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BIOTECHNOLOGY AND IN VITRO DIAGNOSTICS

Microarray evaluation of specific IgE to allergen components in elite athletes

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Abstract

Background: Allergic sensitization and diseases have been reported to have a very high and increasing prevalence in elite athletes. Over 80% of allergic athletes are poly-sensitized.

Objective: This study aims at evaluating the potential diagnostic added value of a microarray technology (ImmunoCAP ISAC, Phadia AB [at present Thermo Fisher Scientific] Uppsala, Sweden which detects IgE antibodies to specific or cross-reacting allergen components.

Methods: Seventy-two poly-sensitized athletes according to skin prick test (SPT) with different allergic phenotypes (asthma $n = 19$; rhino-conjunctivitis $n = 20$; food allergy and/or oral allergy syndrome $n = 13$; no clinical symptoms $n = 20$) and two different control populations (20 poly-sensitized sedentary subjects with respiratory allergy and 20 healthy athletes with negative SPT) were studied for detecting specific IgE (sIgE) both to allergen extracts (ImmunoCAPsIgE) and to allergen components (ImmunoCAP ISAC).

Results: ImmunoCAP ISAC detected the presence of sIgE in 90% of poly-sensitized athletes – in 96% with symptoms and in 75% without symptoms – and in 100% of allergic controls. The pattern of positivity towards the 103 components tested differed from subject to subject, even in those with the same sensitization to allergen extract SPT or sIgE. Based on the ISAC results, poly-sensitized athletes were classified into the following prototypical patterns, differently represented in the clinical phenotypes studied ($P = 0.03$): (1) One single predominant specific allergen positivity; (2) sIgE to two or more non-cross-reacting allergens; (3) sIgE to cross-reacting allergens; and (4) sIgE to components potentially responsible for severe allergic reactions.

Conclusions: The ImmunoCAP ISAC represents a useful additional tool for diagnosis and management of poly-sensitized athletes.

Allergic sensitization and diseases occur with a very high and increasing incidence in elite athletes (1). In fact, the prevalence of asthma in athletes is higher than in the general population, ranging from 3.7% to 22.8% in summer sports and up to 54.8% in winter sports (2). Furthermore, the risk for asthma appears to be closely associated with atopy (3). Rhino-conjunctivitis also occurs very frequently in athletes, being its prevalence dependent on the criteria used for diagnosis in the different studies (4). As for asthma, the prevalence of allergic

rinitis seems to increase, as the reported prevalence in the 1980s (8.0%) resulted more than doubled in 1996 (19.6%) (5). Allergic rhinitis has been shown to have negative effects on quality of life and physical performance, particularly over the spring season (6). Food and drug allergy has been far less investigated than respiratory allergy in athletes. However, the most severe form of allergy in sports medicine – exercise-induced anaphylaxis – is often related to sensitization to food ingested shortly before an intense exercise (7).

Preliminary data of a multicenter European study of athletes participating at the Beijing Olympics (www.GA2LEN.net) have shown that 56.5% of athletes from the Italian delegation had positive skin tests to at least one of the most common inhalant allergens. In 34.0% of athletes, sensitization was associated with one or more allergic diseases. Interestingly, 84.4% of the allergic athletes had positive skin tests to more than one allergen (8). The high prevalence of allergic subjects sensitized to several allergens represents a phenomenon also observed in the general population (9). Certainly, the increasing incidence of multiple sensitizations over a short period of time cannot be ascribed to genetic factors. More likely, still undefined environmental factors or genetic/environmental interactions may be responsible for this finding. The number and quality of potential allergens and/or agents favouring a polyclonal IgE activation have been suggested as possible causes of this phenomenon.

The isolation and characterization of allergen components, as well as their production by recombinant techniques, has determined a significant progress in allergy diagnosis. This approach, named by Valenta and co-workers component-resolved diagnostics (10), allows the detection of IgE to specific or cross-reacting molecular allergens responsible for the clinical sensitization to the extract-based complex allergen sources used in SPT or in common specific IgE tests. Recently, a microarray technology – Immuno Solid-phase Allergen Chip (ISAC), ImmunoCAP ISAC, Phadia AB (at present Thermo Fisher Scientific), Uppsala, Sweden – became available to detect IgE antibodies to 103 molecular components of allergens (11). These include:

- Molecules specific for a given allergen indicating a genuine sensitization (e.g. Phl p 1,2,4,5,6,11 for grass; Der p 1 and Der p 2 for mites; Amb a 1 for *Ambrosia*; etc.)
- Cross-reacting molecules present in different allergen sources (pathogenesis-related protein family 10 proteins; profilins; tropomyosins; cross-reacting carbohydrate determinants; calcium binding proteins).
- Molecular markers of sensitizations potentially responsible for severe allergic reactions, particularly to foods (Ara h 1,2,3; Pru p 3; Cor a 8; omega-5 gliadin; lipid transfer proteins) (12, 13).

This study aims at evaluating whether the ImmunoCAP ISAC microarray technology may provide additional useful information to those obtained by conventional IgE tests in identifying different sensitization profiles in poly-sensitized allergic athletes.

Patients and Methods

Subjects

Among the 435 athletes selected for the Beijing Olympics, on the basis of clinical history, results of a specific allergy questionnaire – AQUA – (14), physical examination, SPT, pulmonary function tests and specific IgE determination, 72 poly-sensitized athletes (more than one sensitization) were selected for being representative of the following atopic phenotypes:

- Allergic asthma ($n = 19$).
- Allergic rhino-conjunctivitis ($n = 20$).

- Food allergy and/or oral allergy syndrome, OAS ($n = 13$).
- No clinical symptoms ($n = 20$).

Athletes with concomitant asthma and rhinitis were considered as asthmatics.

Athletes were selected for practicing both aerobic and nonaerobic disciplines, anyway requesting an intense training and cardiovascular demand.

Two age- and sex-matched control groups were included in the study:

- Twenty poly-sensitized (according to SPT) sedentary subjects with respiratory allergy.
- Twenty healthy athletes with negative SPT.

Sera from all groups were tested for total IgE and a standard panel of extract-based specific IgE by ImmunoCAP as well as for specific IgE to molecular allergen components by the ImmunoCAP ISAC microarray in the framework of a large National Study (12). Informed consent was obtained by all athletes and allergic control patients. The study was approved by the Internal Review Board and was performed in respect of privacy requirements.

Skin prick test

SPT was performed according to the methodology and panel of allergen extracts validated by the Global Asthma and Allergy European Network, GA2LEN (15) which includes the following allergens: cat, dog, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Blatella*, birch, hazel, alder, olive, cypress, ragweed, grass mix, *Artemisia*, *Parietaria*, *Alternaria* and *Aspergillus*.

Lung function

Pulmonary function tests were performed according to the standards set by the European Respiratory Society and the American Thoracic Society (16).

Blood sampling and testing

Blood samples were collected and sera, obtained by centrifugation, were divided in aliquots and sent to a central laboratory (G. Gaslini Hospital, Genoa, Italy) for being tested. Aliquots were stored at -20°C until testing according to the procedures described in details below.

ImmunoCAP ISAC microarray (ISAC)

ISAC tests were performed according to manufacturer's recommendations (Phadia AB [at present Thermo Fisher Scientific] Uppsala, Sweden). Briefly, 0.02 ml of serum was incubated on the microchip containing 103 allergen spots. After 60 min of incubation, slides were washed, and a anti-IgE antiserum labelled with Alexa Fluor 420 was added and incubated for 60 min. Chips were analysed using a Laser Scan Confocal microarray reader (LuxScan 10K/A; Capital-Bio, Beijing, China). Analysis of the results was automatically performed using a Microarray Image Analyser SW. All

samples were identified using a single bar code, and the results were automatically calculated by the software. All slides were visually scored to detect the presence of defects (e.g. smears of fluorescence or drops interfering with the reading). Only in these cases, a manual correction (or a refusal of the result) was decided. The ISAC score was reported as ImmunoCAP ISAC Standardized Units (ISU) with a detection range from 0.3 to 100 ISU. Clinical data of samples tested were not disclosed to researchers performing the test, and privacy rights of all subjects studied were protected by a coding system open only to the principal investigator.

ImmunoCAP total (tIgE) and specific IgE (sIgE)

Total and specific serum IgE (tIgE and sIgE) were measured by a commercial immune-enzymatic method (ImmunoCAP; Phadia AB [at present Thermo Fisher Scientific] Uppsala, Sweden) using, for sIgE, the same allergens employed for skin tests. Detection range was 2–5000 kU/l for tIgE and 0.1–100 kU_A/l for sIgE.

Statistical analysis

The correlation between ISAC and ImmunoCAP test was analysed for four different airborne allergens. The IgE values measured by ImmunoCAP for grass pollen mix, *Parietaria judaica*, *Felis domesticus* and *Dermatophagoides pteronyssinus* were compared with those measured by ISAC for the related molecular components, including both species-specific and cross-reacting ones (Table 1). ISAC values below the detection limit (<0.3 ISU) were set to zero. The ability of ISAC to detect a sensitized patient was evaluated for two different cut-off levels of the extract-based ImmunoCAP test: (1) the limit of detection (LoD) of 0.1 kU_A/l and (2) an arbitrary chosen cut-off of 1 kU_A/l. To evaluate the ISAC sensitivity, the percentage of subjects who were positive on ISAC (>0.3 ISU) out of all subjects who were positive on ImmunoCAP Specific IgE either at > 0.1 kU_A/l or at > 1 kU_A/l was calculated (positive percent agreement, PPA). For evaluation of the ISAC specificity, the percentage of subjects who were negative on ISAC (<0.3 ISU) out of all subjects who were negative on ImmunoCAP Specific IgE (<0.1 kU_A/l) was calculated (negative percent agreement, NPA).

Confidence intervals (CI) of the agreements were estimated with the Clopper–Pearson method. The PPA and NPA for assessing the correlation between ISAC and SPT were calcu-

lated in a similar way. Positivity for SPT was considered according to the GA2LEN methodology (15).

Differences in the sensitization pattern classes among the different groups of subjects were tested with a conditional permutation test, based on an approximate exact distribution of the test-statistic under null hypothesis, suitable for small samples (using a Monte Carlo method with 999 999 iterations) (17). Statistical calculations were carried out by using the R system for statistical computing version 2.12.0 (18) with the Coin extension version 1.0-17 (17).

Results

ImmunoCAP ISAC detected the presence of IgE antibodies against at least one allergen component in 65/72 (90%) poly-sensitized athletes – in 50 of 52 (96%) with symptoms, in 15 of 20 (75%) without symptoms and in 100% of allergic controls.

Sera from nonallergic athletes showed ISAC sIgE values above the LoD (0.3 ISU) in 17 of the 2060 molecular allergen determinations (0.08%), in six cases for Equ c 3 (horse), in three cases for Bla g 1 (cockroach) and Gly m 5 (soy), in one case for Bla g 7 (cockroach), Alt a 1 (*Alternaria*), Asp f4, Asp f 6 (*Aspergillus*) and Cor a 1 (hazelnut). In two determinations only (Equ c 3), values were above 1 ISU.

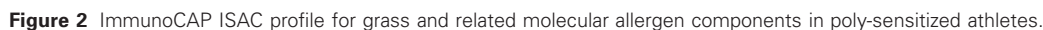
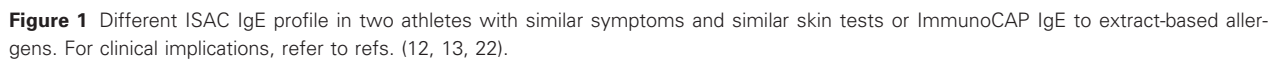
The pattern of positivity towards the 103 components tested was different in all ISAC positive athletes, even in those with the same sensitization to extract-based SPT or sIgE. An example of the ISAC results in two poly-sensitized athletes with the same sensitizations at SPT and in extract-based sIgE is presented in Fig. 1. The ISAC profiles for grass and cat and related molecular allergens are shown in Figs 2 and 3, respectively.

The correlation between ISAC and SPT or ImmunoCAP sIgE was evaluated for the four allergens most frequently responsible for sensitization: grass mix, *Parietaria judaica*, *Felis domesticus* and *Dermatophagoides pteronyssinus*. The PPA of ISAC vs SPT ranged from 46% to 87% depending on the allergen considered (Table 2). The PPA of ISAC vs ImmunoCAP sIgE ranged from 49% to 81% at the cut-off of 0.1 kU_A/l, while it was 85–95% at the cut-off of 1 kU_A/l. The specificity, expressed as NPA, at the cut-off of 1 kU_A/l, was 89–100% (Table 2).

Based on the ISAC results, the sensitized subjects were classified into the following prototypical sensitization patterns:

Table 1 Species-specific and cross-reacting allergen components tested for specific IgE with ISAC and corresponding extract-based allergens in ImmunoCAP igE

Extract-based ImmunoCAP test	Corresponding ImmunoCAP ISAC components	
	Species-specific components	Cross-reacting components
Grass (g1,g6)	Cyn d1, Phl p1, Phl p2, Phl p4, Phl p5, Phl p6, Phl p11	Phl p12 (profilin), Phl p7 (polcalcin), Ana c2 (CCD)
<i>Parietaria judaica</i> (w21)	Par j2	Mer a1 (profilin), Phl p7 (polcalcin), Ana c2 (CCD)
<i>Felis domesticus</i> (e1)	Fel d1, Fel d4	Fel d2 (serum albumin)
<i>Dermatophagoides pt</i> (d1)	Der p1, Der p2	Der p10 (tropomyosin)



- Subjects with one predominant sensitizing allergen (ISAC values > 1 ISU to one allergen only): *Monosensitized*.
- Subjects with IgE abs to two or more non-cross-reacting allergens: *True poly-sensitized*.
- Subjects with IgE abs to cross-reacting allergens: *False poly-sensitized*.
- Subjects with IgE abs to components potentially responsible for severe allergic reactions: *at high risk for severe allergic reactions*.

The prevalence and distribution of the sensitization patterns in the different study groups is reported in Table 3. Overall, there was a statistically significant difference in sensitization patterns among the four groups of poly-sensitized athletes (asthma, rhino-conjunctivitis, OAS, no symptoms; $P = 0.03$). Monosensitization seemed to occur more frequently in athletes with rhino-conjunctivitis, true poly-sensitization in asthmatics and false poly-sensitization or high-risk sensitization in athletes with food allergy and/or OAS.

SPT	slgE	ANIMALS					TROPOMYOSIN					SERUM ALBUMIN					MITES				
		dog	dog	cat	cat	mouse	anisakis	shrimp	shrimp	cockroach	mite	cow	dog	cat	mite	mite	mite	mite	mite	mite	mite
CAT	CAT	Can f 1	Can f 2	Fel d 1	Fel d 4	Mus m 1	Ani s 3	Pen a 1	Pen i 1	Blag 7	Der p 10	Bos d 6	Can f 3	Fel d 2	Der f 1	Der f 2	Der p 1	Der p 2	Eur m 2		
pos	12.10	0	0	10.16	0	0	0	0	0	0.59	1.06	0	0	0	0	5.86	1.32	3.57	0	0	0
pos	7.66	0	0	11.48	0	0	0.26	0	0	0	0	0	0	0	0	0.71	0	0.48	0	0	0
pos	7.38	0	0	1.96	0	0	0	0	0	0	0	0	0	0	0.94	6.92	2.15	8.77	0	0	0
pos	4.35	0	0	3.08	0	0	0	0	0	0	0	0	0	0	1.48	14.13	2.15	9.87	0	0	0
pos	4.05	0	0	3.52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
pos	2.96	0	0	1.96	0	0	0	0	0	0	0	0	0	0	4.69	2.99	4.16	3.55	0	0	0
pos	2.72	0	0	0	0	0	0	0	0	0	0	0	0	0	3	28.51	4.73	18.99	0	0	0
pos	2.53	0	0	2.65	0	0	0	0	0	0	0	0	0	0	1.9	4.57	2.13	1.85	0.73	0	0
pos	2.45	0	0	3	0	0	0	0	0	0	0	0	0	0	5.79	18.06	6.95	19.25	3.86	0	0
pos	2.40	0.68	0	2.08	0	0	0	0	0	0	0	0	0	0	0.49	0	0	0	0	0	0
pos	1.74	0	0	1.96	0	0	0	0	0	0	0	0	0	0	0	0.66	0	0	0	0	0
pos	1.62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9.59	0	3.33	0.32	0	0
pos	1.38	0	0	1.22	0	0	0	0	0	0	0	0	0	0	1.14	11.36	4.09	11.03	0	0	0
pos	0.84	0	0	0.78	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
pos	0.76	0	0	1.44	0	0	0	0	0	0	0	0	0	0	0	19.6	0	17.35	1.23	0	0
pos	0.59	0	0	0	0	0	0	0	0	0	0	0	0	0	0.66	7.84	1.3	4.49	1.54	0	0
pos	0.57	0	0	0.63	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
pos	0.45	0	0	0.55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
pos	0.34	0	0	0.35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
pos	0.32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21.55	0	14.04	1.32	0	0
pos	0.24	0	0	0.27	0	0	0	0	0	0	0	0	0	0	0	8.29	0	8.96	1.6	0	0
pos	0.14	0	0	0	0	0	0	0	0	0	0.28	0	0	0	0	8.01	0	3.45	1	0	0
pos	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.88	0.38	1.08	0	0	0
pos	0.06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
pos	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
pos	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.63	9.67	4.63	7.32	0.66	0
pos	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
pos	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.28	3.36	0.44	2.1	0	0
pos	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14.44	0	22.7	1.08	0	0
pos	0.04	0	0	0	0	0	0	0	0	0.22	0	0	0	0	0	9.53	30.48	19.95	30.14	1.49	0
pos	0.03	0	0	1.19	0	0	0	0	0	0	0	0	0	0	0	1.5	15.67	0.79	8.24	1.82	0
pos	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
pos	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.61	5.69	2.12	1.31	0	0
pos	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4.64	13.05	5.25	9.19	1.34	0
pos	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
pos	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.58	0	0	0	0	0
pos	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
neg	0.80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
neg	0.45	0	0	0.55	0	0	0	0	0	0	0	0	0	0	0	6.09	7.83	1.17	7.08	0	0
neg	0.32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
neg	0.21	0	0	0.32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
neg	0.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.81	17.65	2.26	8.12	1.37	0
neg	0.11	0	0	0	0	0	0	0	0	0	0.8	0	0	0	0	2.26	3.75	2.59	2.89	0.87	0
neg	0.10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
neg	0.06	0	0	0	0	0	0	0	0	0	0.2	0	0	0	0	0	0	0	0	0	0
neg	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
neg	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
neg	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.77	0.23	0.78	0	0	0
neg	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.61	0	0.55	0	0	0
neg	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27.89	0	9.82	2.47	0	0
neg	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
neg	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.6	0	3.9	0	0	0
neg	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	3.55	0.48	2.59	0	0	0
neg	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
neg	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
neg	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
neg	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
neg	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
neg	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
neg	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
neg	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.39	0	0.78	0	0	0
neg	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
neg	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.26	0	1.88	0	0	0
neg	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
neg	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.32	0	0	0	0	0
neg	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.72	0	0	0	0	0
neg	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.54	0	1.18	0	0	0
neg	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.43	0	0.94	0	0	0

Figure 3 ImmunoCAP ISAC profile for cat and related molecular allergen components in poly-sensitized athletes.

Table 2 Positive percent agreement (PPA) and negative percent agreement (NPA) of ImmunoCAP ISAC compared with ImmunoCAP Specific IgE and skin prick test (SPT)

Allergen	Comparison	Athletes (n)	PPA%(CI)	NPA%(CI)
Grass (g2, g6)	ISAC vs slgE 1.0 kU _A /l	72	95 (82–99)	
	ISAC vs slgE 0.1 kU _A /l	72	81 (69–91)	89 (65–99)
	ISAC vs SPT	70	87 (75–95)	100 (80–100)
<i>Parietaria judaica</i> (w21)	ISAC vs slgE 1.0 kU _A /l	69	95 (74–100)	
	ISAC vs slgE 0.1 kU _A /l	69	49 (34–64)	95 (77–100)
	ISAC vs SPT	72	59 (42–74)	97 (83–100)
<i>Felis domesticus</i> (e1)	ISAC vs slgE 1.0 kU _A /l	70	85 (55–98)	
	ISAC vs slgE 0.1 kU _A /l	70	62 (42–79)	98 (87–100)
	ISAC vs SPT	72	46 (29–63)	94 (81–99)
<i>Dermatophagoides pt</i> (d1)	ISAC vs slgE 1.0 kU _A /l	72	90 (76–97)	
	ISAC vs slgE 0.1 kU _A /l	72	67 (53–79)	100 (81–100)
	ISAC vs SPT	72	69 (55–82)	91 (72–99)

Table 3 Phenotype distribution of subjects studied

Group	Allergic phenotype	Sensitization pattern					Total
		Mono-sensitized (%)	True poly-sensitized (%)	False poly-sensitized (%)	At high risk (%)	Negative (%)	
Athletes	Asthma	2 (11)	13 (68)	3 (16)	0 (0)	1 (5)	19
	RC	4 (20)	10 (50)	5 (25)	1 (5)	0 (0)	20
	OAS	1 (8)	4 (31)	5 (38)	2 (15)	1 (8)	13
	Total with symptoms	7 (13)	27 (52)	13 (25)	3 (6)	2 (4)	52
	No symptoms	6 (30)	7 (35)	1 (5)	1 (5)	5 (25)	20
	Total	13 (18)	34 (47)	14 (19)	4 (6)	7 (10)	72
Allergic controls	Respiratory symptoms	3 (15)	4 (20)	6 (30)	7 (35)	0 (0)	20

However, the pair of patterns causing the statistical difference could not be found ($P > 0.14$ for each pair).

The difference in the sensitization pattern distribution between poly-sensitized athletes with allergic symptoms ($n = 52$) and poly-sensitized athletes with no allergic symptoms ($n = 20$) was statistically significant ($P = 0.01$). The difference in sensitization pattern distribution between poly-sensitized athletes with allergic symptoms ($n = 52$) and poly-sensitized sedentary controls with respiratory symptoms ($n = 20$) was also statistically significant ($P = 0.02$).

Allergic athletes and allergic controls had serum total IgE values significantly higher than nonallergic athletes ($P < 0.01$). On the other hand, no statistical difference was observed for total IgE serum values in athletes with different allergy phenotypes or different sensitization patterns.

Discussion

This study shows that specific IgE determination using the ImmunoCAP ISAC microarray technology may provide useful additional information to those provided by conventional IgE tests in athletes with multiple positive skin tests.

The correlation between ISAC and ImmunoCAP sIgE or skin tests was quite high for the four main allergens considered. In fact, PPA between ISAC and ImmunoCAP sIgE ranged between 85% and 95% in case of a clearly positive ImmunoCAP sIgE (above 1 kU_A/l), while the NPA ranged between 89% and 100% in case of a clearly negative ImmunoCAP sIgE (<0.1 kU_A/l). PPA between ISAC and SPT was also high (94–100%), while NPA was lower (range: 46–87%).

A positive ISAC value above 0.3 ISU was found in some nonallergic control athletes only for a few molecular allergens in the lack of any clinical symptom. This should prompt the manufacturing company to check the nonspecific binding of some molecular allergens included in the ISAC microarray (mainly Equ c 3, Bla g 1 and Gly m 5).

However, rather than focusing on the already reported very good correlation between ImmunoCAP ISAC and skin tests or ImmunoCAP specific IgE, we prefer to focus on the potential significance of discordant cases, as well as on the potential added value of distinguishing among different patterns of sensitization.

The correlation between the presence of specific IgE to at least one ISAC allergen and a positive skin test to the corresponding extract-based allergen was higher in athletes with clinical symptoms (96%) than in athletes with positive skin tests but who were asymptomatic (75%). This might indicate a higher sensitivity of skin tests to detect sensitization but a higher specificity of ImmunoCAP ISAC to detect clinical allergy.

The correlation of ImmunoCAP ISAC vs skin tests or ImmunoCAP specific IgE for detecting specific IgE against a major specific allergen component varied among allergens. This might imply a different prevalence of sensitization to major allergen-specific components for different allergens and the fact that crude allergen extracts commonly include also other known and unknown specific or nonspecific components. For instance, PPA was higher for grass (represented on the chip by almost all major allergen components) than for *Parietaria*, which contains several allergen components not present on the chip apart from Pari2, profilin and CCD.

However, a more interesting and unique advantage of component-resolved diagnostics stands in the possibility of providing a fingerprint of sensitization in subjects with a similar sensitization to multiple allergens at skin tests or at other extract-based assays of specific IgE. In fact, not all subjects with multiple sensitizations at SPT and/or at ImmunoCAP sIgE were shown to be really poly-sensitized at ISAC. Of the 72 athletes with positive skin tests to multiple complete extract-based allergens, 13 (18%) had a predominant mono-sensitization and 14 (19%) had IgE antibodies to cross-reacting allergens, mainly PR-10 and profilins.

Although, possibly due to small size of the different phenotype subgroups studied, the pair of patterns causing statistical difference in the phenotype distribution was not found, the prevalence of true poly-sensitizations in athletes seemed to be higher in asthma (13/19, 68%) than in both rhinoconjunctivitis (10/20, 50%) and poly-sensitized without allergic symptoms (7/20, 35%). Poly-sensitized athletes without allergic symptoms (6/20, 30%) were in fact predominantly mono-sensitized. Interestingly enough, a predominant profile of cross-reacting allergens were particularly frequent in athletes with respiratory symptoms and OAS (13/52, 25%).

Accordingly, ISAC, but not serum total IgE levels, may help in distinguishing among subjects with multiple positive

skin tests between the polyclonal IgE activation leading to true poly-sensitizations and a predominant monosensitization or the presence of cross-reacting allergens. This can be of special relevance when deciding about the optimal treatment strategy in poly-sensitized athletes with multiple sensitizations, particularly when deciding about the appropriateness of preventive environmental measures or immunotherapy.

The limited number of observations and the deliberate selection of subjects with a poly-sensitization do not allow any speculation on the finding of a different prevalence of sensitization to individual allergen components (such as Phl p 1, Der p 2 and tropomyosin) in allergic athletes vs allergic controls. Accordingly, the relatively small number of athletes, with different allergy phenotypes, prevented from a reliable multivariate analysis of the influence of the sport discipline practiced on the type of sensitization. However, the similar prevalence of different sensitizations in the entire Italian Olympic Delegation ($n = 435$) compared with the general population (8) seems to indicate that the reported high prevalence of sensitizations and allergic diseases in athletes cannot be ascribed to peculiar 'occupational' sport allergens, but rather to the effect of an intense physical training in skewing the immune response to a Th2 phenotype (2, 3, 19) and/or in causing epithelial damage in the target organs (20).

Allergy in athletes may trigger potentially life-threatening symptoms such as severe asthma attacks or exercise-induced anaphylaxis (7, 21). The detection of IgE antibodies to allergens at risk for severe allergic reactions, although less frequent than in allergic controls, was found in a relevant percentage of athletes (4/72, 6%), mostly in athletes with OAS, but even in asymptomatic athletes. Therefore, Immu-

noCAP ISAC might represent a useful additional allergy test to detect athletes at risk for severe allergic reactions or for an adequate allergy diagnosis as well as to adopt preventive measures in those who experienced these dangerous events.

Athletes have a peculiar life-style with reference to diet, exposure to different environments as a result of frequent travelling, and physical and emotional stress. The high prevalence of sensitization and allergic diseases in athletes should prompt to include allergy diagnosis among routine procedures in medical screening of all those who exercise, at both competitive and noncompetitive level.

In athletes with respiratory allergy and multiple sensitizations – and particularly in those with symptoms suggestive of food allergy (22) – the ImmunoCAP ISAC microarray technology may provide a useful additional diagnostic tool to better define the pattern of sensitization and to implement the optimal preventive and therapeutic management.

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Conflicts of interest

S. Bonini, G.W. Canonica and G. Melioli were part of the speaker panel bureau at symposia sponsored by Phadia AB (at present Thermo Fisher Scientific). Other authors have no interest to disclose in relation to the present manuscript.

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