Diagnosis of occupational asthma and rhinitis: usefulness of recombinant allergens (component resolved diagnosis), metabolomics and other new aspects

Monika Raulf
Allergy diagnostic

- Anamnesis
- Skin test
- In vitro test
- Specific provocation test
In vitro-test systems

Specific IgE detection

- Allergen extracts
  - Single allergens
    - recombinant
    - nativ
- Current standard
  - CRD*1

Cellular tests

- PBMC*2
  - CAST
  - FlowCAST
  - LTT
  - histamine release
- Basophils
- from eosinophils
  - ECP
- from mast cells/basophils
  - tryptase

Soluble mediators

- from peripheral blood mononuclear cells

Complementary tests

*1 component-resolved diagnosis
*2 peripheral blood mononuclear cells
Occupational allergen sources

- Flour dust
- Isocyanates
- Wood dust
- Laboratory animals
- Enzymes
- Natural rubber latex
- Mold
- Cow hair
- (Storage-)mites
„Allergen“ - is an ambiguous term

**Allergen source:** raw material from which allergenic extracts are obtained

**Allergen extract:** mixture of allergenic and non-allergenic molecules solubilized from a defined (usually) source

**Allergen molecule:** Proteins or glycoproteins identified with serum IgE of sensitized or allergic patients
What about recombinant allergens?
From the allergen source to the allergen molecule

Allergen source → Allergen extract → Specific allergen component → Cross reacting allergen component

Referring to Huss-Marp/Thermo Fisher Scientific
Purified and recombinant allergens can be used

- alone (Singleplex method)
- in combination with CRD e.g. in the Microarray (Multiplex method)
- spiked in extracts
- combined as extract surrogate (so far not in use)
Methodical reasons for a molecular allergy diagnosis

Allergen source/extract (A, B, C)

Reasons for the use of allergen molecules

Allergen molecules

Effects for the test:

Variations:

Missing or barely available

Defined clinical role (e.g. risk)

IgE cross-reactivity

Genuine (primary) sensitization

1. Limit of quantitation (LoQ)
2. Increasing of test sensitivity
3. Analytical specificity
4. Limit of quantitation (LoQ)

Marker allergen of cross-reactivity

Marker allergen of species-specific sensitization

modifiziert nach Kleine-Tebbe/Jakob, Allergo J Int 2015; 24: 185
Allergens associated with a higher or lower risk

Higher risk

nsLTPs
(Pru p 3, Cor a 8, Ara h 9, Tri a 14)

Storage proteins
(Ara h 1, Ara h 2, Cor a 9, Gly m 5, Gly m 6)

PR-10
(Gly m 4)

Wheat ω5-gliadin
(Tri a 19)

Lower risk

Profilin
(Cor a 2, Pru p 4, Mal d 4, Cuc m 2, Dau c 4)

PR-10
(Ara h 8, Cor a 1, Mal d 1, Cuc m 1, Dau c 1)
What can we learn from the “Latex Story“?
Latex allergy diagnostic “State of the art”

- Allergens are characterized, Hev b 1 - 15
- rHev b 1, 3, 5, 6.01, 6.02, 7, 8, 11 available
- Sensitized patients clearly recognized major allergens (HCW versus SB)
- Spiking of the latex extract with the stable rHev b 5 improved the in-vitro diagnostic
- Latex SPTs are no longer available but
- Component-resolved in-vitro diagnostic is possible (diagnostic workflow)
Marker allergens for latex allergy

If positive, clinical relevant latex allergy; latex allergen prevention necessary

“True” latex allergens Hev b 1, 3, 5, 6

In asymptomatic patients, if Hev b 8 + and ‘true’ latex allergens −, avoidance of latex contact is not necessary; no clinical relevant latex allergy

Latex profilin Hev b 8, CCD
Cross-reactive Carbohydrate Determinants (CCD)

- Most allergens, particularly of plant origin, reveal a glycan-associated IgE reactivity.
- Glycan epitopes may share significant structural elements with allergens of other, non-related protein families. This feature predestinates them to be an important cause of a large variety of cross-reactions.

**Diagram: Cross-reactive Carbohydrate Determinants (CCD)**

- **MMXF³**
  - Monosaccharides: Fucose, Xylose, Mannose
  - sugars: N-acetyl-glucosamine, Sialic acid

- **MUFX³**
  - Monosaccharides: Fucose, Xylose, Mannose
  - sugars: N-acetyl-glucosamine, Sialic acid

Monika Raulf - 6th Jack Pepys Workshop Toronto
Diagnostic workflow

Natural rubber latex (NRL) extract (SPT or k82)

- IgE-mediated NRL-sensitization approved
  - Application of CRD (Hev b 5, Hev b 6.01, Hev b 1, Hev b 3)
    - slgE to Hev b 5, Hev b 6.01 or Hev b 1, Hev b 3, respectively (slgE to major NRL-allergens)
      - Clinical-relevant NRL-sensitization most likely
        - Patient care: Avoidance of latex products is necessary; Allergy passport including information about latex and cross-reactivity to fruits
    - Application of CCD (e.g. HRP) and Hev b 8
      - Testing with minor relevant allergens (e.g. Hev b 11)
        - Clinical relevance of NRL-allergy unlikely; cross-reactivity with other plants possible
          - Patient care: Information about possible cross-reactivity to fruits; avoidance of latex products is not necessary
      - CCD-slgE >> NRL-slgE or CCD inhibited NRL-slgE or Hev b 8
        - Clinical relevance of NRL-allergy unlikely; IgE-reactivity based on cross-reactive carbohydrate (CCD) determinants or Hev b 8
          - Patient care: Avoidance of latex products is not necessary
  - NRL-sensitization unlikely
Latex-sIgE vs. provocation

Latex k82

Latex k82

Sum Hev b 5 and Hev b 6.01

<table>
<thead>
<tr>
<th></th>
<th>slgE-Cut-Off [kU/L]</th>
<th>Sensitivity [%]</th>
<th>Specificity [%]</th>
<th>PPV</th>
<th>NPV</th>
<th>Youden-Index</th>
<th>Likelihood ratio</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latex k82</td>
<td>≥ 1.12</td>
<td>85 (76-92)</td>
<td>76 (55-91)</td>
<td>92 (84-97)</td>
<td>61 (42-78)</td>
<td>0.61 (0.42-0.81)</td>
<td>3.6</td>
<td>0.84 (0.75-0.94)</td>
</tr>
<tr>
<td>Sum Hev b 5 and Hev b 6.01</td>
<td>≥ 1.497</td>
<td>79 (69-87)</td>
<td>88 (69-97)</td>
<td>96 (88-99)</td>
<td>56 (40-72)</td>
<td>0.67 (0.5-0.84)</td>
<td>6.61</td>
<td>0.86 (0.77-0.94)</td>
</tr>
</tbody>
</table>

Vandenplast, …, Raulf et al. Allergy 2016
Baker's asthma

- One of the oldest recognized occupational diseases (First described by Bernardino Ramazzini (1633-1714) in “De Morbis artificum diatriba”)
- One of the most common forms of occupational asthma

Examples:

in France: Incidence of baker's asthma among young bakers ranges from 0.3 to 2.4 cases per 1000 person-years [Remen et al. 2010]

in Norway: Incidence of occupational asthma among male bakers 2.4 and female 1 case per 1000 person-years [Leira et al. 2005]

in Germany: Incidence of occupational asthma among bakers ~2 cases per 1000 person-years [BGN, personal communication]

~ 10 % of all bakers develop asthma during their working life period
Potential allergens in bakeries

- Wheat flour
- Rye flour
- Further cereal flours (e.g. barley)
- Enzymes (α-amylase, cellulase etc.)
- Soy, Lupine flour
- Storage mites
- Flour pests (including flour worm, flour moth)
- Moulds
- Egg yolk and white, sesame seed, nuts, poppy etc.
The most relevant allergenic wheat fractions for baker’s asthma are the water-/salt-soluble albumins and globulins.

Which allergens are important?
Molecular allergy diagnosis for baker’s asthma

Proteomic approach

- 2D-electrophoresis and
- 2D-immunoblotting

e.g. 1995 Posch et al.
1997 Weiss et al.
2001 Sander et al.

High number of proteins/peptides with IgE-binding capacity were identified

High interindividual variation of 2D IgE-binding profiles in patients with baker’s asthma
Identified wheat flour allergens

- **α-amylase inhibitors MW 14-14 KDa**
  - Tri a 15   Monomer (Wheat) alpha-amylase inhibitor 0.28 (WMAI-0.28)
  - Tri a 28   Dimeric alpha-amylase inhibitor (WDAI-0.19)
  - Tri a 29.01 Tetrametric alpha-amylase inhibitor (WTAI-CM1)
  - Tri a 29.02 Tetrametric alpha-amylase inhibitor (WTAI-CM2)
  - Tri a 30   Tetrametric alpha-amylase inhibitor (WTAI-CM3)

- **Thiol reductase homologue 27 KDa**
  - Tri a 27

- **Serine protease inhibitors**
  - Tri a 39 SPILA – Serine protease inhibitor-like protein 9.9 KDa
  - Tri a 33 – Serpin 40-43 KDa
### Identified wheat flour allergens II

<table>
<thead>
<tr>
<th>Tri a</th>
<th>Name</th>
<th>Molecular Weight</th>
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<tbody>
<tr>
<td>12</td>
<td>Profilin</td>
<td>14 KDa</td>
</tr>
<tr>
<td>14*</td>
<td>Wheat nonspecific lipid transfer protein 1</td>
<td>9 KDa</td>
</tr>
<tr>
<td>18</td>
<td>Agglutinin isolectin 1</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Omega-5 gliadin, seed storage</td>
<td>65 KDa</td>
</tr>
<tr>
<td>21</td>
<td>Alpha/beta gliadin</td>
<td>32.7 KDa</td>
</tr>
<tr>
<td>25</td>
<td>Thioredoxin H</td>
<td>13.4 KDa</td>
</tr>
<tr>
<td>26</td>
<td>High molecular weight glutenin</td>
<td>88 KDa</td>
</tr>
<tr>
<td>31</td>
<td>Triosephosphate-isomerase (TPIS)</td>
<td>27 KDa</td>
</tr>
<tr>
<td>32</td>
<td>1-cys-peroxiredoxin</td>
<td>23.9 KDa</td>
</tr>
<tr>
<td>34</td>
<td>Glyceraldehyde-3 phosphate-dehydrogenase</td>
<td>36.5 KDa</td>
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<tr>
<td>35</td>
<td>Dehydrin</td>
<td>12.4 KDa</td>
</tr>
<tr>
<td>36</td>
<td>Low molecular weight glutenin GluB 3-23</td>
<td>40 KDa</td>
</tr>
<tr>
<td>37</td>
<td>Alpha purothionin</td>
<td>12 KDa</td>
</tr>
</tbody>
</table>

*isoforms: Tris a 14.01.101 (nsLTP 9.1); Tri a 14.0201 (nsLTP 9.7)
Limitation of the studies to evaluate the importance of single allergens in baker’s asthma

- Different patients (from different countries, different exposure)
- Different methods (SPT, ELISA, Dot-blot, (1D/2D-)Western-blot, microarray, etc.)
- Tested with single or only few purified proteins in natural or recombinant forms

Using a (complete) panel of identified wheat flour allergens in recombinant form for determination of the IgE-binding profile
Characteristics of the study group

101 Bakers  (40 German, 37 Dutch, 24 Spanish)

f4: 7.47 kU/L; (0.55 - 83.3 kU/L)
gx1: 1.79 kU/L; (0.01 - >100 kU/L)

29 Controls  (10 German, 10 Dutch, 9 Spanish; 59% asthma, 72% rhinitis, grass pollen positive and wheat positive)

f4: 1.14 kU/L; (0.36 – 9.1 kU/L)
gx1: 81.3 kU/L; (3.63 - 705 kU/L)

19 Recombinant wheat flour allergens and 2 CCDs

- Tri a 15 (WMAI-0.28)
- Tri a 28 (WDAI-0.19)
- Tri a 29.01 (WTAI-CM1)
- Tri a 29.02 (WTAI-CM2)
- Tri a 30 (WTAI-CM3)
- Tri a 12.0102 (Profilin)
- Tri a 14.02 (nsLTP)
- Tri a 34 (GAPDH)
- Tri a 33 (Serpin)
- Tri a 31 (TPIS)
- Tri a 21 (αβ-Gliadin)
- Tri a 25 (Thioredoxin H)
- Tri a 32 (1-cys-Peroxiredoxin)
- Tri a 27 (Thiol reductase)
- Peroxidase 1
- Tri a 35 (Dehydrin)
- Tri a 39 (SPILA)

- HRP- Horse Radish Peroxidase (MMXF)
- MUXF (Glucan Bromelain)
- Tri a 19 (ω-5-Gliadin)*
- Tri a 14.01 (nsLTP)* (*ThermoFisher)

Binding to Streptavidin ImmunoCAPs

MBP or TRX-His-S were also tested

according to
Sensitization profile of 101 symptomatic bakers and 29 controls

- Tri a 27 (Thiolreductase) 27%
- Tri a 28 (WDAI-0.19) 0%
- Tri a 29.01 (WTAI-CM1) 7%
- Tri a 30 (WTAM) 0%
- Tri a 29 (SPILA) 0%
- Tri a 31 (TPIS) 0%
- Tri a 25 (Thioredoxin H) 0%
- Tri a 14.02 (WTAB-CM2) 0%
- Tri a 32 (1-cys-Per) 0%
- Tri a 32.01 (WTAI-CM1) 0%
- Tri a 30.01 (WTAI-CM2) 0%
- Tri a 29.02 (WTAI-CM3) 0%
- Tri a 14.01 (nsLTP9.1) 0%
- Tri a 15 (WMAI-0.28) 0%
- Tri a 31 (TPIS) 0%
- Tri a 33 (Serp) 0%
- Tri a 31 (TPIS) 0%
- Tri a 12.0102 (Profilin) 0%
- Tri a 34 (GAPDH) 0%
- Tri a 14.02 (nsLTP) 0%
- Peroxidase 1 0%
- Tri a 35 (Dehydrin) 0%
- Tri a 35 (Dehydrin) 0%
- Tri a 19 (ω5-Gliadin) 0%
- HRP 0%
- MUXF 0%

No major allergen detectable
For bakers, in most cases the wheat flour CAP values were higher than the sum of IgE to single allergens, whereas for control subjects the sum was higher.
Baker’s asthma and CRD

- All soluble fractions contain allergens; the most relevant for baker's asthma are the **albumins** and **globulins**.
- In 2D-immunoblots of the salt-/water-soluble fraction more than **100 different allergen spots** were detected.
- The allergen spectrum differs individually.
- So far, no **common major wheat allergen** could be identified, but **Tri a 27** and **Tri a 28** are most frequent.
- **Tri a 19** (**ω-5-Gliadin**; marker allergen for WDEIA) is not a relevant allergen for baker’s asthma.
- So far, for routine diagnosis allergen **specific IgE tests with whole wheat flour extracts** remain mandatory because of superior diagnostic sensitivity.
- **Component-resolved diagnosis** might improve the diagnosis of baker’s asthma and help to **differ between grass pollen, respiratory wheat flour and wheat-induced food allergy** (differentiation of occupational sensitization and sensitization caused by cross-reactivity is possible).
Laboratory animal allergens

As common for the most mammalian inhalant allergens, the major allergens from mouse, rat, guinea pig, hamster and rabbit are lipocalins.
Laboratory animal allergy (LAA)

• Allergen-specific IgE to the suspected animal allergen extracts is the common and recommended step. Specific IgE determination in the case of LAA based on extracts prepared from epithelia, serum-/urine protein as mixture or alone.

• Dual sensitization to rat and mouse urinary allergens reflects cross-reactive molecules rather than atopy and therefore the determination of the primary sensitizer is difficult.

• There is not enough evidence to advice the use of single molecules for in-vitro diagnosis. Single animal allergens, relevant for LAA, are not commercially available so far for routine testing.

• Impact of individual molecules on severity of symptoms is still unknown.
Molecular approach for Hypersensitivity pneumonitis (HP)

Example:

Farmer’s lung disease
Proteomic – 2D-Electrophoresis – detection of immune reactive *S. rectivirgula* proteins

Barrera et al, 2014; Proteomics Clin
• Identification of **25 farmer’s lung-specific proteins** via MS (e.g. proteases SR2, SR4, SR14, SR21; glycosidase SR9)

• Production of **17 recombinant proteins with immune reactivity**

• **ELISA-testing**  
  *with sera from 41 farmer’s lung-patients*  
  and from  
  *43 healthy, exposed controls*  
  (from France and Switzerland)

• Evaluation if the proteins can contribute to a **differentiation between diseases and exposed patients**
ELISA-testing with recombinant proteins

Combination of SR1FA + SR17 + SR22 in ELISA
83% sensitivity and 77% specificity
Perspectives

• Immune precipitation with S. rectivirgula-extract 40% sensitivity
• ELISA with S. rectivirgula-extract 70% sensitivity
• ELISA with SR1FA, SR17, SR22 83% sensitivity + 77% specificity

➤ but not all responded to actinomycetes
➤ in the future: production of a panel of recombinant proteins of the four species S. rectivirgula, W. sebi, Lichtheimia corymbifera, Eurotium amstelodami (A. vitis) for the diagnosis of farmer’s lung disease
Summary

• Wide range of occupational allergens, often individual case reports ⇒ only few allergen sources are characterized on the molecular level; but an essential prerequisite for the production of suitable allergen extracts is knowledge about relevant allergens.

• Molecular allergy diagnostic only useful for latex. For baker’s asthma and for LAA no relevant single allergens are commercially available so far.

• Component-resolved diagnosis might help to differ between occupational sensitization and sensitization caused by cross-reactivity between environmental allergens.

• CCD-reagents can be used as in vitro screening-tools to discriminate between ‘true’ allergy and clinical not relevant cross-reactivity.

• Complementary tests (e.g. BAT (FlowCAST, CAST), inhibition test, serum or urine biomarkers etc.) may be helpful, but they need further validation.

• There is a broad range of unmet needs in the case of OA to improve diagnosis and therapy.
Thank you for your attention!

Bochum, Germany