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

Ole D. Wolthers1,*

1Asthma and Allergy Clinic, Children's Clinic Randers, Randers, Denmark

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.

Keywords: Allergy, asthma, component resolved diagnosis, eczema, molecular allergy diagnostics, peanut, rhinitis, urticaria.

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 recently 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 age...
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 anaphylaxis 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, Cor a1, Cor a8 | 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, Cor a1, Cor a8; 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, Cor a1, Cor a8 | 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, Cor a1, Cor a8; 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 prick testing inconclusive | Phi l, Phi 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 | Phi l, Phi 5 | Phi l 441, Phi 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.

FUNDING
This research did not receive any specific grants from funding agencies in the public, commercial, or not-for-profit sectors.

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

ACKNOWLEDGEMENTS
The authors would like to thank study nurses Anne Karina Kjaer and Signe Dreier for helping with managing patients and protocol; and Dr Benjamin Ole Wolthers for helping with data processing.

REFERENCES
[1] Matricardi PM, Kleine-Tebbe J, Hoffmann HH, Valenta R, Hilger C, Hoffmaier S, et al. EAACI M Allergy User’s Guide. PAI 2016; (S23): 1-250. [http://dx.doi.org/10.1111/pai.12563]
[2] Canonica GW, Ansetegui IJ, Pawankar R, et al. A WAO - ARIA - GA²LEN consensus document on molecular-based allergy diagnostics. World Allergy Organ J 2013; 6(1): 17. [http://dx.doi.org/10.1186/1939-4551-6-17] [PMID: 24090398]
[3] Kennedy GC, Chudova DI, Wang ET, Wilde JT. Algorithms for disease diagnostics. US20180127832 (2018).
[4] Valenta R, Campana R, Mothes-luksch N. Method for determining the type of allergy. EP2078960 (2009).
[5] Sampson HA. Prognostic allergy test. EP1939628 (2017).
[6] Borres MP, Ebisawa M, Eigenmann PA. Use of allergen components begins a new era in pediatric allergology. Pediatr Allergy Immunol 2011; 22(5): 454-61.
[7] Wolthers OD. Resolved component diagnostics in pediatrics. ISRN Pediatrics 2012. [http://dx.doi.org/10.5420/2012/806920]
[8] Jensen-Jarolim E, Jensen AN, Canonica GW. Debates in allergy medicine: Molecular allergy diagnosis with ISAX will replace screenings by skin prick test in the future. World Allergy Org J 2017; 10(1): 33. [http://dx.doi.org/10.1186/s40413-017-0162-3] [PMID: 28959378]
[9] Gradman J, Wolthers OD. Allergic conjunctivitis in children with asthma, rhinitis and eczema in a secondary outpatient clinic. Pediatr Allergy Immunol 2006; 17 (7): 524-26. [http://dx.doi.org/10.1111/j.1399-3038.2006.00429.x] [PMID: 17014628]
[10] Ewan PW, Coote D. Evaluation of a capsulated hydrophilic carrier polymer (the immunoCAP) for measurement of specific Ig antibodies. Allergy 1990; 45(1): 22-9. [http://dx.doi.org/10.1111/j.1399-9995.1990.tb01080.x] [PMID: 2309986]
[11] Yman L. Standardization of in vitro methods. Allergy 2001: 56(Suppl. 67): 70-74. [http://dx.doi.org/10.1111/j.1399-9995.2001.00921.x] [PMID: 11298014]
[12] Oppenheimer J, Durham S, Nelson H, Wolthers OD. Allergic Diagnostic Testing. WAO, WAO 2014 July; http://www.worldallergy.org/professional/allergic_diseases_center/allergy_diagnostic/ [Accessed November 24 2017].
[13] Lindvik H, Lodrup Carlsen KC, Mowinckel P, Navaratnam J, Borres MP, Carlsen KH. Conjunctival provocation test in diagnosis of peanut allergy in children. Clin Exp Allergy 2017; 47(6): 785-94. [http://dx.doi.org/10.1111/cea.12899] [PMID: 28160326]
[14] R Core Team. R: A Language and Environment for Statistical Computing 2017. https://www.r-project.org/.
[15] Skamstrup Hansen K, Poulsen LK. Component resolved testing for allergic sensitization. Curr Allergy Asthma Rep 2010; 10(5): 340-8. [http://dx.doi.org/10.1007/s11882-010-0133-2] [PMID: 20628838]
[16] Masthoff LN, Blom WM, Rubingh CM, Klemans RJB, Remington BC, Bruijnzeel-Koomen CAFM, et al. Sensitization to Cor a 9 or Cor a 14 has a strong impact on the distribution of thresholds to hazelnut. J Allergy Clin Immunol Pract 2018; 6(6): 2112-2114.e1. [http://dx.doi.org/10.1016/j.jaip.2018.04.040] [PMID: 29782938]