than 70% sequence identity. Proteins having less than 50% sequence identity are very seldom cross-reactive [14]. In general, the term co-sensitization underlies the presence of IgEs against epitopes that are not shared between allergenic sources or molecules [15]. In most conditions, the allergic agents are completely different.

There are several different tests including skin prick testing, patch testing, intradermal testing and *in vitro* testing (for specific IgE antibodies) used in the possible identification of individual allergens [16]. We characterized human IgE antibodies raised against airborne and food allergens in serum using a semi-quantitative immunoblot assay in this study. It is a non-invasive method for the detection of 20 allergens per strip. The allergens pre-coated on the strip were purified biochemically characterized antigens which were specific to IgE. Antigen-binding of allergen-specific IgE were not inhibited by other immnunoglobulins such IgG and IgM. The automatic system provided quantitative results for each sIgE after evaluation by the EUROLineScan program.

In this study, Trait sIgE, the specific immune agent, is in the highest concentrations in poly-sensitized patients. The diagnostic efficiency of trait sIgEs in allergic diseases was also evaluated. The role of B cells and plasma cells is not well known in IgE associated allergic diseases. In order to investigate the mechanism, several immunoglobulins which were not related to allergic diseases were detected.

Materials and methods

Subjects

2409 serum samples were collected from hospitalized and out-patients at the First Affiliated Hospital of Dalian Medical University from June 2015 to May 2016. These patients were clinically evaluated by their physicians to have allergic diseases including allergic dermatitis (534, 22.17%), atopic asthma (500, 20.76%), urticaria (729, 30.26%), allergic rhinitis (259, 10.75%) and others (387, 16.06%). Information such as gender, age, time to hospitalization and diagnosis were collected for each sample. 1002 male and 1407 female patients (aged 34.53±20.24 years) blood samples were collected before they received any anti-allergic therapy. 44 healthy individuals (aged 39.84±18.25 years) blood sample were also evaluated as a control during the same period, these patients had to have no prior history of allergic disease. Among all these samples, 55 samples of allergic diseases and 42 samples of control group were collected randomly to detect several different immunoglobulins. This study was approved by the Ethics Committee of Dalian Medical University.

Human sIgE to inhalation and food allergens test

Blood samples (3 mL) were collected from 2409 patients and 44 healthy individuals.

Table 1. The type and classes of sIgE reactivity in response to the 20 allergens tested

Class	Concentration (kU/L)	Allergen code	Allergen name	Allergen code	Allergen name	Allergen code	Allergen name
0	<0.35	ts20	Tree mix2	i6	Cockroach	f27	Beef
1	0.35~0.70	w1	Common ragweed	ms1	Mould mix1	f88	Mutton/lamb
2	0.70~3.50	w6	Mugwort	u80	Hop	fs33	Sea fish mix1
3	3.50~17.50	ds1	House dust mite mix1	f1	Egg white	fs34	Fresh water fish mix1
4	17.50~50.00	h1	House dust	f2	Cow's milk	f24	Shrimp/Prawn
5	50.00~100.00	e1	Cat	f13	Peanut	f23	Crab
6	>100.00	e2	Dog	f14	Soybean	Ind	Indicator band

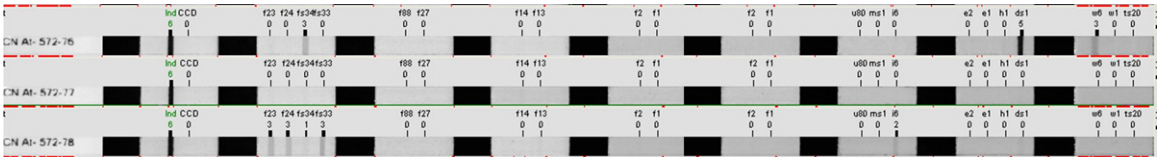

Figure 1. Result of strips after detection. The result of the first strip (CN At-572-76): fs34 class 3, ds1 class 5, w6 class 3, the result of the second strip (CN At-572-77): negative, the third strip (CN At-572-78): f23 class 3, f24 class 3, fs3 class 4, fs33 class 3, i6 class 2.

Serum was separated after centrifugation at 2630 × g for 10 min. The EUROLINE atopy (China) test kit (EUROIMMUN medizinische Labor diagnostika AG, Lübeck, Germany) was used for the test. It was a semi-quantitative *in vitro* assay for human sIgE to inhalation and food allergens in serum. The test strips were pre-coated with parallel lines of 20 different allergen extracts. They were first moistened and then incubated in the first reaction step with patient serum (diluted 1:11 with the pre-diluted universal buffer) for 16 h. If samples were positive, specific antibodies of class IgE would bind to the allergens. To detect the bound antibodies, a second incubation was carried out using an enzyme-labeled monoclonal anti-human IgE (enzyme conjugate) catalyzing a color reaction. In the test kit, the enzyme conjugate was alkaline phosphatase labeled anti-human IgE (mouse). Substrate solution was nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolylphosphate. For automated incubation with the EUROBlotMaster select the program Euro12 Allerg16h (version b). The whole experimental process was performed according to the procedure provided by EUROBlotMaster.

Assessment

The EUROLineScan program was used for digital evaluation of the strips once dry. The intensity of the bands was calculated in EAST (Enzyme-Allergo-Sorbent Test) classes of 0-6. EAST values are determined with respect to the concentration grades used in the well-known RAST system (Radio-Allergo-Sorbent Test) used in allergy diagnostics. The test strips were coated with parallel lines of 20 different allergen extracts (**Table 1**). The concentration of each sIgE and their classes are also shown in **Table 1**.

Calculation of observed proportion of mono-sensitization (OPM) and theoretical proportion of mono-sensitization (TPM)

Calculation of the observed proportion of mono-sensitization: OPM=(number of mono-sensitized samples/total number) × 100%

Calculation of theoretical proportion of mono-sensitization: Principle: In this test, there were 20 allergens which were random variants, for example one sample, if only one allergen was positive and 19 others were negative, the result was considered mono-sensitized. In other words, the probability of mono-sensitization was calculated on the basis of a joint probability: one positive variant and 19 negative variants had to be found in a single sample to qualify as mono-sensitized. According to the principle of probability theory, joint probability is the product of individual probabilities. Theoretically, mono-sensitization probability for an allergen (A) is equal to the product of positive probability of allergen A and

Table 2. Overall sIgE reactivity to the 20 allergens tested in 2409 patients

Allergenz	Positive number	Positive rate (%)	Class (n)											
			1	Proportion	2	Proportion	3	Proportion	4	Proportion	5	Proportion	6	Proportion
f23	83	3.45	33	39.76	22	26.51	9	10.84	14	16.87	5	6.02	0	0.00
f1	137	5.69	101	73.72	22	16.06	8	5.84	4	2.92	2	1.46	0	0.00
f13	241	10.00	187	77.59	36	14.94	8	3.32	6	2.49	4	1.66	0	0.00
f27	37	1.54	30	81.08	3	8.11	3	8.11	1	2.70	0	0.00	0	0.00
f88	27	1.12	10	37.04	8	29.63	8	29.63	0	0.00	1	3.70	0	0.00
fs34	170	7.06	60	35.29	49	28.82	32	18.82	22	12.94	7	4.12	0	0.00
fs33	212	8.80	86	40.57	55	25.94	32	15.09	33	15.57	6	2.83	0	0.00
f2	52	2.16	38	73.08	9	17.31	3	5.77	2	3.85	0	0.00	0	0.00
f14	97	4.03	45	46.39	24	24.74	15	15.46	10	10.31	2	2.06	1	1.03
f24	85	3.53	56	65.88	19	22.35	4	4.71	4	4.71	2	2.35	0	0.00
w1	70	2.91	24	34.29	20	28.57	16	22.86	6	8.57	4	5.71	0	0.00
w6	265	11.00	45	16.98	45	16.98	50	18.87	50	18.87	74	27.92	1	0.38
ds1	492	20.42	79	16.06	84	17.07	93	18.90	107	21.75	114	23.17	15	3.05
e1	125	5.19	34	27.20	24	19.20	24	19.20	20	16.00	22	17.60	1	0.80
e2	71	2.95	20	28.17	14	19.72	9	12.68	16	22.54	12	16.90	0	0.00
u80	61	2.53	24	39.34	18	29.51	12	19.67	5	8.20	1	1.64	1	1.64
ts20	67	2.78	36	53.73	15	22.39	11	16.42	2	2.99	3	4.48	0	0.00
h1	96	3.99	52	54.17	26	27.08	12	12.50	5	5.21	1	1.04	0	0.00
i6	90	3.74	50	55.56	27	30.00	9	10.00	4	4.44	0	0.00	0	0.00
ms1	22	0.91	11	50.00	7	31.82	3	13.64	1	4.55	0	0.00	0	0.00

Note: ts20 Tree mix2 (willow, poplar, elm), w1 Common ragweed, w6 Mugwort, ds1 House dust mite mix1 (Der. pteronyssinus/Der. farinae), h1 House dust, e1 Cat, e2 Dog, i6 Cockroach, u80 Hop, f1 Egg white, f2 Cow's milk, f13 Peanut, f14 Soybean, f27 Beef, f88 Mutton/lamb, fs33 Sea fish mix1 (codfish, lobster, scallop), fs34 Fresh water fish mix1 (salmon, perch,carp), f24 Shrimp/Prawn, f23 Crab, ms1 Mould mix1, Ind Indicator band.

Table 3. Proportion and number of different sIgEs in 2409 patients

SIgE	0	1	2	3	4	5	6	7	8	9	10	>10	Total
Number	1211	550	339	152	63	49	23	8	8	2	3	1	2409
Proportion (%)	50.27	22.83	14.07	6.31	2.62	2.03	0.95	0.33	0.33	0.08	0.12	0.04	100
Intake	Number	730		30.3%									
Inhaled	Number	803		33.3%									
Compound	Number	335		13.9%	TPC*	10.10%	P<0.001						

Note: TPC: theoretical value for the proportion of compound sensitization; *P<0.001, significant difference compared to observed proportion of compound sensitization (Non-parametric Binomial tests).

negative probability of all other allergens. $\sum_{n=1}^{20}[(1-A_n)\frac{\prod_{n=1}^{20}A_n}{A_n}]$, A_n is negative probability of allergen A. A_n=(number of negative of allergen A/total number) × 100%

Definition of trait sIgEs

If more than one allergen were found positive in one sample, the sIgE with the highest concentration was considered as the trait sIgE [17]. If there were several positive allergens with the same concentration, they were all considered traits IgEs. They were all taken into account for our statistics. For example, if there were the same concentrations of egg white and peanut sIgEs and both of them were the highest concentration in a sample, then both were considered as trait sIgEs. For statistical analysis, this sample is put into the egg white group and also into the peanut group.

Calculation of observed proportions of compound sensitization (OPC) and theoretical proportion of compound sensitization (TPC)

Compound sensitization refers to the existence of food allergen(s) and inhaled allergen(s) in one sample.

Table 4. Number of sIgEs in mono-sensitized patients in response to these 20 allergens

Allergen	Positive number	Monosensitization number	Monosensitization proportion (%)	Allergen	Positive number	Monosensitization number	Monosensitization proportion (%)
f23	83	5	6.02	w1	70	4	5.71
f1	137	39	28.47	w6	265	67	25.28
f13	241	72	29.88	ds1	492	163	33.13
f27	37	6	16.22	e1	125	12	9.6
f88	27	1	3.7	e2	71	5	7.04
fs34	170	68	40	u80	61	2	3.28
fs33	212	59	27.83	ts20	67	13	19.4
f2	52	3	5.77	h1	96	1	1.04
f14	97	22	22.68	i6	90	1	1.11
f24	85	7	8.24	ms1	22	0	0
OPM	22.8	TPM*	38.6	P	<0.001		

Note: ts20 Tree mix2 (willow, poplar, elm), w1 Common ragweed, w6 Mugwort, ds1 House dust mite mix1 (Der. pteronyssinus/Der. farinae), h1 House dust, e1 Cat, e2 Dog, i6 Cockroach, u80 Hop, f1 Egg white, f2 Cow's milk, f13 Peanut, f14 Soybean, f27 Beef, f88 Mutton/lamb, fs33 Sea fish mix1 (codfish, lobster, scallop), fs34 Fresh water fish mix1 (salmon, perch, carp), f24 Shrimp/Prawn, f23 Crab, ms1 Mould mix1, Ind Indicator band. OPM: observed proportions of mono-sensitization; TPM: theoretical proportion of mono-sensitization. *P<0.001, significant difference compared to OPM (Non-parametric Binomial tests).

Table 5. Comparison of OPM and TPM values for different gender, age, season, disease and pathway groups

	Group	Total number	Number of mono-sensitization	OPM (%)	TPM (%)	*p*-value
Gender	Female	1407	321	21.11	37.38*	<0.001
	Male	1002	229	20.65	37.73*	<0.001
Age	0-6	299	80	23.20	35.30*	<0.001
	7-17	219	59	23.30	38.30*	<0.001
	18-28	498	106	19.14	38.31*	<0.001
	29-44	598	154	24.62	38.32*	<0.001
	45-59	472	88	16.20	37.10*	<0.001
	60-	323	63	19.77	37.40*	<0.001
Season	Summer	617	158	20.10	38.61*	<0.001
	Autumn	546	167	23.99	38.16*	<0.001
	Winter	359	85	18.66	37.17*	<0.001
	Spring	500	140	20.20	36.00*	<0.001
Disease	Allergic dermatitis	534	90	16.85	38.02*	<0.001
	Atopic asthma	500	124	24.80	37.95*	<0.001
	Urticaria	729	132	18.11	37.22*	<0.001
	Allergic rhinitis	259	77	29.73	39.20*	<0.001
	Others	387	127	32.82	34.62	0.248
Pathway	Intake	2409	282	11.71	31.04*	<0.001
	Inhaled	2409	268	11.12	35.09*	<0.001

Note: OPM: observed proportions of mono-sensitization; TPM: theoretical proportion of mono-sensitization. *P<0.001, significant difference compared to OPM (Chi-squared test).

OPC=(number of observed compound sensitization samples/total number) × 100%

TPC=(number of food sensitization only samples/total number) × (number of inhale sensitization only samples/total number) × 100%

Detection of IgG antibodies to hepatitis C virus (HCV) and Treponema pallidum (Syphilis TP assay)

The chemiluminescent microparticle immunoassay (ARCHITECT i2000SR, Abbott Labo-

ratories, Abbott Park, IL, USA) was used for the detection of HCV IgG and Syphilis TP assay. The ARCHITECT System calculates the cutoff (CO) using the mean chemiluminescent signal (RLU). S/CO=Sample RLU/Cutoff RLU. Specimens with S/CO value ≥1.0 were considered reactive.

Detection of IgG antibody to human immunodeficiency virus (HIV)

ELISA was conducted using diagnostic kit for antibody to HIV (Zhu Hai Livzon diagnostics Inc, Zhuhai, China) with Addcare ELISA 600 immunoassay workstation (Yantai Addcare Bio-Tech Co.Ltd. Yantai, China). Specimens with S/CO value≥1.0 were considered reactive.

Detection of IgM antibodies to Toxoplasma gondii (Toxoplasma), Cytomegalovirus (CMV) and Rubella virus (RV)

IgM antibodies to *Toxoplasma*, CMV and RV were analyzed using a *Toxoplasma* IgM test kit (F.Hoffmann-La Roche, Ltd, Basel Switzerland) and a CMV IgM test kit (F.Hoffmann-La Roche, Ltd, Basel Switzerland) and a RV IgM test kit (F.Hoffmann-La Roche, Ltd, Basel Switzerland) with eletrochemiluminescense assay system (Cobas e 601, F.Hoffmann-La Roche, Ltd, Basel Switzerland). Reference ranges (value of S/CO) for healthy Chinese population are as follows: *Toxoplasma* IgM(0.8-1.0), CMV IgM(0.7-1.0), RV IgM(0.8-1.0).

Statistical analysis

All data were analyzed using SPSS (Statistical Package for Social Sciences) 17.0 software (Chicago, IL, USA). Chi-squared test was used to determine the between-group differences of numerical data. Non-parametric Binomial tests were used to evaluate the observed proportion and theoretical proportion. ROC analysis was carried out to evaluate the diagnostic efficacy for trait sIgEs. Two independent samples test of non-parametric tests was used to determine the difference of IgG antibodies to HCV, Treponema pallidum and human immunodeficiency virus and IgM antibodies to *Toxoplasma gondii*, Cytomegalovirus and Rubella virus between allergic disease group and control group. Data were considered to be statistically significant when P≤0.05.

Results

General results of strips for inhalation and food allergens

In this study, 1198 (positive rate 49.7%) subjects tested positive among 2409 patients who were diagnosed as suspected allergic reactions (**Figure 1**). Among the sIgEs to inhalation allergens, House dust mite mix1 (20.42%), Mugwort (11.00%) and cat (5.19%) were the three predominant positive sIgEs. Among the food allergens, Peanut (10.00%), Sea fish mix1 (8.80%) and Fresh water fish mix1 (7.06%) were the three predominant positive sIgEs (**Table 2**).

The number of positive allergen types in one sample was termed clone (0-10). If only one sIgE was positive, it was called mono-sensitization (22.8%, **Table 3**). If more than one sIgEs were found positive, it was called poly-sensitization (26.9%, **Table 3**). The proportion of mono-sensitization for different allergens was different (**Table 4**). For each allergen, the proportion of mono-sensitization was much less than proportion of poly-sensitization. The poly-sensitization proportion of u80 and ms1 were over 95%. When the OPM (22.8%) was compared with the TPM (38.6%), it was significantly lower (P<0.001, **Table 4**).

In different disease groups, there were statistical differences found for groups 1-4 (Allergic dermatitis, atopic asthma, urticaria, allergic rhinitis, P<0.001). There was no statistical difference found in the fifth group (others, P=0.25). There was a statistical difference between OPC (13.91%) and TPC (10.10%) (P<0.001, **Table 3**).

Positivity rates of sIgEs to 20 allergens in both genders

There were 1407 female subjects and 1002 male subjects in this study. Of these 556 male subjects and 642 female subjects were found to be positive in our test. The positive rate of males (55.49%, 556/1002) was significantly different from that for females (45.63%, 642/1407) (X^2=22.759, P<0.001). TPM and OPM values were both significantly different between these two groups (P<0.001) (**Table 5**). The top three trait sIgEs were to House dust mite mix1, Mugwort and Fresh water fish mix in female individuals. The top three traits IgEs

Table 6. Positive result for trait sIgEs in different gender, age, season and disease groups

Allergen	Gender		Age						Season				Disease				
	F	M	0-6	7-17	18-28	29-44	45-59	>59	Sum	Aut	Win	Spr	Dermatitis	Asthma	Urticaria	Rhinitis	Others
f23	14	10	1	2	3	8	8	2	10	3	3	8	2	8	5	3	6
f1	34	35	29	3	9	9	9	10	21	19	4	25	12	15	20	8	14
f13	55	64	10	5	28	34	22	20	69	35	1	14	23	26	28	10	32
f27	8	4	6	0	2	1	2	1	7	2	1	2	2	5	3	1	2
f88	1	2	0	1	0	1	1	0	2	0	0	1	1	1	0	1	0
fs34	84	38	27	7	21	33	23	11	21	27	29	45	21	27	31	17	26
fs33	58	62	5	6	20	29	31	29	37	35	19	29	22	26	29	13	30
f2	2	6	6	0	2	0	0	0	2	4	0	2	1	1	2	1	3
f14	24	13	6	2	6	9	8	6	9	13	8	7	4	12	7	6	7
f24	7	4	2	1	2	2	3	1	7	3	0	1	3	0	3	1	4
w1	4	4	0	1	4	2	0	1	1	3	1	3	3	2	1	0	2
w6	89	66	8	19	40	42	30	16	32	78	18	27	34	39	33	17	32
ds1	192	199	90	85	74	84	35	23	117	133	74	67	64	100	79	61	87
e1	25	27	1	6	15	19	9	2	14	12	8	18	8	9	13	6	16
e2	11	7	3	0	5	8	2	0	2	7	4	5	3	2	4	1	8
u80	5	7	2	2	4	2	2	0	5	6	1	0	1	4	3	0	4
ts20	11	5	3	1	4	5	2	1	6	4	1	5	2	6	5	0	3
h1	18	17	1	3	9	7	7	8	8	10	6	11	5	9	3	9	9
i6	14	7	1	3	5	6	2	4	6	6	3	6	4	2	8	2	5
ms1	3	3	1	0	2	2	0	1	2	0	2	2	3	0	1	0	2
Total	659	580	202	147	255	303	196	136	378	400	183	278	218	294	278	157	292

Note: ts20 Tree mix2 (willow, poplar, elm), w1 Common ragweed, w6 Mugwort, ds1 House dust mite mix1 (Der. pteronyssinus/Der. farinae), h1 House dust, e1 Cat, e2 Dog, i6 Cockroach, u80 Hop, f1 Egg white, f2 Cow's milk, f13 Peanut, f14 Soybean, f27 Beef, f88 Mutton/lamb, fs33 Sea fish mix1 (codfish, lobster, scallop), fs34 Fresh water fish mix1 (salmon, perch, carp), f24 Shrimp/Prawn, f23 Crab, ms1 Mould mix1, Ind Indicator band.

Table 7. Comparison of IgM, IgG between allergic disease and control

Disease	Percentiles	*Toxoplasma*-IgM (S/CO)	CMV-IgM (S/CO)	RV-IgM (S/CO)	TP-IgG (S/CO)	HCV-IgG (S/CO)	HIV-IgG (S/CO)
Allergic disease	25th	0.199	0.221	0.234	0.050	0.050	0.035
	50th	0.215	0.254	0.257	0.060	0.070	0.035
	75th	0.245	0.328	0.303	0.080	0.090	0.036
Control	25th	0.188	0.193	0.236	0.030	0.050	0.035
	50th	0.254	0.213	0.265	0.040	0.060	0.035
	75th	0.306	0.250	0.313	0.063	0.090	0.036
p value		0.111	0.000	0.810	0.000	0.485	0.739

Note: *Toxoplasma: Toxoplasma gondii;* CMV: Cytomegalovirus, RV: Rubella virus, HCV: hepatitis C virus, TP: Treponema pallidum, HIV: human immunodeficiency virus.

were to House dust mite mix1, Mugwort and peanut in male individuals (**Table 6**).

Positive rates of sIgE to 20 allergens in six age groups

The whole study population was divided into six age groups (**Table 5**). The statistical differences between OPM and TPM values were all significant (P<0.001). The positive rates of trait sIgEs for each age group were different. The positive rates of House dust mite mix1, Mugwort and Fresh water fish mix were higher in all age groups, but the order of magnitude for the concentration of each allergen's sIgE were different. The top three trait sIgEs in the 0-6 age group were to House dust mite mix1, Egg white and Fresh water fish mix. The top three trait sIgEs in the 7-17 age group were to House dust mite mix1, Mugwort and Fresh water fish

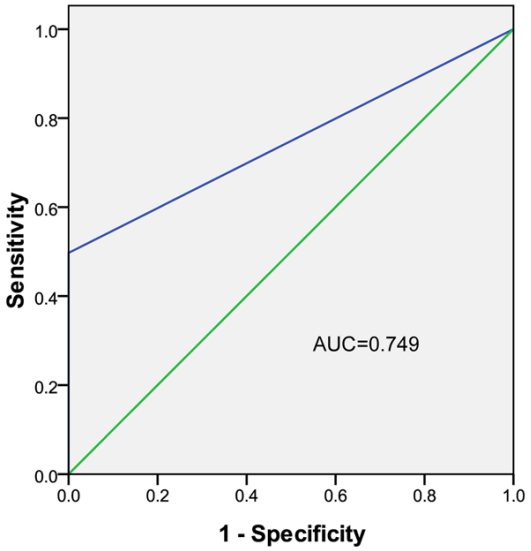

Figure 2. ROC analyses of trait sIgE detection.

mix. The top three trait sIgEs in the 18-28 and 29-44 age groups were House dust mite mix, Mugwort and Peanut. The top three trait sIgEs in the 45-59 age group were to House dust mite mix, Sea fish mix and Mugwort. The top three trait sIgEs in the over 60 age group were Sea fish mix, House dust mite mix and peanut (**Table 6**).

Positivity rates of sIgE to 20 allergens in four season groups

Samples in this study were collected from June 2015 to May 2016, OPM and TPM were significantly different (P<0.001) in each of the four seasons (**Table 5**). The trait sIgEs were different in different seasons. In spring (from March to May), the top three trait sIgEs were House dust mite1, Fresh water fish mix and Sea fish mix. In summer (from June to August), the top three trait sIgEs were House dust mite1, peanut and Sea fish mix. In autumn (from September to November), the top three trait sIgEs were House dust mite1, Mugwort, peanut and Sea fish mix1. In winter (from December to February), the top three trait sIgEs were House dust mite1, Fresh water fish mix and Sea fish mix (**Table 6**).

Positivity rates of sIgEs to 20 allergens in two pathway groups

There are 10 inhalation allergens and 10 food allergens detected in this study. TPM and OPM in both pathways were significantly different (P<0.001) (**Table 5**).

Results of IgM for Toxoplasma, CMV and RV, IgG for TP, HCV, HIV

Results of IgM for *Toxoplasma*, CMV and RV, IgG for TP, HCV, HIV in allergic disease were in **Table 7**. When the data were analyzed, *p* value was<0.05 in anti-CMV IgM group and anti-TP group.

Evaluation of diagnostic efficacy

The Receiver operating characteristic curve (ROC curve) analyses used the concentration of trait sIgEs as an indicator for the healthy control group and allergic disease group were shown in **Figure 2**. The area-under-the-curve (AUC) was found to be 0.749.

Discussion

In this study, frequencies of sIgE classes were different for all 20 allergens. Most of the common sIgEs belonged to class one. For example, the highest proportion (92.53%) of sIgE to peanuts were composed of class one (77.59%) and class two (14.94%). While for sIgEs to House dust mite mix1 and Mugwort, the highest proportions of sIgEs were class five which suggested a very high concentration of this sIgE. These sIgE classes are helpful in predicting whether or not an individual will become symptomatic of a specific disease. Asymptomatic sensitization can be seen in some individuals having class one or two sIgEs [18, 19].

Proportions of mono-sensitization were all lower than proportion of poly-sensitization for sIgEs for these 20 allergens. Further investigation showed the same results when gender, age, season and disease were taken into account. There was a debate about high poly-sensitization caused by cross reactivity or co-sensitization. Inhaled allergens and food allergens are considered two very different allergen subsets with completely unique epitopes reducing the likelihood for cross-reactivity. The observed proportion of compound sensitization was higher than the theoretical proportions of compound sensitization. This suggests that poly-sensitization is probably caused by co-sensitization instead of cross reactivity. It is unknown whether it is related to specific activa-

tion of plasma cells in response to an antigenic stimulus. Further investigation is needed to reveal the mechanism of co-sensitization.

The proportion of poly-sensitization was 26.9% for allergic disease patients in this study. Here the 20 allergens were pre-coated on an immunoblot strip and it is unknown whether the proportion of poly-sensitization will increase if more allergens are included. There was a study using Immuno Solid-phase Allergen Chips (ISAC), which was commercially available microarray-based IgE detection chips which might allow for a more thorough examination. They allow for the measurement of specific IgE antibodies to 112 allergen components of 46 major allergens per measurement with 30 μL serum blood or serum [20]. However, there was still controversy about the sensitivity of this microarray method when compared with traditional IgE tests when we embarked on this study. The ISAC is known to have higher variability when the serum IgE level is low. Variable results in the analysis of certain allergens and some limitations in the types of allergen sources have also been shown [21]. Positivity rates differ between allergens and methods. It was earlier described that sensitivity to mold was more accurately detected in the skin prick test than any other type of detection method [22].

Despite the different positivity rate for poly-sensitization in different countries or areas, poly-sensitizations were all higher than mono-sensitization reactions. However, it is the first time anyone has described a difference in the predicted value for mono-sensitization and the observed proportion of mono-sensitization using probability theory and the given mathematical formula. Using this method, we consistently observed lower OPM values than TPM values even when we looked at different age, gender, diseases and allergic pathway groups. Poly-sensitization was more prevalent (81%) than mono-sensitization when using either the skin prick or sIgE detection methods in the United States of America [23].

In allergic disease, the elevation of different class antibodies suggested that polyclonal antibodies were not limited in IgE class. Other antibodies of IgG class and IgM class to pathogens for infectious diseases were also affected. We have a hypothesis that more antibodies would be found increased, if the scope for detection was extended. Different classes of immunoglobulin were all produced by plasma cells. Mechanisms regulating IgE production are poorly understood. Recent studies suggest that membrane IgE receptors provided a signal mediating antigen-independent chronic activation that influences the further differentiation of IgE class-switched B cell [24]. But further investigation was needed for mechanism of different classes of immunoglobulin increased in allergic diseases.

There is an debate about allergen immunotherapy in poly-sensitized patients. For subcutaneous allergen immunotherapy, in Europe most formulations are single-allergen extracts, while in the United States it contains an average of 8 different components [25-27]. In this study, the concept of trait sIgEs was proposed for the first time. The sIgE with the highest concentration was regarded as the most troublesome allergen that must be treated. In the evaluation of efficiency of trait sIgE, the AUC was 0.749. Trait sIgE could prove to be a valuable new indicator for diagnosis of allergic disease and a good indicator for treatment.

In our study, most common allergens in different diseases were shown to have similar sIgE profiles. Overall, the top three allergens are House dust mite mix1, Mugwort and Peanut. The trait sIgEs in females and males are different. The positivity rates of sIgEs in males are higher than females which is similar to other studies. Males were more likely to be atopic than females, with the total IgE concentration being higher for males [28]. Total serum IgE concentrations were strongly correlated with the sum of the allergen-specific IgE measurements. For males with current asthma the correlation was 0.88 and for females with current asthma it was 0.74 [29].

The specific mechanisms for these differences are unclear, but female sex hormones have been implicated [30, 31]. It seems likely that sex hormones contribute to this pattern. Use of hormonal contraceptives may reduce asthma exacerbation and number of care episodes [32].

In this study, for the 0-6 years age group, the second most abundant sIgE is to egg white which is different from other age groups. Eggs are widely used as an early childhood food sup-

plement in China, making exposure in this group significantly higher than others which may explain the increase in egg white specific IgE. There were several other sIgEs described in this age group that might be explained when you consider the diet of various age groups and the food exposure of these patients. It was found that atopy was associated with age, gender and some reversible lifestyle-related factors including alcohol, smoking and education level [33].

Allergic diseases are major public health issues, with high prevalence and significant burden. In this study, proportions of mono-sensitization were all lower than proportions of poly-sensitization for sIgE to 20 allergens. OPM values were all lower than TPM values when gender, age, season and disease were taken into account. The OPC was lower than TPC suggesting that co-sensitization and not cross-reactivity may be the mechanism of high poly-sensitization in allergic diseases. Further research about the differentiation of B cells and plasma cells related to poly-sensitization is needed.

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Disclosure of conflict of interest

None.

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References

[1] Linneberg A. The increase in allergy and extended challenges. Allergy 2011; 66 Suppl 95: 1-3.

[2] Gershon AS, Guan J, Wang C, To T. Trends in asthma prevalence and incidence in Ontario, Canada, 1996-2005: a population study. Am J Epidemiol 2010; 172: 728-736.

[3] de Marco R, Cappa V, Accordini S, Rava M, Antonicelli L, Bortolami O, Braggion M, Bugiani M, Casali L, Cazzoletti L, Cerveri I, Fois AG, Girardi P, Locatelli F, Marcon A, Marinoni A, Panico MG, Pirina P, Villani S, Zanolin ME, Verlato G. Trends in the prevalence of asthma and allergic rhinitis in Italy between 1991 and 2010. Eur Respir J 2012; 39: 883-892.

[4] Patel TR, Sur S. IgE and eosinophils as therapeutic targets in asthma. Curr Opin Allergy Clin Immunol 2017; 17: 42-49.

[5] Olivieri M, Heinrich J, Schlunssen V, Anto JM, Forsberg B, Janson C, Leynaert B, Norback D, Sigsgaard T, Svanes C, Tischer C, Villani S, Jarvis D, Verlato G; European Community Respiratory Health Survey II Verona and Pavia, Italy Neuherberg, Germany, Aarhus, Denmark, Barcelona, Spain, Umea and Uppsala, Sweden, Paris, France, Bergen, Norway, and London, U.K. The risk of respiratory symptoms on allergen exposure increases with increasing specific IgE levels. Allergy 2016; 71: 859-868.

[6] Dougherty RH, Fahy JV. Acute exacerbations of asthma: epidemiology, biology and the exacerbation-prone phenotype. Clin Exp Allergy 2009; 39: 193-202.

[7] Lebman DA, Coffman RL. Interleukin 4 causes isotype switching to IgE in T cell-stimulated clonal B cell cultures. J Exp Med 1988; 168: 853-862.

[8] Punnonen J, de Vries JE. IL-13 induces proliferation, Ig isotype switching, and Ig synthesis by immature human fetal B cells. J Immunol 1994; 152: 1094-1102.

[9] Jiang H, Harris MB, Rothman P. IL-4/IL-13 signaling beyond JAK/STAT. J Allergy Clin Immunol 2000; 105: 1063-1070.

[10] Iciek LA, Delphin SA, Stavnezer J. CD40 cross-linking induces Ig epsilon germline transcripts in B cells via activation of NF-kappaB: synergy with IL-4 induction. J Immunol 1997; 158: 4769-4779.

[11] Shapiro-Shelef M, Calame K. Regulation of plasma-cell development. Nat Rev Immunol 2005; 5: 230-242.

[12] Bousquet PJ, Castelli C, Daures JP, Heinrich J, Hooper R, Sunyer J, Wjst M, Jarvis D, Burney P. Assessment of allergen sensitization in a general population-based survey (European Community Respiratory Health Survey I). Ann Epidemiol 2010; 20: 797-803.

[13] Arbes SJ Jr, Gergen PJ, Elliott L, Zeldin DC. Prevalences of positive skin test responses to 10 common allergens in the US population: results from the third National Health and Nutrition Examination Survey. J Allergy Clin Immunol 2005; 116: 377-383.

[14] Aalberse RC. Structural biology of allergens. J Allergy Clin Immunol 2000; 106: 228-238.

[15] Ferreira F, Hawranek T, Gruber P, Wopfner N, Mari A. Allergic cross-reactivity: from gene to the clinic. Allergy 2004; 59: 243-267.

[16] Tourlas K, Burman D. Allergy testing. Prim Care 2016; 43: 363-374.

[17] Ningyu Z, Ying Z, Hui L. Establishment of a simple model for hepatitis B virus infection status based on trait marker from quantitative measurement of serum markers. J Med Virol 2015; 87: 1008-1012.
[18] Sampson HA, Ho DG. Relationship between food-specific IgE concentrations and the risk of positive food challenges in children and adolescents. J Allergy Clin Immunol 1997; 100: 444-451.
[19] Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. J Allergy Clin Immunol 2001; 107: 891-896.
[20] Jung JH, Kang IG, Kim ST. Comparison of component-resolved diagnosis by using allergen microarray with the conventional tests in allergic rhinitis patients: the first using in Korea. Clin Exp Otorhinolaryngol 2015; 8: 385-389.
[21] Canonica GW, Ansotegui IJ, Pawankar R, Schmid-Grendelmeier P, van Hage M, Baena-Cagnani CE, Melioli G, Nunes C, Passalacqua G, Rosenwasser L, Sampson H, Sastre J, Bousquet J, Zuberbier T. A WAO - ARIA - GA(2)LEN consensus document on molecular-based allergy diagnostics. World Allergy Organ J 2013; 6: 17.
[22] Kespohl S, Maryska S, Bunger J, Hagemeyer O, Jakob T, Joest M, Knecht R, Koschel D, Kotschy-Lang N, Merget R, Mulleneisen NK, Rabe U, Roseler S, Sander I, Stollewerk D, Straube H, Ulmer HM, van Kampen V, Walusiak-Skorupa J, Wiszniewska M, Wurpts G, Bruning T, Raulf M. How to diagnose mould allergy? Comparison of skin prick tests with specific IgE results. Clin Exp Allergy 2016; 46: 981-991.
[23] Craig TJ, King TS, Lemanske RF Jr, Wechsler ME, Icitovic N, Zimmerman RR Jr, Wasserman S. Aeroallergen sensitization correlates with PC(20) and exhaled nitric oxide in subjects with mild-to-moderate asthma. J Allergy Clin Immunol 2008; 121: 671-677.
[24] Yang Z, Robinson MJ, Chen X, Smith GA, Taunton J, Liu W, Allen CD. Regulation of B cell fate by chronic activity of the IgE B cell receptor. Elife 2016; 5.
[25] Calderón MA, Cox L, Casale TB, Moingeon P, Demoly P. Multiple-allergen and single-allergen immunotherapy strategies in polysensitized patients: looking at the published evidence. J Allergy Clin Immunol 2012; 129: 929-934.
[26] van Cauwenberge P, Bachert C, Passalacqua G, Bousquet J, Canonica GW, Durham SR, Fokkens WJ, Howarth PH, Lund V, Malling HJ, Mygind N, Passali D, Scadding GK, Wang DY. Consensus statement on the treatment of allergic rhinitis. European academy of allergology and clinical immunology. Allergy 2000; 55: 116-134.
[27] Cox L, Jacobsen L. Comparison of allergen immunotherapy practice patterns in the United States and Europe. Ann Allergy Asthma Immunol 2009; 103: 451-459; quiz 459-461, 495.
[28] Paula Couto TA, Falsarella N, Mattos Cde C, Mattos LC. Total IgE plasma levels vary according to gender and age in Brazilian patients with allergic rhinitis. Clinics (Sao Paulo) 2014; 69: 740-744.
[29] Rajendra C, Zoratti E, Havstad S, Nicholas C, Wegienka G, Cross MT, Johnson CC, Ownby D. Relationships between total and allergen-specific serum IgE concentrations and lung function in young adults. Ann Allergy Asthma Immunol 2012; 108: 429-434.
[30] Baibergenova A, Thabane L, Akhtar-Danesh N, Levine M, Gafni A, Leeb K. Sex differences in hospital admissions from emergency departments in asthmatic adults: a population-based study. Ann Allergy Asthma Immunol 2006; 96: 666-672.
[31] Haggerty CL, Ness RB, Kelsey S, Waterer GW. The impact of estrogen and progesterone on asthma. Ann Allergy Asthma Immunol 2003; 90: 284-291; quiz 291-283, 347.
[32] Nwaru BI, Sheikh A. Hormonal contraceptives and asthma in women of reproductive age: analysis of data from serial national Scottish Health Surveys. J R Soc Med 2015; 108: 358-371.
[33] Skaaby T, Husemoen LL, Thuesen BH, Jorgensen T, Linneberg A. Lifestyle-related factors and atopy in seven danish population-based studies from different time periods. PLoS One 2015; 10: e0137406.