Application of molecular diagnosis in precision medicine: biotinylation of recombinant allergens and their coupling to Streptavidin ImmunoCAP – a method for measuring specific IgE antibodies

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- **Competitive research**
- **Advanced technologies based on recombinant allergens**
- **Multidisciplinary team**

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Background

Detection of allergen-specific IgE antibodies in sera from allergic patients plays a key role in the diagnosis of IgE-mediated allergy. Currently, there are numerous extracts and allergen components from various allergenic sources available for diagnosis. If no validated test is available, it is possible to develop new allergen tests, usually by coupling or binding the allergens to a solid phase. For example, Streptavidin-conjugated ImmunoCAP allows the coupling of self-prepared biotinylated recombinant allergens for measuring specific IgE antibodies (sIgE).

Objectivs

This study aimed to assess the diagnostic accuracy of biotinylated recombinant allergen coupled to Streptavidin ImmunoCAP in comparison with commercially available ImmunoCAP with natural allergen.

Methods

Ten patients allergic to *Artemisia vulgaris* (mugwort), with positive response to natural major allergen nArt v 1 in the ImmunoCAP system, with different sIgE levels, were selected for this study. Streptavidin ImmunoCAPs were loaded and incubated on a Phadia 100 system, using 5µg/CAP of the biotinylated rArt v 1. The ImmunoCAPs were transferred to the Phadia 250 system, where measurements of allergen-specific IgE antibody binding were performed according to the manufacturer's instructions. Specific IgE levels were then measured on the Phadia 100 and Phadia 250 Immunoassay Analyzers (Figure 2).

rArt v 1 was bound to Streptavidin ImmunoCAPs by first incubating the protein overnight in buffer (1M NaCl, 0.1M $NaHCO_3$, pH 4.5). Afterwards the protein was incubated in 5x molar excess of Biotin for 3h RT. The biotinylated protein was dialyzed in PBS. The protein concentration after dialysis was measured using BCA Protein Kit and biotinylation was verified by ELISA using streptavidin labeled with HRP and ABTS for detection (Figure 1).

The ImmunoCAPs were then prewashed and 50 µL of biotinylated rArt v 1 (conc. 100µg/mL) was added to each CAP and incubated for 30 minutes.

Results

The sIgE levels obtained to biotinylated rArt v1 were similar to sIgE levels to nArt v 1. Spearman correlation indicates a strong positive correlation between nArt v 1 and rArt v 1, (ρ=0.988, p<0.001, n=10).

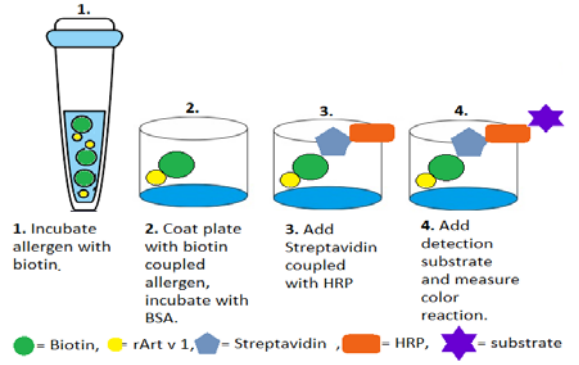
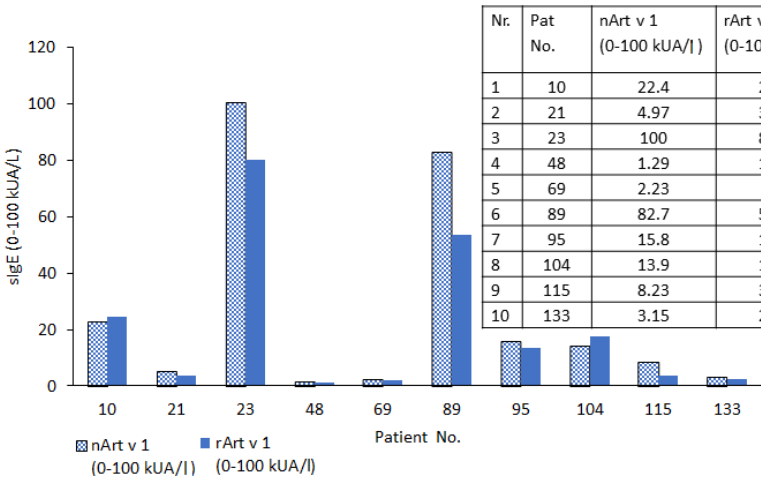


Figure 1: Biotinylation of recombinant allergen



Nr.	Pat No.	nArt v 1 (0-100 kUA/l)	rArt v 1 (0-100 kUA/l)
1	10	22.4	24.3
2	21	4.97	3.71
3	23	100	80.2
4	48	1.29	1.31
5	69	2.23	2.1
6	89	82.7	53.4
7	95	15.8	13.3
8	104	13.9	17.6
9	115	8.23	3.73
10	133	3.15	2.31

Figure 2: sIgE recognition of Art v 1 for 10 patients with initial response in ELISA

Conclusions

Streptavidin ImmunoCAP is a valuable research tool for specific and sensitive measurement of IgE binding using various recombinant allergens and increasing the accuracy of molecular allergy diagnosis

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