

Article

Atopic Status in Children with Asthma and Respiratory Allergies—Comparative Analysis of Total IgE, ImmunoCAP Phadiatop/fx5 and Euroimmun Pediatric Immunoblot

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Abstract: Introduction: An atopic status assessment (skin prick test or specific immunoglobulin (sIgE)) in asthmatic children is considered a milestone in identifying potential risk factors and triggers provoking loss of asthma control and asthma exacerbation. Objective: The study aims to perform a comparative analysis of different laboratory methods for a serological assessment of an atopic status in asthma and respiratory allergies in children. Material and methods: A total of 86 children were included, all of whom were diagnosed with bronchial asthma, aged from 5 to 17 years and screened for total IgE level using enzyme-linked immunosorbent assay (ELISA). In 48 randomly selected children, we performed a semi-quantitative serological in vitro assessment of the specific IgE antibodies against food and aeroallergen, using two different laboratory methods—Euroimmun Immunoblot and ImmunoCAP (Phadiatop/fx5). Results: In 70% of the children with a history of allergies, and 65.3% without clinically manifested allergies, multiscreen test ImmunoCAP Phadiatop/fx5 showed positivity and confirmed atopy. Our results showed a significant moderate to strong correlation between multiscreen ImmunoCAP Phadiatop/fx5, and Euroimmun specific IgE titers against aeroallergens—cats, mites, tree mix and food allergens—soy, wheat ($p = 0.006$), rice, $p = 0.090$), apple $p = 0.007$ and peanut. A sensitivity of 63% and specificity of 73.5% was observed for EUROIMMUN Pediatric (food allergens, IgE titer > 1) compared with the gold standard ImmunoCap/fx5. The mean value of total IgE is significantly higher in children with asthma and concomitant with allergic rhinitis compared to those without allergic rhinitis (mean 202.52 U/mL, IQR 102.50 (24.20–363.95) vs. 316.68, IQR 261.00 (109.20–552.50), $p = 0.005$). Conclusion: Establishing the spectrum of the most common respiratory and food allergens is an essential factor for maintaining asthma control, both through a strategy to avoid allergen exposure and by developing a recommendation plan. The immunoblotting technique is easily applicable in daily clinical and laboratory practice. It is also a cost-effective and reliable alternative to the “gold standard” ImmunoCAP Phadiatop/fx5 in diagnosing atopy in children.

Keywords: atopic status; allergic rhinitis; total IgE; specific IgE; childhood asthma; immunoblot; ImmunoCAP

Citation: Lazova, S.; Baleva, M.; Priftis, S.; Naseva, E.; Velikova, T. Atopic Status in Children with Asthma and Respiratory Allergies—Comparative Analysis of Total IgE, ImmunoCAP Phadiatop/fx5 and Euroimmun Pediatric Immunoblot. *Sinusitis* **2022**, *6*, 1–14. <https://doi.org/10.3390/sinusitis6010001>

Received: 25 August 2021

Accepted: 21 December 2021

Published: 29 December 2021

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1. Introduction

In epidemiological studies, atopy and allergy have been associated as risk factors for bronchial hyperreactivity and asthma in children and adults [1,2]. Atopy is defined as a

tendency to produce IgE antibodies in response to allergen exposure. This increases the risk of developing diseases such as asthma, allergic rhinitis (AR) or atopic dermatitis (AD) [3]. Atopy can range from asymptomatic sensitization to one or more allergens with no clinical presentation to clinically manifested atopic disease. During childhood, sensitization is predominantly an immunological phenomenon [4,5]. In clinical practice, atopy is often equated with the presence of serum allergen-specific IgE antibodies or positive skin prick test (SPT). However, a positive SPT is not always associated with clinical manifestations of established sensitization. In fact, some “atopic” children do not develop any allergic diseases [6]. Skin allergy testing is generally more sensitive than *in vitro* studies [6]. However, serum-specific IgE (sIgE) determination provides quantitative information. The main disadvantage of the available extract-based methods such as SPT is the variability of the crude extracts from different manufacturers available in the market, concordance limitations and cross-reactivity [7]. In children with persistent asthma, the characterisation of an atopic status is complex and often requires *in vivo* and *in vitro* tests [7,8].

There is still no consensus on the underlying pathophysiological basis of childhood asthma. Underlying chronic inflammation is often characterised by eosinophilic activity and allergic inflammation, but non-allergic asthma is not uncommon in childhood [9,10]. In addition, childhood asthma poses a significant clinical challenge in the diagnostic process, prognosis, and follow-up [10,11]. Inflammatory biomarker testing may provide additional information for the clinical evaluation and monitoring of children with asthma and respiratory allergies [7,12]. Therefore, at school age, the combined use of atopy biomarkers—sensitisation to allergens (SPT or serum sIgE), FeNO (fractional exhaled nitric oxide) and blood eosinophilia, can determine the risk of developing asthma in school-age and evaluate the severity of childhood asthma [2,12–14].

In children with asthma, the presence of atopy complicates achieving and maintaining good control [1,15]. On the other hand, allergic sensitization is a dynamic condition [16]. Additionally, monosensitized children may become polysensitized over time [16,17]. Therefore, experts recommend testing for inhalation and food allergens at least once per year (SPT and or serum sIgE), or more often in case of a new clinical allergic presentation [1,15]. The Monitoring asthma in children European Respiratory Society (ERS) Task Force recommends an active screening for allergen exposure, emerging sensitisation, or related changes in the clinical course of allergic disease [15]. The presence of allergies in patients with asthma (identified by SPT or serum sIgE) may help to identify the risk factors provoking asthma symptoms. A diagnostic clarification of atopy against particular food or aero-allergens is necessary, especially in the presence of suboptimal asthma control and before undertaking a change in therapy [1].

Historically, radioallergosorbent tests (RAST) have been the first to detect serum allergen-specific antibodies [6,18]. The first generation of quantitative tests (RAST, MAST—Multiple-antigen simultaneous test and EAST—Enzyme Allergosorbent Test) evolved into second-generation semi-quantitative IgE tests (AutoCAP, Alastat, HYTech, Matrix, MagicLite) to modern quality third-generation autoanalysers [6,19]. Two widely used third-generation immunological methods are the ImmunoCAP System (Phadia, Thermo Scientific, Uppsala, Sweden) and Immulite 2000 (Diagnostic Products Corporation, Los Angeles, CA, USA). The chemical analysis is similar to the original RAST, but non-isotopic markers are used; the study is faster, with improved precision, accuracy, and analytical sensitivity [20].

Multi-allergen screening tests are designed to measure sIgE against multiple allergens in a single assay. Allergens from different groups (house dust, animal epidermis, grass, tree pollen, fungal spores) or multiple allergens from the same group (e.g., mould—*Penicillium*, *Cladosporium*, *Alternaria*, *Aspergillus*) are embedded in a standard solid-phase [21]. Multi allergen screening for aero-allergens (Phadiatop), combined with a food allergen mix (fx5), is more effective than measuring individual allergen-specific IgE in characterising the atopic march of children with asthma [22]. These two tests showed a positive predictive value of 97.4% for any suspected allergic disease (asthma, AR, AD/eczema syndrome, food allergy) in children older than four [23,24]. Therefore, the combination of ImmunoCAP

Phadiatop (aeroallergens) with ImmunoCAP fx5 (food allergens) is officially accepted as the “gold standard” in clinical practice and in research determining the atopic status in childhood [25,26]. In addition, ImmunoCAP Phadiatop/fx5 has the highest predictive value for determining atopy from any available laboratory test (Phadiatop for children over 12 and in combination with fx5—under 12 years) [7,15].

2. Objectives

The study aims to perform a comparative analysis of the three laboratory methods for the serological assessment of the atopic status (the “gold standard” RAST multi-screening test ImmunoCAP Phadiatop/fx5, EAST Euroimmun pediatric immunoblot and total IgE ELISA) in children with bronchial asthma. Furthermore, we aimed to assess the clinical significance of atopic status determination in the enrolled children, based on serological testing (total serum IgE and sIgE) and history data for clinical manifestations of an allergy.

3. Materials and Methods

3.1. Subjects

In the study, 86 children with diagnosed bronchial asthma aged from 5 to 16 years were enrolled, in which 28 girls (33%) and 58 boys (67%), were hospitalised due to asthma exacerbation.

Before inclusion in the study and the collection of blood samples, all parents and children over 12 years of age signed a written Informed Consent, following the Commission on Ethics of Research at Medical University Sofia (Ethical approval No. 5/17.04.2013, scientific project identification code 23D/2013).

3.2. Study Design

All patients had a detailed medical history and were tested for total serum IgE antibodies by ELISA, EUROIMMUN Medizinische Labordiagnostica AG and specific IgE antibodies (Euroline Allergy Profile Pediatrics, Enzyme Allergo Sorbent Test (EAST) of Euroimmune® (Medizinische Labordiagnostica, AG, 2014, Luebeck, Germany). Additionally, the blood samples of 48 randomly selected children (18 girls and 30 boys with a mean age of 10.65 ± 4.14 SD) were assessed with a semi-quantitative in vitro assay of specific human IgE antibodies against a complex of food and aero-allergens in serum, by two different laboratory methods: Euroimmune® EUROLINE Pediatrics (Medizinische Labordiagnostica, AG, 2014, Luebeck, Germany) and Phadiatop/fx5 (multi-screening test for atopy) ImmunoCAP, Phadia, Thermo Fischer Scientific Inc., (Phadia AB®, Uppsala, Sweden) (Figure 1). The laboratory tests were performed by two independent certified laboratories in clinical immunology—Euroimmun Euroline Allergy Profile Pediatrics and total IgE in the Laboratory of Clinical Immunology of the University Hospital “St. Ivan Rilski” and ImmunoCAP Phadiatop/fx5 in the Laboratory of the Clinic of Clinical Immunology, University Hospital “Alexandrovska”.

We defined the children as allergic and non-allergic according to the medical history data for diagnosed allergic disease, or clinically allergic manifestation prior to enrolment. Using an in vitro atopy diagnostic panel (serum total IgE and sIgE), we determined the children as atopic and non-atopic.

3.3. Laboratory Immunological Methods

- o Determination of serum levels of total IgE by ELISA (enzyme-linked immunosorbent assay), EUROIMMUN Medizinische Labordiagnostica AG

IgE concentration is determined using a calibration curve. The reading is performed at a 450/630 nm wavelength with four standards (calibrators) 0 U/mL, 10 U/mL, 100 U/mL, and 500 U/mL. Results are quantitative and are presented in U/mL. Normal values were determined relative to the upper limit of normal according to age. (Table S1).

- o Euroimmun EUROLINE Pediatric (complex of the most common food and aero-allergens in childhood).

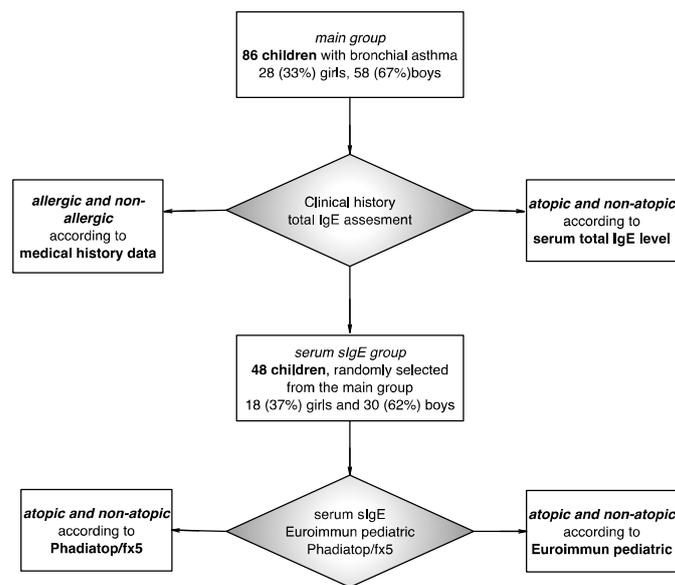


Figure 1. Study design.

EUROLINE test kits provided a semi-quantitative in vitro assay of human IgE antibodies in serum or plasma. It is a comprehensive screening profile with essential inhalation and food allergens for childhood allergies. The test strips are first activated with a universal buffer and then incubated at the first reaction with the patient's sera. If there are specific IgE antibodies in the test serum, they bind to the allergen. A second incubation is performed with enzyme-labeled monoclonal human IgE (enzyme conjugate) to visualise the bound antibodies, catalysing the enzymatic reaction. At the bottom of each test, there is a strip that acts as an indicator bar, representing the internal laboratory quality control. The colour reaction of the control indicator strip only becomes visible when the incubation is carried out correctly.

The EUROLINE test is a semi-quantitative method. The scale for reporting the results is expressed in the EAST system in seven classes—from 0 to 6 (<0.35 kU/L EAST class 0 to >100 kU/L EAST class6) (Table S2). Digital reporting of the results is performed with a scanning device (Canon[®], Tokyo, Japan) and a licensed software product EUROLIneScan program.

Allergens contained in the test membrane strips, include EUROLINE Pediatric (11 aero-allergens, 15 food allergen and CCD (cross-reactive carbohydrate determinants) marker) (Figure 2). Aero-allergens: gx grass mix 2 (timothy grass, cultivated rye), t3 birch, w6 mugwort, d1 *der. pteronyssinus*, d2 *der. farinae*, e1 cat, e2 dog, e3 horse, m2 *cladisporium her.*, m3 *aspergillus fum.*, m6 *alternaria* alt. Food allergens include f1 egg white, f75 egg yolk, f2 cow's milk, f3 codfish, f76 α -lactoalbumin, f77 β -lactoglobulin, f78 casein, e204 bovine serum albumin, f4 wheat flour, f9 rice, f14 soybean, f13 peanut, f17 hazelnut, f31 carrot, f35 potato, f49 apple, CCD marker, indicator band. CCDs can be found in various allergens from plant and animal origin. As a result of significant structural similarity, CCDs can cause strong cross-reactivity.

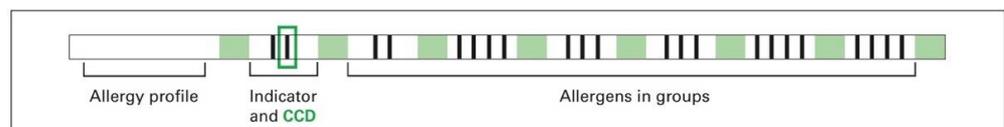


Figure 2. The principal structure of the membrane strip with allergen Euroimmun blot.

In order to perform a comparative analysis, we divided Euroimmun EUROLINE Pediatric into two groups—Euroimmun aero and Euroimmun food, depending on the inhaled and alimentary allergens.

- o Specific IgE—multi-screening atopy test—ImmunoCAP, Phadia (Thermo Fischer Scientific Inc, Phadia[®] AB, Uppsala, Sweden), Phadiatop (aeroallergen complex), and fx5 (MultiCAP food mix)

A Phadiatop test is a mixture of the following allergens: micro-mites (d), moulds (m), wood (t) and grass (g) allergens, weeds (w), animal allergens—dogs and cats (e). MultiCAP food mix (fx5) is the most commonly used pediatric multi-screening test for food allergy. It includes the following six food allergens—cow’s milk protein, chicken egg white, white flour, fish, peanuts, and soy protein, which account for 90% of IgE-mediated food allergies in childhood.

The results of ImmunoCAP specific allergen mixtures are presented qualitatively (positive/negative/borderline) and quantitatively (Specific IgE Class 0 to 6). Values between the lower limit of antibody detection and 0.35 kUA/l may indicate the presence of low IgE levels (class 1). Values ≥ 0.35 kUA/l indicate the occurrence of specific IgE antibodies against one or more allergens included in the multiallergen mixture. Healthy individuals have low levels of specific IgE in the peripheral blood, normally below 0.35 kUA/l (class 0). Sensitized patients show elevated levels, i.e., ≥ 0.35 kUA/l (class 1 to 6). The higher the value of the reported IgE kUA/l, the stronger the allergenic sensitisation (Table S3).

3.4. Statistical Methods

Raw data processing was performed with SPSS[®], IBM 2009, version 19 (2010) and Excel (v. 2010). We used the methods of descriptive statistics to describe demographic and clinical characteristics of patients and the studied immunological parameters. We used a correlation analysis—between category characteristics (χ -square for more than two groups of one of the variables and Fisher’s Exact test for tables with dimension 2×2); between categorical and quantitative features (Analysis of variance—ANOVA) and between quantitative features (correlation and regression analysis) to determine the existence of a relationship (associative or causal) between two or more indicators, its strength, shape and direction. When studying the specificity and sensitivity of quantitative diagnostic tests, we applied the method of ROC curves (Receiver operating characteristic), which represent the relationship between sensitivity (really positive values) as a function of 1-specificity (false positive values) Graphically.

4. Results

4.1. Demographic Characteristics

A total of 86 children were enrolled in the study and included in the main study group. All of them were diagnosed with bronchial asthma and hospitalised due to the exacerbation of symptoms. The children are 5 to 16 years old (mean age 10.18 (SD 3.54). Girls are 28 (33%) with a mean age of 9.98 (SD 3.41), and boys—58 (67%), mean age of 10.54 (SD 3.75).

Children aged 7 to 15 years predominate; 12.9% are pre-school age, and 8% are over 15 years old. Family history for bronchial asthma was reported in almost half of the children—48.8% (n 42), and 25.6% (n 22) of the children were in the first line relatives (mother, father, siblings). In 16 children, there was evidence of bronchial asthma in more than one family member. Approximately half of the children (48.8%) in the main group had medical history for allergy symptoms and/or a diagnosis prior the enrollment (AD, AR, food, drug, venom allergy, urticaria, angioedema). (Table 1) We defined these children as allergic. Concomitant AR was diagnosed in 46.4%.

Table 1. Children with atopic symptoms and or concomitant AR in the main study group.

According to the Medical History	Girls N 28	Boys N 58	Total N 86
Prior allergy symptoms	14 (50.0%)	28 (48.2%)	42 (48.8%)
Concomitant AR	13 (46.4%)	34 (58.6%)	47 (54.7%)

4.2. Total Serum IgE Determination (ELISA)

Serums of 86 children (main study group) were tested for total IgE level, 28 of whom were girls (33%) and 58 were boys (67%). Elevated IgE above the age-adjusted upper limit of normal was found in 64% ($n = 55$) of children: 67.8% ($n = 19$) girls and 62% ($n = 36$) boys. Very high levels of total IgE (titre ≥ 300 U/mL) were found in 35 children (40.1%).

The mean value of total IgE increases parallel with age in the studied group of asthmatic children, except for those older than 16 years, whose confidence interval was wide and uninformative (Figure 3). The mean value of total IgE in girls is slightly higher than that in boys, without significance ($p < 0.05$) (Table S4).

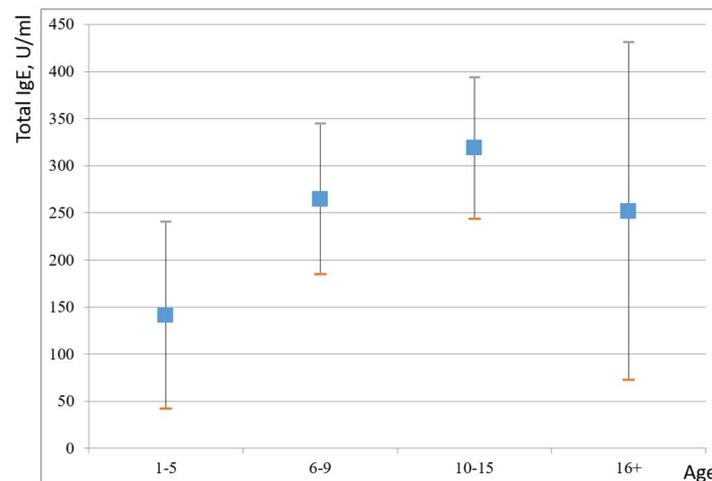


Figure 3. Serum level of total IgE.

- Comparative analysis of serum total IgE and sIgE, assessed by two methods (ImmunoCAP Phadiatop/fx5 and Euroimmun pediatric immunoblot)

In the serum sIgE group, 75 children (87%) showed elevated specific IgE (EUROIMMUN Pediatric). In addition, 27 children (32%) had normal total IgE antibodies (below age-related upper limit of normal—ULN) but at least one positive sIgE according the multiscreen (Phadiatop/fx5 and or Euroimmun pediatric). Of the children with elevated total IgE ($n = 55$), only 3 (5.5%) were negative for the studied sIgE panels (food and aeroallergens).

We constructed an ROC curve to determine the specificity and sensitivity of the serum level of total IgE in detecting children with atopy, defined as the positivity of specific IgE antibodies by both methods—EUROIMMUN Pediatric and ImmunoCAP Phadiatop/fx5. The ROC curve of total IgE against the multiscreen allergy test ImmunoCAP Phadiatop/fx5 (“gold standard”) has an area under the curve of 0.667 (95% CI 0.500–0.834, $p = 0.073$, stand.err—0.085) (Figure 4a). The ROC curve of total IgE against the Euroimmun pediatric (right) has an area under the curve (AUC) of 0.760 (95% CI 0.618–0.903, $p = 0.004$, stand.err—0.073) (Figure 4b).

At a threshold for total IgE above 368.5 U/mL, the method showed the best combination of sensitivity (50.5%) and specificity (81.8%), with 53.1 positive predictive value (PPV) and 86.6 negative predictive value (NPV) relative to the “gold standard” ImmunoCAP Phadiatop/fx5. However, even at a high titer of total IgE, the sensitivity remains relatively low (50.5%).

The ROC curve of total IgE compared to the Euroimmun paediatrics has better diagnostic characteristics, with a larger area under the AUC curve of 0.760 (95% CI 0.618–0.903). The threshold for total IgE over 142.5 combines best sensitivity (75%) and specificity (66.7%) in predicting the presence of positive specific IgE with 83.2 PPV of 51.7.

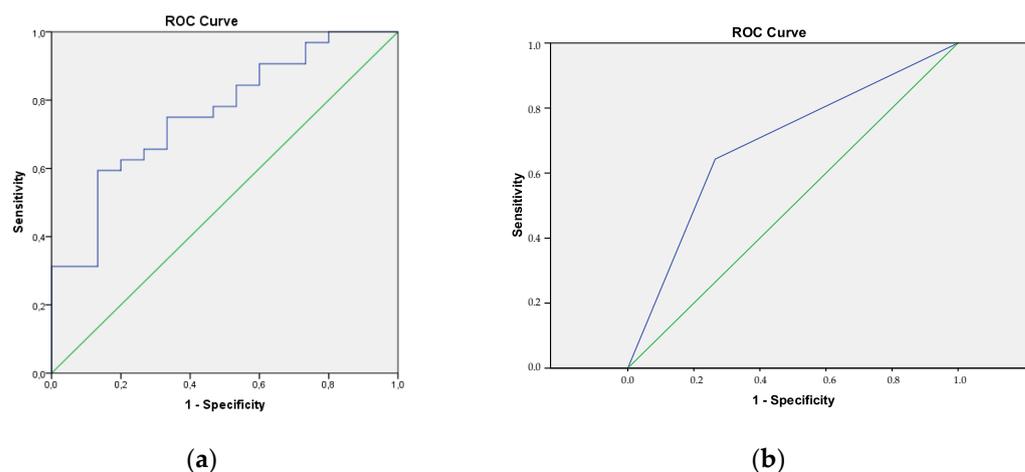


Figure 4. ROC curve to assess the specificity and sensitivity of total IgE vs. ImmunoCAP Phadiatop/fx5 (a) and total IgE vs. Euroimmun paediatrics (b).

The elevated specific IgE against *D. pteronyssimus* ($p < 0.0001$), *D. farinae* ($p < 0.0001$) for titers above 1 EAST class, and against cat fur with titers above 3 EAST class, correlate with the level of total IgE ($p = 0.009$) (Table S5).

- Comparative analysis of serum sIgE against aero-allergens, assessed by two methods (ImmunoCAP Phadiatop and Euroimmun aero)

We constructed an ROC curve to determine the sensitivity and specificity of EUROIMMUN aero to ImmunoCAP Phadiatop (the 'gold standard') (Figure 5). The ROC curve shows AUC—0.958 (Stand.error—0.026, 95% CI (0.000–1.000), $p = 0.000$).

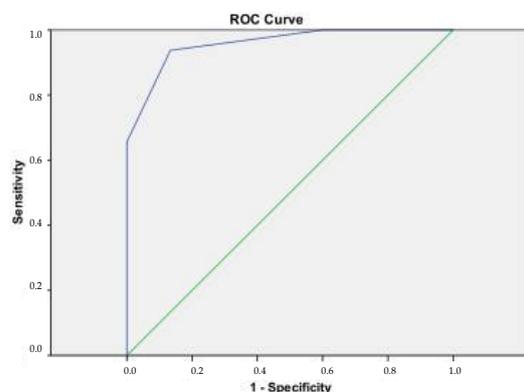


Figure 5. ROC curve for assessing the specificity and sensitivity of Euroimmun aero (as a panel positive/negative) vs. ImmunoCAP Phadiatop.

In multiple analyses for single aero-allergens included in EUROIMMUN aero, the ROC curves remain significant. Aero-allergens d2, e1 and gx are characterised by low sensitivity (between 40 and 60%) and high specificity (above 80%) at EAST class 1 (Figure 6 and Table S6a,b).

Other aero-allergens included in EUROIMMUN aero (dog, birch, and wild wormwood) did not show good predictive value with an area under the curve of below 0.5 (Table S6a,b).

There was a moderate to strong correlation between sIgE titer (e1, d1, d2 and gx) assessed by ImmunoCAP Phadiatop and Euroimmun aero. (Table S7).

- Comparative analysis of specific IgE against food allergens, assessed by two methods (ImmunoCAP fx5 and Euroimmun food)

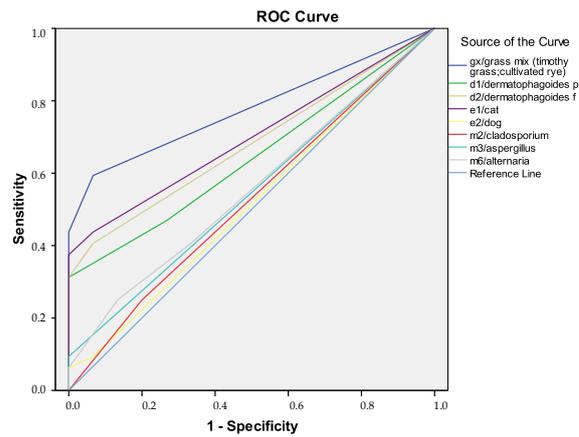


Figure 6. ROC curve for assessing the specificity and sensitivity of EUROIMMUN aero (distinct sIgE) vs. ImmunoCAP Phadiatop.

There was a moderate to strong correlation between the sIgE titer determined with ImmunoCap fx5 (food mix) and those with Euroimmun food—soy (soybean), flour ($p = 0.006$), rice ($p = 0.090$), apple ($p = 0.007$), as well as a weak correlation for peanut. However, no statistically significant correlation was found between the two methods for other food allergens included in the fx5 mix (Table S8).

We constructed an ROC curve to determine the sensitivity and specificity of EUROIMMUN food to ImmunoCAP fx5. Values of EUROIMMUN food above 1 EAST class have a sensitivity of 64.3% and a 73.5% specificity with AUC—0.689, stand. error—0.087, 95% CI (0.518–0.860), $p = 0.041$ (Figure 7a). To check which IgE to use from the EUROIMMUN food to predict the ImmunoCAP fx5 positivity, we performed a multiple analysis with an ROC curve (Figure 7b) and (Table S9).

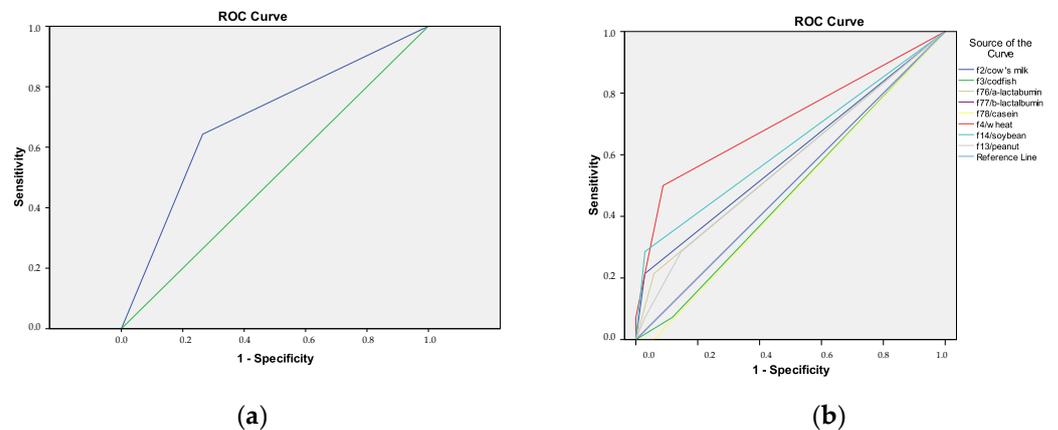


Figure 7. ROC curve for assessment of the specificity and sensitivity of EUROIMMUN food (as a panel positive/negative) to ImmunoCAP fx5 (a), ROC curve for evaluation of the specificity and sensitivity of EUROIMMUN food (distinct sIgE) to ImmunoCAP fx5 (b).

When combining milk allergens (f2, f76, f77, f78) in one variable, the predictive value increases (Table 2).

Table 2. Characteristics of the ROC curve—multiple analysis, cow milk as a combined variable.

Tested Allergen	AUC	SE	p	95% CI	
				Lower Limit	Upper Limit
f2,f76,f77,f78	0.620	0.095	0.196	0.434	0.805

- Clinical significance of medical history, total IgE and sIgE, assessed by two methods

No significant difference was found between mean titer level of total IgE and Phadi-atop/fx5 in children with and without a family history of atopy ($p > 0.05$). For total IgE, the difference is borderline but not significant ($p = 0.077$). In addition, no significant difference was found in mean serum levels of total IgE in the groups of children with and without history data for a clinically manifested allergy (allergic children) ($p > 0.05$).

In the studied population of children, the family history for asthma was associated with clinical symptoms of allergic diseases (AD, drug and food allergies). ($p = 0.048$) Additionally, family history for atopy shows a tendency to correlate with positivity for sIgE antibodies against cat. ($p = 0.054$).

There was a significant difference in total IgE levels in children with and without AR, but not in groups with and without other allergic symptoms/diseases. The mean value of total IgE is significantly higher in children with asthma and AR than in those without concomitant AR. (mean 202.52, IQR 102.50 (24.20–363.95) vs. 316.68, IQR 261.00 (109.20–552.50) ($p = 0.005$).

There was a significant difference in mean serum levels of total IgE in the group of atopic and non-atopic children according to Euroimmun paediatrics sIgE. Atopic children have a significantly higher total IgE titer than non-atopic children (mean 52.4, IQR 39.50 (8.50–83.40) vs. mean 293 IQR (261.00 (91.30–549.60) ($p < 0.0001$).

The chi-square analysis showed that among the group of polysensitized (more than 2 positive sIgE) children, more patients with high or borderline IgE were observed than non-atopic or monosensitized patients (at least one positive sIgE) ($p = 0.003$) (Figure S1).

Overall, 70% (N 14) of children who were defined as allergic according to the medical history had a positive Phadiatop/fx5 result, and 35.7% of the non-allergic according to their history had a negative test result.

Phadiatop/fx5 identified 36 children as atopic and 16 as non-atopic. In 9 children (56%) identified as non-atopic with Phadiatop/fx5, at least one positive sIgE antibody was detected with EUROIMMUN Pediatric (7 of them with EAST class 1, only two with EAST class 2). On the other hand, two children (5.6%) identified as atopic with Phadiatop/fx5 showed a negative result with the EUROIMMUN Pediatric. (only food allergens EAST class 1).

Medical history for allergy symptoms (drug, food, venom allergy, urticaria, AD, angioedema) were reported in 20 children (41%). In two (10%) of the children, atopy was not confirmed with EUROIMMUN Pediatric and ImmunoCAP. The total IgE was increased in 52% ($n = 25$) of children and defined the children as atopic. Using the Phadiatop/fx5, 75% of the tested children ($n = 36$) were classified as atopic, and 85.4% ($n = 41$) with EUROIMMUN Pediatric.

In the serum sIgE group, 28 (58.3%) children had concomitant AR. According to medical history, 46%, are allergic according to the total IgE titer—71% are atopic, Phadiatop/fx5—86%, and EUROIMMUN Pediatric—92.8% (Figure S2).

5. Discussion

Serological diagnosis of atopy began in 1968, and demonstrated a link between the body's sensitisation and the newly discovered class of human immunoglobulins called IgE [27]. Serum concentrations of total IgE are known to have high specificity but low sensitivity in determining atopic status. Thus, in the presence of elevated total IgE, the patient is likely to be atopic, but if normal, atopy cannot be ruled out [12,15]. This was supported by our results. Both analyses of ROC curves, using the two methods for detecting sIgE as a reference, showed excellent specificity but low sensitivity even at high titers for total IgE (>142.5 U/mL and >368.5 U/mL, respectively).

Total IgE levels among atopic children correlate with the size of the target organ, with the lowest values reported in individuals with AR, the highest in those with AD and intermediate for asthmatics [27]. According to our results, the total IgE value is significantly higher in children with asthma and AR than in those without concomitant AR. ($p = 0.005$). Using an χ square, we observed more patients with high or borderline IgE in the group of polysensitized children than in the non-atopic or monosensitized. ($p = 0.003$).

In healthy subjects, total IgE levels increase from birth (0–1 KU/l) to adolescence, decreasing slowly until reaching a plateau at 20–30 years [6,18,28]. Our results confirmed a significant increase in total IgE mean value with age, except for those over 16 years, whose confidence interval is uninformatively wide [6].

In order to define the allergen avoiding strategy, international guidelines include sIgE assessments to identify the subject's comorbidity [29,30]. However, literature data indicate that multi-allergens screening methods detecting aeroallergen in combination with a food allergen mix are more effective than measuring individual allergen-specific IgE in characterising the atopic status of children with asthma [7].

Allergen-specific IgE have a significant advantage, especially in detecting children with clinically undiagnosed allergies. In the studies population, 73% of children had positive sIgE. However, only half of them have a positive history of allergies. Furthermore, a higher percentage of children are sensitised to aero-allergens (64%) than those sensitised against food allergens (43%). This ratio corresponds to literature data for the tested age group. In contrast, atopic sensitisation for food allergens predominates in infants [31,32].

In a recently published study, Chang et al. investigated the sensitivity and specificity of Phadiatop and total IgE levels in hospitalised adults and children with clinical symptoms, suggesting persistent allergic rhinitis. In the study, 576 children were enrolled. The authors report sensitivities and specificities of 86.3% and 77.4% of the total IgE levels to predict positive allergens using Phadiatop in children, and 65.7% and 85.7%, respectively, in adults [33]. Pierotti et al. performed a cross-sectional study of Brazilian children, who were tested for total IgE using Phadiatop and Phadiatop infant. They found a significantly higher mean total serum level of IgE among allergic children, especially those with asthma/rhinitis, as confirmed by our results [34]. Khasawaneh et al. estimated the sensitivity and specificity of total IgE as 77.4% and 92.5%, respectively, when using sIgE as a standard test. The authors tested 80 patients between 1 year and 77 years, 32 of whom were children. Specific IgE were tested with immunoblot EUROLINE. Our result has shown lower sensitivity (50.5%) and similar specificity (81.8%) of the total IgE (the threshold for total IgE above 368.5 U/mL) to predict a positive ImmunoCap Phadiatop result. We found better sensitivity (75%) and lower specificity (66.%) in the total IgE as a predictor for positive Euroimmun paediatrics, using 142.5 U/mL as an LLN for the IgE total (larger area under the AUC curve of 0.760) [23].

When compared the sensitivity and specificity of EUROIMMUN aero vs. the "gold standard" ImmunoCAP Phadiatop, we found 100% sensitivity, but only 40% specificity (PPV 68.1%, NPV 100%) for EUROIMMUN aero using EAST class 1 as a threshold and 98.3% sensitivity and 87, 6% specificity (PPV 94.4%, NPV 96%) for values greater than EAST class 2, which is similar to the results reported in the literature [23,25]. ROC curves remain significant only for *D. Farinae*, cat, and grass mix in multiple aero-allergens analyses. For all other aero-allergens included in the panel (dog, birch, and wild wormwood), EUROIMMUN aero did not show good predictive properties. According to the manufacturer data, the sensitivity of EUROLINE to the Phadia CAP system („gold standard“) for timothy (grass) and birch is 90%, for *D. Pteronyssimus* and *D. Farinae* it is 83% and 84%, respectively, for cat it is 91% and for horse it is 100%. Thus, the two methods have similar indicators in terms of general sensitivity and specificity [35].

Compared to ImmunoCAP fx5 food mix (cow's milk protein, wheat, chicken egg white, fish, peanuts, and soybeans), EUROIMMUN food showed weaker but acceptable sensitivity at 64.3% and specificity of 73.5%, including for lower titers (EAST class 1). In the multiple analysis, the highest predictive power had an sIgE against flour and soy and them combined, but a single sIgE against allergens in cow's milk (f2, f76, f77, f78).

The presence of positive sIgE using the Euroimmun aero was detected in 9 children, which result was negative when using the ImmunoCAP Phadiatop. Seven of them had a sIgE titer corresponding to Euroimmun immunoblot EAST class 1 and only two to EAST class 2. The main sIgE positive with both methods are from the mould group—*Cladosporium*

and *Alternaria*. According to data from patient history, 41.7% of the children are atopic (with clinically manifested allergy).

Total IgE levels showed an increased age-related serum titer in 52% of the tested children, and serological tests for sIgE determined an additional 23 to 30% of children as atopic. According to the Phadiatop/fx5 result, 75% of the children are atopic, using EUROIMMUN Pediatric the percentage of atopic children was found to be 85.4%. In children with concomitant AR, the detectability of atopy by serological methods in children without a history of allergies is even more significant. Singh et al. found Phadiatop/fx5 to reveal that physicians' diagnosis of IgE mediated allergy is accurate in only 59% of cases in the Indian study of allergen sensitisation prevalence. We found that the case history alone reveals atopic children with asthma and concomitant AR in 46% and even in lower per cent 41.7 in the group of asthmatic children without AR [36]. According to data cited by the manufacturer, ImmunoCAP Phadiatop demonstrated 91% specificity, 89% efficacy, and 91% correct classification of patients (atopic/non-atopic) in a study of 836 patients with allergy-related symptoms in six different centres (Italy, Spain, Germany, the Netherlands, Sweden, Great Britain) [20,37]. The study used a threshold for the specific IgE calibrator with a value of 0.35 kUA/l, used in the present study [30].

The results of our study showed an excellent correlation between the two serological methods in terms of sensitivity and specificity. EUROIMMUN Pediatric demonstrated an advantage over the "gold standard" in detecting children with atopy. Using correlation, regression, and factor analyses, we found that ImmunoCAP Phadiatop/fx5 missed positive results in 19% of patients compared to the EUROIMMUN Pediatric results. This error is weaker for fx5 than Phadiatop, probably due to the broader aero-allergenic spectrum embedded in the EUROIMMUN panel. ImmunoCAP Phadiatop and fx5 generate a dichotomous positive/negative value and relative positivity semi-quantitatively (kilo units per litre). Thus the summary result does not define which of the included sIgE is positive. On the other hand, EUROIMMUN Pediatric offers a broader range of tested allergens—inhaled and food allergens with comparable sensitivity and the ability to detect the specific allergen(s) responsible for the positive result. Additionally, EUROIMMUN Pediatric provides a titer for each particular sIgE (semi-quantitative, expressed in EAST class system), not a summary result as provided by ImmunoCAP Phadiatop/fx5.

Skevaki et al. compared the three commonly used technologies for sIgE detection—ImmunoCAP™ sx1 and fx5 mixes, the ImmunoCAP ISAC™ 112 microarray and a Euroline™ panel. Euroline identified the highest percentage of positive samples out of 12 comparable allergens. However, the authors found that, when considering the overall positive samples, Euroline suffers from a higher background signal. In addition, it detects the highest number of sensitisations at threshold 1, but not at a higher and more stringent threshold [38].

Another advantage of EUROIMMUN pediatric immunoblot is the user-friendly and straightforward methodology, which does not require specialised and expensive equipment, easily applicable in daily clinical and laboratory practice. Combined with the excellent specificity and sensitivity of the method and the reliability of results, it is a reasonable alternative to the ImmunoCAP methods. Additionally, we confirmed the correlation between elevated total IgE and the history data for symptoms suggestive of allergic disease. However, total IgE titer is significantly less sensitive than serological multiscreen atopy tests (sIgE panels).

6. Limitation of the Study

We must acknowledge that the results are based on a small number of patients positive for specific IgE against food allergens, which may affect the objectivity of the analysis. Additionally, significant titers (>3.5 IU/mL) were detected only for aero-allergens by both methods (ImmunoCAP, EUROIMMUN). In addition, sIgE detected against food allergens had low titers—EAST class 1 and 2, less than 3.5 kUA/L; kU/L). Lastly, we included children with asthma with/without AR, but we did not have healthy controls. Therefore,

we believe that the study's limitations do not make it invalid, but they are opportunities to inspire future research.

7. Conclusions

Contact with provocative allergen(s) in sensitised children with bronchial asthma and respiratory allergy is a risk factor for exacerbation/hospitalisation. Allergen-specific IgEs have a significant advantage, especially in the detection of children with clinically undiagnosed allergies. Identifying sIgE for particular allergens is essential for the effective management of asthma and other allergic diseases—prevention, diagnosis, and treatment. EUROIMMUN blot methodology does not require specialised equipment and is easily applicable in everyday clinical and laboratory practice. Combined with its high reliability, it makes it an affordable alternative to the “gold standard” ImmunoCAP.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/sinusitis6010001/s1>, Table S1: Reference values for specific IgE in serum, Table S2: EAST scale for reporting the results, Table S3: ImmunoCAP reading scale (kUA/l) * The expected values for specific IgE are not age-dependent, Table S4: Comparison of mean total IgE (U/ml) titer values by sex (girls/boys), Table S5: Comparison of the mean values of total IgE between positive specific IgE against cats and house dust mites against children with a negative test, Table S6: a. ROC curve characteristics of EUROIMMUN aero vs ImmunoCAP Phadiatop, b. ROC curve characteristics of EUROIMMUN aero vs. ImmunoCAP Phadiatop, Table S7: Correlation analysis between ImmunoCAP Phadiatop and Euroimmun aero, Table S8: Correlation analysis between ImmunoCAP Phadiatop and Euroimmun food, Table S9: ROC curve characteristics (assessment of the specificity and sensitivity of EUROIMMUN food to ImmunoCAP fx5), Figure S1. Comparison of total IgE titer in the group of non-atopic, mono- and polysensitised patients, Euroimmun pediatrics, $p = 0.003$, Figure S2. Classification of children with bronchial asthma and AR as atopic / non-atopic (N = 28).

Author Contributions: Conceptualisation, S.L. and T.V.; Data curation, M.B. and E.N.; Formal analysis, S.L., M.B., S.P. and E.N.; Funding acquisition, S.L.; Investigation, S.L. and T.V.; Methodology, S.L., M.B. and T.V.; Project administration, S.L.; Resources, T.V.; Software, S.P. and E.N.; Supervision, T.V.; Validation, S.L. and E.N.; Visualization, S.P. and T.V.; Writing—original draft, S.L. and T.V.; Writing—review and editing, S.L., S.P. and T.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by a grant from the Medical University of Sofia (Council of Medical Science, project no. 35D/2013, grant no. 23D/2013).

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee on Scientific Research at Medical University of Sofia (ethical approval No. 5/17.04.2013, scientific project identification code 23D/2013).

Informed Consent Statement: Before the study enrolment, all parents and children over 12 years old signed written informed consent and child assent, according to the Ethics Committee on Scientific Research requirements at the Medical University of Sofia.

Data Availability Statement: Data available on request due to restrictions, e.g., privacy or ethics.

Acknowledgments: We want to thank Diana Hristova, clinical allergologist for the help in the review process.

Conflicts of Interest: The authors declare no conflict of interest.

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