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RESEARCH ARTICLE

Molecular Allergy Diagnostics as an Adjunct to Conventional Diagnostics in a Secondary Pediatric Referral Center

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Abstract: ***Background***: Several compositions for determination of specific molecular components in allergens have recently been patented. The role of Molecular Allergy (MA) diagnostics in suspected IgE mediated allergic conditions is currently debated. Guideline reports have concluded that population-based studies involving evaluation of the usefulness of MA diagnostics are needed.

Objective: To evaluate the usefulness of MA diagnostics in a secondary pediatric referral center.

Methods: A total of 961 children and adolescents aged 0.2-18.8 (mean 7.0) years was included in a prospective observational survey. Inclusion criterion was a suspected diagnosis of an IgE mediated condition based on history and clinical symptoms and signs. If a specific diagnosis could not be reached from conventional investigations suspected peanut allergy, birch pollen allergy and associated cross-reactivity, insect allergy and triggering allergens for specific immunotherapy were assessed by MA diagnostics.

Results: Based on conventional work-up a diagnostic conclusion was established in 946 patients (98.4%). MA diagnostics were performed in 15 individuals (1.6%), 7 girls and 8 boys aged 3.2 to 17.8 (mean 10.6) years. In 8 cases a specific diagnosis was established based on MA diagnostics; in 7 cases MA diagnostics could not improve diagnosis. MA were most frequently (N = 7 (14%)) used in children with peanut allergy (N = 50).

Conclusion: Most patients in a secondary pediatric referral center with suspected IgE mediated allergy can be managed by conventional diagnostic methods. MA diagnostics may be useful in small and selected subgroups as in patients with suspected peanut allergy, however, may not be helpful in all cases.

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1. INTRODUCTION

Conventional diagnostics in suspected allergic disease include history and a clinical examination (first line approach), skin prick testing and/or assessment of specific IgE antibodies to allergens in the blood (second line investigation), and organ provocation or elimination-provocation-elimination tests (third line evaluation) [1, 2]. As in medicine in general, in the second line investigation of allergic conditions these years focus is increasingly on methods for molecular profiling [3]. Several compositions for the determination of specific components in allergens have been patented [4, 5]. Such methods measure IgE antibodies to specific components of allergens [6, 7]. The methods have been designated as component resolved diagnosis or molecular diagnostics [7].

Whether molecular diagnostics may be alternatives to conventional diagnostics or whether they should be considered to be adjuncts to conventional specific IgE tests is currently debated [1, 2, 8]. The paucity of data to settle this has been highlighted [1, 2]. Recent guidelines and consensus reports have suggested that molecular based allergy diagnostics may be used as third-line work-up adjuncts in selected cases of suspected peanut allergy, birch pollen allergy and associated cross-reactivity, insect allergy and in determining triggering allergens for specific immunotherapy [1, 2]. Such reports, however, have also concluded that population-based studies involving evaluation of the usefulness of molecular diagnostics are needed [2]. The aim of the present study was to evaluate the usefulness of molecular allergy as an adjunct to conventional diagnostics in a secondary pediatric referral center.

2. MATERIALS & METHODS

The design was a prospective, observational study. During a 4-year period children and adolescents 0-19 years of

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age were included from the prospective Asthma and Allergy in a Secondary Pediatric Referral Center Study (AASP) [9]. Inclusion criterion was a suspected diagnosis of an IgE mediated condition (eczema, bronchial asthma, hay fever, food allergy, urticaria) based on the history and clinical symptoms and signs. Conventional work-up including skin prick testing and assessment of specific IgE panels in the blood were performed in all children. A suspicion of food allergy based on history and screening panel test results was followed by an oral provocation test. If a specific diagnosis could not be established from conventional investigations, suspected peanut allergy, birch pollen allergy and associated cross-reactivity, insect allergy and triggering allergens for specific immunotherapy were assessed by molecular allergy diagnostics. No valid data were available for calculation of study population size, however, based on admission rates during a 2-year period prior to study start it was stipulated that 1000 children and adolescents would be entered into the study during a 4-year period.

Serum IgE inhalant and food allergen screening test panels were analysed by Phadia CAP assays at the clinic's reference laboratory (Regional Hospital Silkeborg, Denmark) according to the manufacturer's instructions (Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden) [10]. The panels included 7 inhalant (birch, timothy, mugwort, cat, dog, horse, dermatophagoides pteronyssinus) and 4 food (milk, peanut, egg, wheat) allergens. The Phadia CAP allergen panels had been defined by an ad-hoc group of pediatricians, allergists and clinical chemists under the auspices of Central Denmark Region. Serum IgE against allergen components were performed at the clinics reference laboratory (Aarhus University Hospital, Denmark) in accordance with the manufacturer's instructions, or at the laboratory of the manufacturer of the method, the ImmunoCAP assay (Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden) [11]. The methodology of the assays has been described in detail previously [10, 11]. IgE levels ≥ 0.35 kUA/L (units of allergen-specific antibodies/L) were considered positive sensitizations [11]. Skin prick testing (Soluprick SQ, ALK-Abello, Hoersholm, Denmark) was performed of all allergens included in the blood IgE screening panels with addition of dermatophagoides farinae, alternaria alternata and cladosporium herbarium to the inhalant and of soy and cod to the food panels, respectively. A skin reaction of ≥ 3cm was considered a positive sensitization [12]. Conventional protocols for organ provocation tests were used [13]. None of the above mentioned institutions or manufacturers were involved in the study.

All data were entered into an electronic data base and processed and analysed using R version 3.3.2 [14].

3. RESULTS

A total of 1002 patients was entered into the study base. However, 41 patients were excluded from the analysis because they were ≥ 20 years of age at the date of blood sampling. So, 961 consecutively referred children and adolescents 0.2-18.8 (mean 7.0) years of age were included in the survey. All children had a blood sample taken; a total of 898 individuals (93.4%) had assessments of IgE inhalant and food allergen screening panels; 48 (5.0%) had assessment of the food allergen panel and 15 (1.6%) of the inhalant allergen screening panel only. In the overall population of 961 children 447 (46.5%) had 497 positive panel test results. In the population of 898 children in whom both screening test panels were assessed 415 (46.2%) had at least one positive test panel result. Of 946 individuals in whom the inhalant IgE allergen test panel was performed 275 (29.1%) had a positive test; of 913 patients who were investigated with the food allergen IgE panel 222 (24.3%) had a positive test. The results in skin prick testing were similar (data not given).

Based on the conventional work-up, a diagnostic conclusion was established in 946 patients (98.4%). Molecular allergy diagnostics were performed in 15 individuals (1.6%), 7 girls and 8 boys aged 3.2 to 17.8 (mean 10.6) years (Table **1**). In 12 cases molecular diagnostics were performed as an alternative to oral provocation testing which the children and/or their parents wanted to avoid. In 7 cases (14%) of suspected peanut allergy (N = 50), in 5 cases (5.1%) of birch allergy and suspected cross reactivity (N = 98), in 2 cases (1.8%) of children who were commenced on immunotherapy (N = 93), and in 1 case (12.5%) of insect venom allergy (N = 8) molecular diagnostics were used. Molecular diagnostics established a specific diagnosis in 8 cases; in 7 cases the assay did not improve diagnosis.

4. DISCUSSION

The present protocol was planned to provide evidence on the clinical use of molecular diagnostics the lack of which has been highlighted by many researchers [1, 2, 6, 7, 14]. Several aspects need further data such as implications for physician´s qualifications in interpreting test results, cost-effectiveness, diagnostic sensitivity and specificity and the usefulness in different clinical settings. Recommendations, however, have been that molecular based allergy diagnostics may be used as third-line work-up in selected cases of suspected peanut allergy, birch pollen allergy and associated cross-reactivity, insect allergy and in determining triggering allergens for specific immunotherapy [1, 2, 7, 12]. Therefore, we planned the present study to answer the question whether third-line molecular diagnostics may be helpful when used as an adjunct in these four well defined conditions in cases in whom conventional third-line diagnostics were not sufficient.

Our findings showed that when used as an adjunct to conventional diagnostics in a secondary pediatric referral center molecular allergy diagnostics were needed in less than 2% of the population which was suspected of an IgE allergic condition. Furthermore, in these few cases, molecular diagnostics did not improve the diagnosis in around 50% of cases. Several reasons may explain the low frequency of use of adjunct molecular diagnostics in our population. First, in our population only less than 50% of the population suspected of IgE mediated allergy proved to have sensitizations. That may be considerably lower than in third center settings from which most of the available data so far have been derived [1]. Secondly, in our population of children and adolescents cross reactivity to birch pollen sensitization may be relatively infrequent and many families would not consider investigation of cross reactivity to be important. Third, insect venom allergy is quantitatively not

Table 1. Molecular Diagnostics in 7 Girls and 8 Boys Aged 3.2 To 17.8 (Mean 10.6) Years with a Suspected IgE Mediated Condition. OP: Oral Provocation; SCIT: Subcutaneous Immunotherapy; SLIT: Sublingual Immunotherapy.

Patient Characteristics	Reason for MA	Components	Results (kUA/L)	Conclusion
Male, 3.5 years	OP hazelnut not conclusive; family wanted to avoid re-OP	Bet v1, Cor a1, Cor a8	All components < 0.20	A subsequent OP indicated a diagnosis of cross reactivity
Male, 1.5 years	OP hazelnut not conclusive; family wanted to avoid re-OP	Bet v1, Cor a1, Cor a8	All components < 0.20	A subsequent OP indicated a diagnosis of anaphylaxis
Female, 10.7 years	Family wanted to avoid OP	Bet v1, Cor a1, Cor a8	All components < 0.20	A subsequent OP indicated a diagnosis of cross reactivity
Female, 3.2 years	Family wanted to avoid OP	Bet v1, Cor a1, Cor a8; Ara h2, Ara h8	Bet v1 1.87, Cor a1 1.95; Cor a8 < 0.20; Ara h2 0.70, Ara h8 < 0.20	Subsequent OPs indicated a diagnosis of anaphylaxis to both allergens
Female, 17.8 years	Re-evaluation of anaphylaxis to peanut; family wanted to avoid re-OP	Bet v1, Cor a1, Cor a8; Ara h2, Ara h8	Bet v1 > 100, Cor a1 11, Cor a8 < 0.20, Ara h2 70, Ara h8 < 0.20	Anaphylaxis to peanut, OP was opted out
Male, 17.7 years	Re-evaluation of anaphylaxis to peanut and hazelnut; family wanted to avoid re-OP	Bet v1, Cor a1, Cor a8, Ara h2, Ara h8	Bet v1 33.1, Cor a1 12.5 , Cor a8 0.61, Ara h2 0.36, Ara h8 < 0.66	OP was planned, however, the patient did not show up at appointments
Male, 9.7 years	Re-evaluation of anapylaxis to peanut; family wanted to avoid re-OP	Bet v1, Cor a1, Cor a8, Ara h2, Ara h8	Bet v1, Cor a1, Cor a8 < 0.20, Ara h2 18, Ara h8 < 0.20	Anaphylaxis to peanut, OP was opted out
Male, 5.9 years	Family wanted to avoid OP	Bet v1, Ara h2, Ara h8	Bet v1 < 0.20, Ara h2 11.6, Ara h8 0.35	Anaphylaxis to peanut, OP was opted out
Female, 8.4 years	Family wanted to avoid OP; suspected cross reactivity	Bet v1, Cor a1, Cor a8; Ara h2, Ara h8	All components < 0.20	OP was opted out. A diagnosis of cross reactivity was not established
Female, 12.1 years	Family wanted to avoid OP	Bet v1, Ara h2, Ara h8	Bet v1 5.9, Ara h2 17.4, Ara h8 2.32	Anaphylaxis to peanut, OP was opted out
Male, 10.3 years	Family wanted to avoid OP; suspected cross reactivity	Bet v1, Ara h2, Ara h8	All components < 0.20	OP was opted out. A diagnosis of cross reactivity was not established
Female, 9.8 years	Family wanted to avoid OP	Bet v1, Ara h2, Ara h8, rGly m4, nGly m5, nGly m6, rTri a14	Bet v1, Ara h2, Ara h8: < 0.20; rGly m4 < 0.20, nGly m5 80.1, nGly m6 81.1; rTri a14 < 0.20	Subsequent OPs negative in peanut; indicated a diagnosis of anaphylaxis to soy
Male, 14.9 years	Negative IgE tests in blood and skin	Phl 1, Phl 5	Both components < 0.20	Immunotherapy was opted out
Male, 14.5 years	No effect of 5 years SCIT and subsequent 3 years of SLIT	Phl 1, Phl 5	Phl 1 441, Phl 5 263	Re-immunotherapy was considered not to be indicated
Male, 11.6 years	IgE tests in blood and skin prick testing inconclusive	Api m1, Ves v5	Both components < 0.20	Re-skin prick testing indicated that SCIT of bee only was indicated

as frequent in children as in adult populations and in most cases specific IgE in blood and/or skin prick testing would establish the diagnosis. Finally, molecular diagnostics were most frequently used in the work-up of potential soy allergy, however, the diagnostic outcome was poorer than previously reported [15]. That may reflect the difference in approach between using molecular diagnostics as an adjunct rather than as an alternative to oral provocation testing.

The present protocol was written in 2011 and it needs to be taken into consideration that more data on sensitivity and specificity of specific molecular components have been provided since then. If the protocol were to be written today the components cor a 9 and cor a 14 would have been included in the assessment of children investigated for hazelnut allergy, since they have been shown recently to be important in assessing the reactivity to hazelnut [16]. Potentially, that might have increased the ratio of children being diagnosed by molecular components, however, it would not have affected the overall number of children in whom the molecular methods were used as an adjunct to conventional diagnostics.

CONCLUSION

Most patients in a secondary pediatric referral center with suspected IgE mediated allergy can be managed by conventional diagnostic methods. Molecular allergy diagnostics may be useful in a small and selected subgroups of children only, in whom they may not be helpful in all cases.

CURRENT AND FUTURE DEVELOPMENTS

Current management guidelines need to consider settings when recommendations for use of molecular allergy diagnostics are given. More large-scale real-life studies of the usefulness of molecular allergy diagnostics are needed. Such studies would be needed to consider settings as well as population characteristics such as age.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The prospective observational study protocol did not need authority approvals or consent.

HUMAN AND ANIMAL RIGHTS

All human rights of the participating children were adhered to.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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