

Introduction

Allergy and autoimmunity are rapidly increasing in all societies world-wide (1, 2). The identification of clinically relevant specific antibodies in patient serum is crucial for current diagnostics of allergy and autoimmunity. *ImmunoCap 250* by Thermo Fisher is a singleplex assay system currently used in clinical labs, which deliver around 400-2000 results per week (3, 4). In this study we have investigated antigen component diagnostics for patients with allergy to egg and peanut and patients with autoimmune coeliac disease, which is characterised by sensitivity to gluten from dietary wheat, barley and rye. Uncontrolled coeliac disease can lead to developmental retardation in children and malnutrition in adults. Unmanaged allergy may lead to fatal anaphylaxis. To address these global health problems biomedical teams are constantly exploring ways to develop current tests. In collaboration with biomedical scientists from NGH, our students investigated if allergen component diagnostics could add value to

Aims

This study was aimed to explore ImmunoCap component diagnostics in allergy and autoimmunity by exploring if EliA IgA Gliadin could complement significantly to current diagnostic tests for coeliac disease as well as to determine if egg and peanut allergen component detection could be more advanced than whole allergen testing.

Methods

Blood samples (31 for allergy and 37 for coeliac disease) were collected into gel vacutainers, which were then centrifuged at 3000rpm for 10 minutes

to separate serum. These samples were then stored at -20°C until needed for testing using the *ImmunoCAP 250*. The samples were used in line with the hospital laboratory protocols, rules and ethical procedures of both DMU and NGH. Samples were incubated with peanut (Ara h 1, 2 and 8) and egg white (Gal d 1, 2 and 3) allergens as well as human recombinant tissue transglutaminase (tT) and synthetic deaminated gliadin peptides to identify specific antibody-

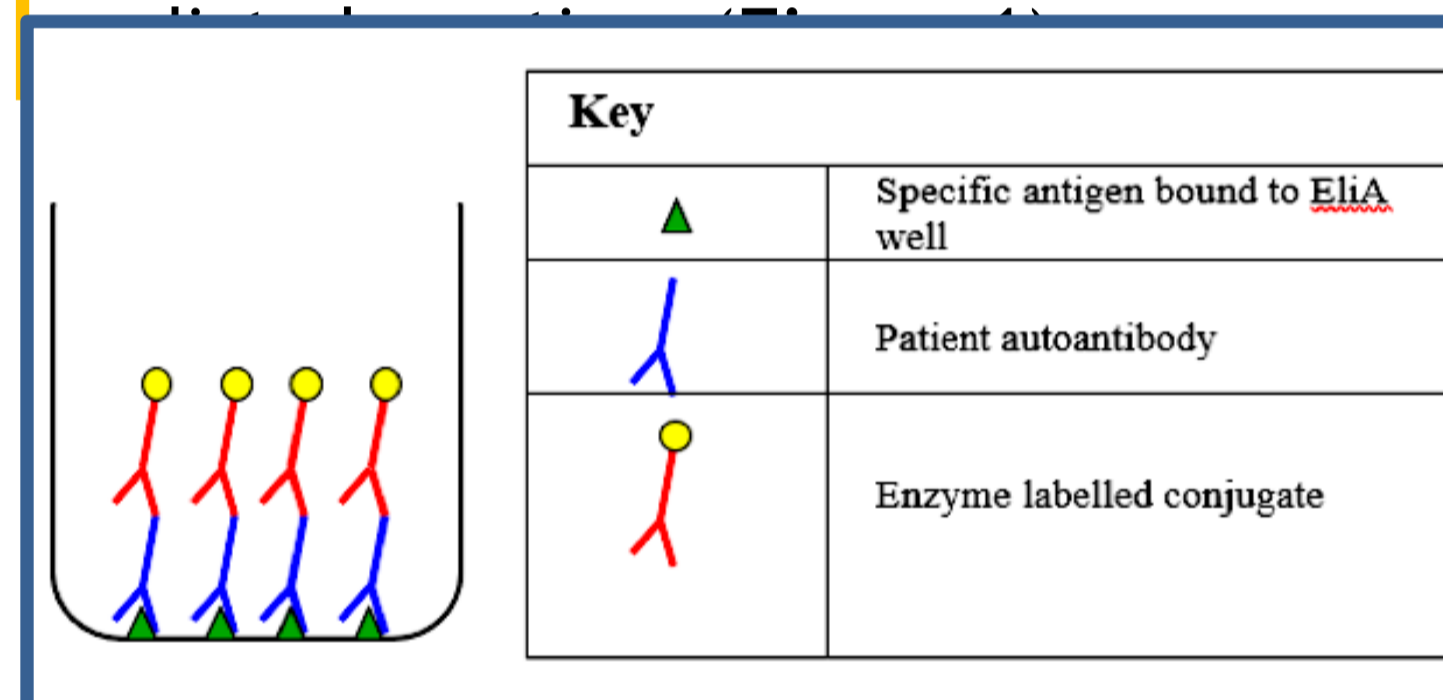


Figure 1. Specific antibodies (IgA for coeliac disease and IgE for allergy) can be detected using *ImmunoCap 250*.

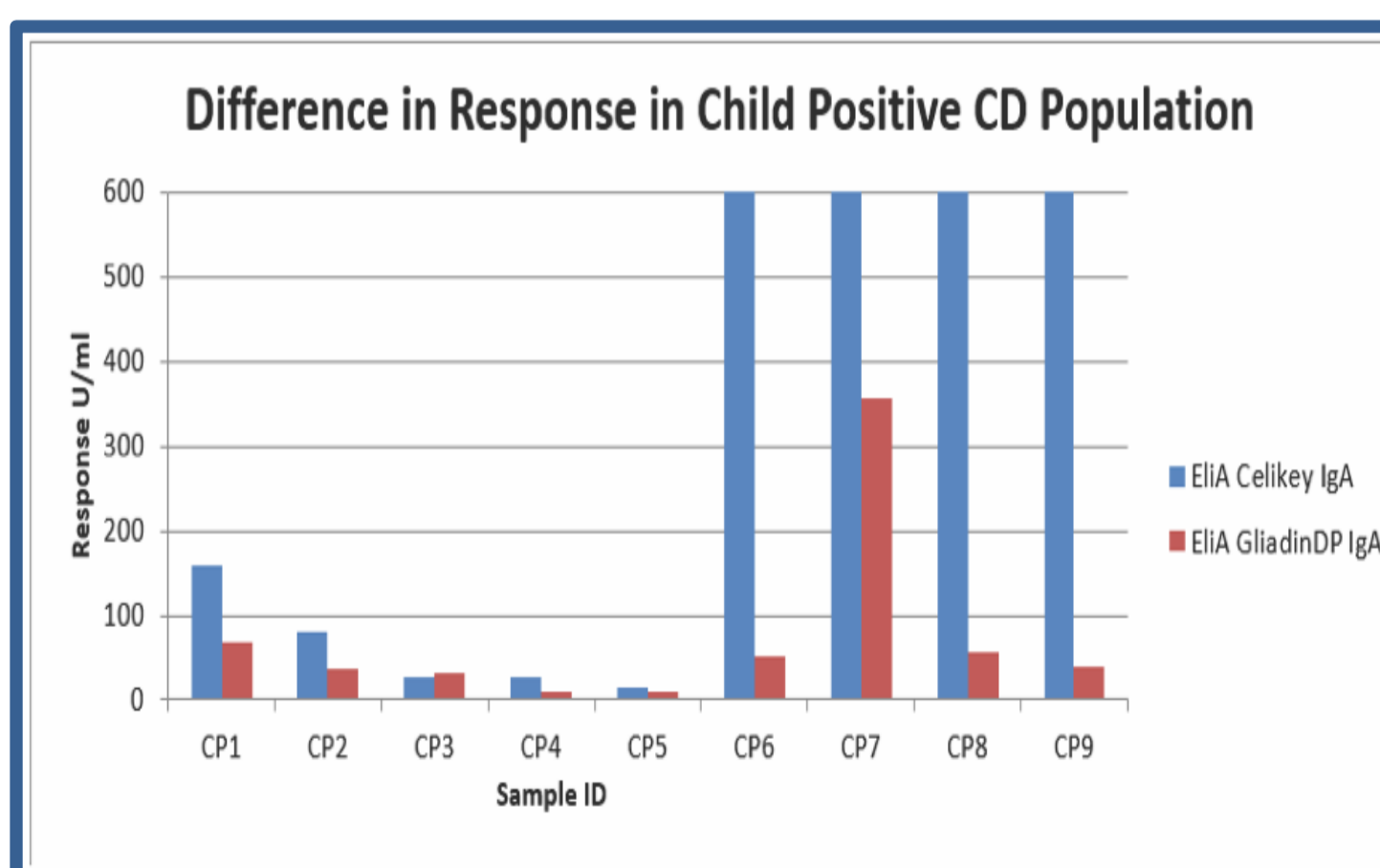


Figure 2. Example of EliA IgA Celikey and EliA IgA Gliadin results in paediatric patients with coeliac disease, showing a greater sensitivity with the EliA IgA Celikey assay.

Results

This study compared the response values given from EliA IgA Celikey and EliA IgA Gliadin^{DP} ImmunoCAP 250 assays in 37 serum samples, including both adult (>16 years) and paediatric populations (≤16 years). The results showed that sensitivity was slightly higher in EliA IgA Celikey (Figure 2), and that there was a positive correlation in the response values between the two tests.

The results of the EliA Gliadin^{DP} assay showed that there is greater sensitivity with the adult coeliac disease positive cohort as compared to the child coeliac disease positive cohort. EliA IgA Gliadin^{DP} assay gave diagnostic sensitivity of 88.23% and all samples were correctly identified as positive with the EliA IgA Celikey assay (diagnostic sensitivity of 100%).

In peanut allergy testing 36% samples had a strong positive reaction with Ara h 1, 2 or both, 7% samples had a positive reaction for all allergen components.

In egg white allergy testing 62.5% samples had a positive reaction with all three allergens, 12.5% samples had a positive reaction to Gal d 1 and Gal d 2, 12.5% samples had a positive reaction to Gal d 2 and Gal d 3, 12.5% had a positive reaction to Gal d 2 alone. Interestingly, all 100% of patients produced a positive reaction with allergen component Gal d

References

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Discussion

One of the hallmark characteristics of allergy and autoimmunity is the presence of specific antibodies of high affinity in patient serum, which can be used in the diagnostics.

The first-line serological test for coeliac disease is an anti-tTG ELISA cap (EliA IgA Celikey). Positive tTG tests are confirmed with a positive EMA (endomysial antibodies) result and a diagnosis of coeliac disease is only made after intestinal biopsy has shown the presence of villous atrophy. Deamidated gliadin assay (EliA IgA Gliadin^{DP}) has been suggested to be a more reliable method of detecting the disease, with increased specificity and a high sensitivity. Our study indicates that it would possibly be of greater benefit to run the EliA IgG Gliadin^{DP} test alongside the EliA IgA Celikey assay, as this should increase sensitivity and potentially remove the need for total IgA testing. Endomysial antibodies are still required as a confirmatory test in those patients with positive EliA IgA Gliadin test. For allergy samples, we found that component diagnosis may complement current diagnostic tests rather than replace them.