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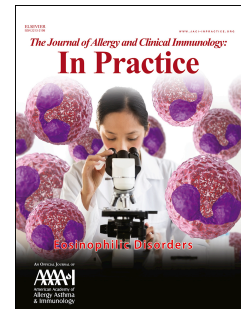
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Allergic sensitization during early life: Concordance between ImmunoCAP and ISAC results

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4 ImmunoCAP and ISAC are both used to detect allergen sensitization. In a multicenter,
5 prospective cohort of infants hospitalized for bronchiolitis, we observed a higher prevalence of
6 sensitization during early life using ImmunoCAP compared to ISAC testing; however, these two
7 methods otherwise yielded highly concordant results.

Early life allergic sensitization may predict development of allergic disease.¹ Various testing methods are used to detect allergen sensitization, including skin prick testing and the measurement of serum specific-IgE (sIgE). Currently, commercially-available methods to measure sIgE include: 1) quantifying sIgE to a single allergen or component attached to a solid phase assay (e.g., ImmunoCAP) and 2) quantifying sIgE to multiple allergen components simultaneously in a microarray (e.g., Immuno Solid-phase Allergen Chip [ISAC]).² While ImmunoCAP has been used for many years in clinical and research settings, ISAC has increasingly been utilized in pediatric cohorts due to the detection of sIgE to a wide array of allergens using a smaller amount of blood.¹ However, it is not known whether ImmunoCAP and ISAC yield concordant results during early life, especially during infancy.

We analyzed data from the ongoing 35th Multicenter Airway Research Collaboration (MARC-35), a multicenter, prospective cohort study of infants hospitalized with bronchiolitis. Briefly, during three consecutive winter seasons from 2011-2014, investigators at 17 sites across the United States enrolled infants hospitalized with an attending physician diagnosis of bronchiolitis. Structured interviews were performed at enrollment and every 6 months, and biospecimens, including blood samples were collected at enrollment and at age 3 year exam, which has been described previously.³ The study was approved by the institutional review boards of participating hospitals.

Blood specimens collected during infancy (age <12 months) and during early childhood (ages 3-5 years) were tested for sIgE via ImmunoCAP and ISAC at the Phadia Immunology Reference Laboratory (Portage, MI, USA). Food sIgE assays to cow's milk, egg white, peanut, cashew nut and walnut were conducted on both the infancy⁴ and early childhood blood specimens. Aeroallergen sIgE assays were conducted only on the early childhood blood

specimens and included grasses, trees, weeds, molds, cat, dog, cockroach, mouse, and dust mites. We defined positive ImmunoCAP results as ≥ 0.35 kU/L. ISAC includes 112 components including 19 food allergens and 10 aeroallergens. We defined positive ISAC results as ≥ 0.30 ISAC Standardized Units (ISU-E).²

We calculated the prevalence of sensitization via ImmunoCAP and ISAC. We statistically examined the concordance of sensitization via ImmunoCAP and the corresponding ISAC components using Gwet's AC_1 , a measure of chance-corrected concordance that is more stable than Cohen's kappa in the context of high agreement.⁵ Because the ImmunoCAP detectable threshold for sensitization has recently lowered from ≥ 0.35 kU/L to ≥ 0.1 kU/L, we also determined the concordance of sensitization via ImmunoCAP and ISAC using a threshold of ≥ 0.1 kU/L for sIgE.² Bland-Altman plots were not used due to different measurement units. We used Spearman's rho (r_s) and scatterplots to assess the correlations of detectable values. Analyses were conducted using Stata 15.1 (College Station, TX). A two-sided $P < 0.05$ was considered statistically significant.

Among 1,016 enrolled participants who provided infant blood specimens, 921 were followed longitudinally. In the longitudinal cohort, 1 child died before age 1 year, and 611/920 (66%) provided an early childhood blood specimen. Cohort characteristics at enrollment and early childhood are summarized in **Table E1** in the Online Repository. At enrollment ($n=1,016$), the median age was 3 months (IQR 2-6), 60% were male, 24% were non-Hispanic black, and 30% were Hispanic. The median age at the early childhood exam was 45 (IQR 41-51) months.

We found a higher prevalence of any food sensitization using ImmunoCAP compared to ISAC during both infancy (17.9% vs. 2.9%) and early childhood (29.0% vs. 4.6%) (**Table I**). The prevalence of sensitization to specific foods via ImmunoCAP ranged from 0% (walnut) to

12.7% (cow's milk) during infancy, and from 3.1% (cashew) to 20.9% (cow's milk) during early childhood. In contrast, sensitization to specific food components via ISAC was rare at both time points; the highest prevalence of sensitization was observed for Gal d 2 (1.7%) during infancy and Ara h 2 (2.3%) during early childhood.

Similarly, for aeroallergen sensitization, we observed a higher prevalence of sensitization during early childhood using ImmunoCAP (27.0%) compared to ISAC (21.7%). The prevalence of sensitization to aeroallergens via ImmunoCAP ranged from 3.9% (mouse) to 12.9% (*D. pteronyssinus*); sensitization to aeroallergen components via ISAC was highest for Fel d 1 (5.4%), Can f 1 (4.6%), and Der p 1 (4.4%).

We found a high concordance between ImmunoCAP and ISAC to foods during infancy, with slightly lower concordance in early childhood, especially for cow's milk and egg white (**Tables I and E2**). Given previous reports of blocking antibodies to cow's milk and egg white,⁶ we performed a sensitivity analysis limited to children who consumed milk or egg at least once per week (per parent report), which yielded similar results. We also found a high concordance between ImmunoCAP and ISAC during early childhood for aeroallergens (**Tables I and E2**), which has previously been described in adults.⁷ We found a lower concordance using a threshold of ≥ 0.1 kU/L for sIgE, especially for egg white and cow's milk (**Table E2**).

We also observed significant correlations of detectable ImmunoCAP and ISAC values, with the exceptions of cat – Fel d 4 and cockroach – Bla g 7 (**Table II**) which may be secondary to Fel d 4 found in cat saliva only and Bla g 7 as a minor allergen.^{8,9} The highest observed correlations were between detectable values of *D. farinae* and Der f 1 ($n=16$, $r_s=0.88$, $P<0.001$) and detectable values of *D. pteronyssinus* and Der p 1 ($n=25$, $r_s=0.83$, $P<0.001$).

In summary, we report a higher prevalence of early life sensitization using ImmunoCAP compared to ISAC; however, these methods yielded mostly concordant results. The difference in prevalence of sensitization may be secondary to the inclusion of only a subset of allergenic epitopes in ISAC compared to ImmunoCAP or to differences in affinity maturation of IgE antibodies against crude food allergens versus specific food proteins during infancy. The observed difference in prevalence of sensitization between the two methods should inform the interpretation of population-based estimates of allergic sensitization, as well as studies using sIgE as a proxy for allergic diseases in early life. Nevertheless, the otherwise concordant results from ImmunoCAP and ISAC values during early life suggest that, for at least certain allergens, these two methods may approximate one another. Although we have analyzed data from a large and diverse cohort, study limitations include that the cohort comprised of infants hospitalized for bronchiolitis, which may differ from the general population. Additionally, ImmunoCAP testing was limited to crude allergen extracts only. Whether these two methods differ in predicting true allergic disease during early life remains to be determined.

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Table I. Prevalence and concordance of sensitization via ImmunoCAP and ISAC

	n	Prevalence of sensitization via ImmunoCAP (%)	Prevalence of sensitization via ISAC (%)	Gwet's AC₁^a
Food sensitization				
Infancy	1,016	17.9	2.9	0.81
Early childhood	611	29.0	4.6	0.64
Infancy or early childhood	611	36.2	6.6	0.53
Aeroallergen sensitization (early childhood)				
Sensitization to any perennial aeroallergen ^b	601	24.6	19.5	0.86
Sensitization to any seasonal aeroallergen ^c	606	10.1	6.1	0.93

Sensitization was defined using thresholds of ≥ 0.35 ku/L (ImmunoCAP) and ≥ 0.3 ISU (ISAC). Food sensitization was defined as sensitization to one or more of the following: cow's milk, egg white, peanut, cashew, and walnut.

^a All $P < 0.001$

Aeroallergen sensitization was defined as sensitization to one or more perennial or seasonal aeroallergen.

^b Perennial aeroallergens included cat, dog, cockroach, *D. farinae*, *D. pteronyssinus*, mouse, and mold mix.

^c Seasonal aeroallergens included grass mix, tree mix, and weed mix.

Table II. Correlations of detectable ImmunoCAP and ISAC values during infancy^a and early childhood^b

ImmunoCAP	ISAC component	n	Spearman's rho	P-value
Egg white ^a	Gal d 2 ^a	16	0.71	0.002
Peanut ^b	Ara h 2 ^b	11	0.70	0.02
Cat ^b	Fel d 1 ^b	30	0.68	<0.001
Cat ^b	Fel d 4 ^b	10	0.30	0.40
Dog ^b	Can f 1 ^b	27	0.74	<0.001
Cockroach ^b	Bla g 7 ^b	10	0.38	0.28
<i>D. farinae</i> ^b	Der f 1 ^b	16	0.88	<0.001
<i>D. farinae</i> ^b	Der f 2 ^b	17	0.61	0.009
<i>D. pteronyssinus</i> ^b	Der p 1 ^b	25	0.83	<0.001
<i>D. pteronyssinus</i> ^b	Der p 2 ^b	20	0.69	<0.001
Mouse ^b	Mus m 1 ^b	10	0.80	0.005