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Performance of basophil activation test and specific IgG4 as diagnostic tools in nonspecific lipid transfer protein allergy: Antwerp-Barcelona comparison

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Abstract

Background: Recent studies show that nsLTP sensitization is not limited to the Mediterranean basin and can present diverse clinical phenotypes. It remains challenging to predict clinical outcome when specific IgE antibodies (slgE) to nsLTPs are present. This study compares both clinical and in vitro allergy characteristics but also diagnostic performance of a basophil activation test (BAT) and slgG4 in nsLTP-sensitized patients from Antwerp (ANT, Belgium) and Barcelona (BCN, Spain).

Methods: Adult subjects with positive slgE rPru p 3 and/or rMal d 3 ≥ 0.10 kU_A/L (n = 182) and healthy controls (n = 37) were included. NsLTP-sensitized individuals were stratified according to clinical symptoms with peach/apple, respectively. BAT rPru p 3 and rMal d 3 were performed and slgG4 antibodies to both components quantified.

Results: In BCN, only ratios of slgG4/slge rMal d 3 and BAT rMal d 3 (0.001 µg/mL) can identify clinically relevant Mal d 3 sensitization (sensitivity of 60%-63% and a specificity of 75%-67%, respectively). In ANT, only the slgE/total IgE rPru p 3 ratio shows added value (sensitivity 60% and specificity 83%). Finally, it appears that symptomatic patients in BCN are more sensitive to lower allergen concentrations compared to ANT. In addition, it was shown that ANT patients were more often sensitized to pollen and that specific pollen sources differed between regions.

Conclusions: NsLTP-related allergy profiles and diagnostic performance differ significantly between regions and are component-specific, which makes extrapolation of data difficult to do. In addition, it seems that basophil sensitivity might show geographical differences. Additional research is needed to confirm these findings.

Abbreviations: AD, atopic dermatitis; ANA, anaphylaxis; ANT, Antwerp; Ara h, *Arachis hypogaeae* (peanut); Art v, *Artemisia vulgaris* (mugwort); AUC, area under the curve; BAT, basophil activation test; BCN, Barcelona; Bet v, *Betula verrucosa* (birch); CO, cut-off; Cor a, *Corylus avellana* (hazelnut); EC50, half maximal effective concentration (50% response); GU/AE, generalized urticaria/angioedema; HC, healthy controls; kU/L, kilounits per litre; Mal d, *Malus domestica* (apple); n, native; nsLTP, nonspecific lipid transfer protein; OAS, oral allergy syndrome; Phl p, *Phleum pratense* (timothy grass); Pla a, *Platanus acerifolia* (plane tree); PR-10, pathogen-related protein family 10; Pru p, *Prunus persica* (peach); r, recombinant; ROC, receiver operator curve; slgE, specific immunoglobulin E antibody; slgG4, specific immunoglobulin G4 antibody; SR, systemic reaction; TFS, Thermo Fisher Scientific; TLP, thaumatin-like protein; TOL, tolerant; Tri a, *Triticum aestivum* (wheat); WAO, World Allergy Organization.

Ine I. Decuyper and Mariona Pascal equally contributed to this manuscript.

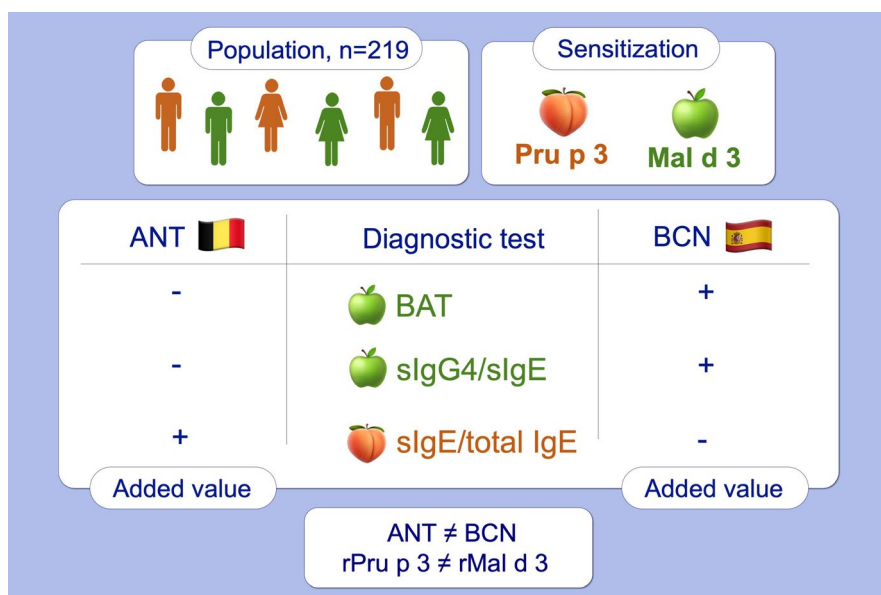
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KEYWORDS

BAT, Mal d 3, nsLTP allergy, Pru p 3, sIgG4



GRAPHICAL ABSTRACT

This study explores the performance of BAT, sIgE/total IgE, sIgG4 and sIgG4/sIgE ratio in Pru p 3- and Mal d 3-sensitized populations from Antwerp (ANT) and Barcelona (BCN). In BCN, only ratios of sIgG4/sIgE rMal d 3 and BAT rMal d 3 can identify clinically relevant Mal d 3 sensitization, whereas in ANT, only the sIgE/total IgE rPru p 3 ratio shows added value. nsLTP sensitization and diagnostic performance cannot be extrapolated from one population to another. BAT, basophil activation test; nsLTP, nonspecific lipid transfer protein.

1 | INTRODUCTION

Patients with nsLTP sensitization often report multiple plant food and pollen allergies, also referred to as “the nsLTP syndrome,” because of the high cross-reactivity between the nsLTPs in different plant sources.¹ nsLTPs are highly ubiquitous in the plant kingdom and have been identified as pan-allergens in different plant foods such as fruits, vegetables, nuts, cereals,² in various pollen species,³ latex⁴ and even in *Cannabis sativa* as recently documented in this journal.⁵ Nowadays, evidence shows that nsLTP sensitization is not limited to the Mediterranean area as initially postulated^{6–8} but also occurs in Northern and Central European regions, Asia and North America.^{4,9–13}

Several studies have looked into nsLTP sensitization and the molecular profile of food allergic patients from different geographic areas. However, to our best knowledge, a comparison of clinical and molecular characteristics of nsLTP-sensitized patients from different geographic areas has not been reported yet.

The available literature on nsLTP-related allergy has demonstrated that the nsLTP syndrome can clinically manifest very heterogeneously and be influenced by cofactors, ranging from asymptomatic sensitization (33%–62% depending on the study^{12,14–16}) to local oropharyngeal complaints and systemic reactions (SR) which

might evolve to life-threatening anaphylaxis.^{17–19} The exact explanation for these distinct clinical phenotypes remains unclear but could relate to differences in sensitization routes, the causative plant source (ripeness, cultivation conditions), inhibitory mechanisms, specific (s)IgG4 response and/or the affinity of sIgE antibodies as well as the presence of co-sensitization to other (plant) allergens.²⁰ In Southern Europe, nsLTP sensitization is believed to be predominantly governed by peach and its main allergen Pru p 3. It has been hypothesized that in this region, co-sensitization to pollen can modulate allergy severity.^{18,21–23} However, whether this also applies to other geographical regions remains elusive.

Although skin prick tests (SPT) and sIgE detection are reliable to detect nsLTP sensitization, in clinical practice, the management and guidance of nsLTP-sensitized patients requires differentiation between clinically irrelevant sensitizations and sensitization associated with (severe systemic) symptoms. In food allergy, it has been demonstrated that both the basophil activation test (BAT) and quantification of sIgG4 antibodies can be helpful in differentiating clinically relevant from irrelevant sIgE results.^{24–31} Preliminary results of our earlier study showed that the BAT with Pru p 3 has good potential in predicting clinical reactivity to peach in an Antwerp population.¹² The diagnostic usefulness of sIgG4 to Pru p 3/Mal d 3 has not been explored yet.

This study aims to explore the differences of several clinical and molecular characteristics in patients sensitized to Pru p 3 and/or Mal d 3 from two different geographical regions: Antwerp (a typical north-western European area in which birch allergy predominates) and Barcelona (a typical Mediterranean area in which nsLTP allergy predominates). In the light of promising preliminary data of BAT Pru p 3 in a north-western European region, this study looked into the diagnostic performance and reliability of BAT, slgG4, slgG4/slgE ratio as well as slgE/total IgE ratio in order to predict clinical outcome to peach and apple in these nsLTP-sensitized populations.

2 | METHODS

2.1 | Patient inclusion

Patients sensitized to rPru p 3 and/or rMal d 3 (serum slgE ≥ 0.10 kU_A/L by FEIA-ImmunoCAP, Thermo Fisher Scientific (TFS), Uppsala, Sweden) were included via outpatient clinics of Allergology of the Antwerp University Hospital (Belgium) and the Allergy Section of the Pneumology Department of the Hospital Clínic de Barcelona (Spain) between September 2015 and December 2017. A total of 219 individuals were included: 182 sensitized to rPru p 3 and/or rMal d 3 and 37 healthy controls (16 (43%) from Barcelona (BCN) and 21 (57%) from Antwerp (ANT), matched for age and sex). Of the nsLTP-sensitized individuals, 73/182 (40%) originated from BCN and 109/182 (60%) from ANT. As shown in Figure 1, patients were stratified according to their clinical response to peach and/or apple ingestion: tolerance (TOL), oral allergy syndrome (OAS), generalized urticaria and/or angioedema (GU/AE) and anaphylaxis (ANA) as defined by the WAO anaphylaxis guidelines.³² Exclusion criteria were if patients had no recollection of peach/apple ingestion, or if these foods had been

avoided for more than a year. Other exclusion criteria were chronic spontaneous urticaria, eosinophilic esophagitis and systemic mastocytosis. Demographic data and allergy history were obtained by trained physicians in both medical centres. The local ethics committees of the University Hospital of Antwerp (B300201524055) and Hospital Clínic de Barcelona (HCB/2016/0361) approved this study.

2.2 | Total and slgE

Serum total IgE, slgE to recombinant (r)Pru p 3 from peach (*Prunus persica*) and rMal d 3 from apple (*Malus domestica*) were quantified by FEIA-ImmunoCAP (TFS, Uppsala Sweden).

Sensitization to other relevant allergens was explored by ImmunoCAP and/or ImmunoCAP ISAC platforms (TFS) according to the manufacturer's instructions: PR-10 (ie quantification of slgE to rBet v 1 from birch (*Betula verrucosa*), rPru p 1 from peach (*Prunus persica*) or rMal d 1 from apple (*Malus domestica*)); profilin (ie rBet v 2 from birch (*Betula verrucosa*), rPhl p 12 from Timothy grass (*Phleum pratense*) or rPru p 4 from peach); pollens from plane tree (ie whole extract and/or rPla a 1, native (n)Pla a 2 (*Platanus acerifolia*); Timothy grass (ie rPhl p 1 and rPhl p 5b); Mugwort (extract and/or nArt v 1 (*Artemisa vulgaris*)) and other nsLTPs (ie nArt v 3 from mugwort, rTri a 14 from wheat (*Triticum aestivum*), rCor a 8 from hazelnut (*Corylus avellana*) and rAra h 9 from peanut (*Arachis hypogaea*)). ImmunoCAP results ≥ 0.10 kU_A/L and ImmunoCAP ISAC microarray results ≥ 0.3 ISU were considered positive as advised by the manufacturer's instructions.

2.3 | slgG4 and slgG4/slgE ratios

rPru p 3 and rMal d 3 slgG4 were measured by FEIA-ImmunoCAP (TFS). Specific IgG4/IgE ratios were determined after conversion of milligrams per millilitre to kilounits per litre (factor 2.4×10^{-3}).

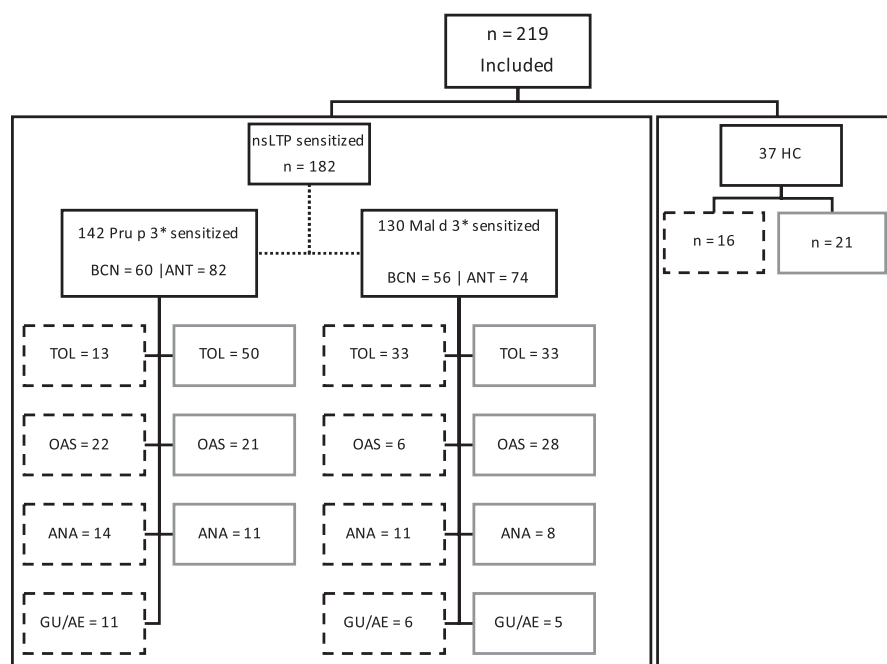


FIGURE 1 Dashed = Barcelona (BCN) patients, grey = Antwerp (ANT) patients. HC = healthy controls, TOL = tolerant, OAS = oral allergy syndrome, ANA = anaphylaxis as defined by WAO²⁹ GU/AE = generalized urticaria and/or angioedema. * Mal d 3 and Pru p 3 sensitizations were based on slgE rPru p 3 and rMal d 3 > 0.10 kU_A/L (ImmunoCAP). Patients with a positive slgE result for both Mal d 3 and Pru p 3 were considered in both analyses

2.4 | Basophil activation tests

Basophil activation tests (BATs) were performed and analysed in both centres strictly employing the same protocol as previously detailed.²⁹ Briefly, prewarmed fresh heparinized blood samples were stimulated with four concentrations (0.001–0.01–0.1–1 µg/mL) of rPru p 3 or rMal d 3 based on the results of a previous pilot study.¹² rPru p 3 and rMal d 3 have been obtained, cloned and characterized as described elsewhere.³³ Anti-human IgE served as a positive control (10 µg/mL, BD Biosciences), and stimulation buffer was used to measure spontaneous CD63 expression in quiescent cells. Analysis of basophil activation was performed using side scatter, anti-IgE and CD203c expression to characterize the basophil population. Within this gate (with at least 500 basophils to consider the test valid), the percentage of activated basophils, that is those expressing CD63, was measured. Results were expressed as net percentages of CD63⁺ basophils, calculated by subtraction of the spontaneous expression from the allergen-induced CD63 expression as recommended elsewhere.³⁴ The technical limit of detection for this technique at 500 gated basophils (the minimum basophil count adhered to in these BATs) was set at 5% CD63⁺ basophils.

All HC individuals (n = 37) were selected as anti-IgE responders in the BAT (≥15% CD63 basophils after stimulation with anti-IgE). In the nsLTP-sensitized patients, 17% (31/182) were found to be non-responsive to the positive control. However, nonresponders were not excluded as 32% (10/31) of them showed a detectable basophil response on allergen-specific stimulation with rPru p 3/rMal d 3.

2.5 | Statistical analysis

IBM SPSS version 24.0 (IBM) software and JMP 13.0 (SAS Institute Inc) were used for data analysis. Dose-finding experiments for BAT were performed using a dose-response curve, and test performance was explored by ROC analyses comparing areas under the curve (AUCs). The area under the curve is a measure for test accuracy with a value of 1 reflecting a perfect accuracy and a value of 0.5 reflecting an accuracy equal to chance. Test performance was calculated by ROC analyses comparing the areas under the curve (AUCs). First of all, patients with a systemic reaction (ANA and GU/AE) were compared to tolerant (TOL) individuals for peach and apple. The ROC analyses were then repeated once more comparing all symptomatic patients (ANA, OAS and GU/AE) to TOL patients for peach and apple, respectively. In addition, for the basophil activation tests, EC50 was calculated by dividing the lowest individual concentration generating maximal CD63 expression by two. (Non-) parametric tests and chi-square analyses were used where appropriate, and correction for multiple testing was applied. A P-value ≤ .05 was regarded as statistically significant.

3 | RESULTS

3.1 | Demographics and clinical characteristics

A total of 219 individuals were included: 182 sensitized to rPru p 3 and/or rMal d 3 and 37 healthy controls. Statistical comparison

demonstrated that Antwerp nsLTP-sensitized patients are significantly younger (mean age ANT = 30 years, BCN = 38 years, $P < .01$), have higher total IgE values (mean total IgE ANT = 1076 kU/L, BCN = 224 kU/L, $P < .01$) and reported atopic dermatitis (AD) in 29% of the cases, whereas none of the BCN patients reported AD. Furthermore, in contrast to the BCN population, the large majority of ANT patients are sensitized to pollen (ANT (94/108) 87% vs. BCN (39/73) 54%, $P < .01$). ANT patients are sensitized mostly to birch (PR10, 73%) and timothy grass (Phl p 1; 77%), less than 5% of the BCN patients are sensitized to these two allergenic sources. On the other hand, patients from BCN are most frequently sensitized to plane tree, 32/37 (86%) of the patients tested. Sex ratio and levels of rPru p 3 and rMal d 3 sIgE do not show any significant difference between the two populations (data shown in Table S1E of the online repository).

3.2 | Characteristics of nsLTP-sensitized individuals

Figure 2 shows the comparison of the different clinical groups in both regions. Additional numerical and statistical comparisons are displayed in the online repository file (Tables S2E–S3E).

A total of 142 rPru p 3-sensitized individuals were included (60 in BCN and 82 in ANT). Although a higher prevalence of pollen sensitization is found in ANT, the different clinical groups in each geographical region had similar proportions of pollen-sensitized patients ($P = .71$ – 1.00) with similar sensitization profiles ($P = .19$ – 1.00). Likewise, no differences are found in the proportions of patients sensitized to five or more different nsLTPs between the clinical groups in each region ($P = .25$ – 0.76). All ANT patients reporting AD are found in the OAS and TOL groups, and these groups also displayed a trend towards higher total IgE values than their BCN counterparts ($P = .11$ – 0.01).

In total, 130 individuals were sensitized to Mal d 3 (56 in BCN and 74 in ANT). Distributions of pollen and nsLTP sensitization, AD and total IgE are in line with the observations for the rPru p 3-sensitized individuals.

3.3 | Test performance

Receiver operator curve analyses are visualized in Figure 3, and corresponding AUCs are shown in Tables S4E–S5E of the online repository. The individual dose responses of the BAT rPru p 3 and rMal d 3 are shown in Figure 4, and sIgG4 and sIgG4/sIgE ratios for rPru p 3 and rMal d 3 are shown in Figure 1E (online repository).

When comparing systemic reactors to asymptomatic individuals, in BCN, only BAT rMal d 3 (0.001 µg/mL, CO = 28%, $P = .005$) demonstrated accuracy (AUC = 0.751, $P = .005$) with a sensitivity of 63% and a specificity of 67%. The BAT rPru p 3's AUC was ≤ 0.5 for all of the tested concentrations ($P = .102$ – 0.943) in BCN. In ANT, neither the BAT rPru p 3 nor the BAT rMal d 3 reached an AUC > 0.5 ($P = .07$ – 1).

In BCN, when examining the ratios of sIgE/total IgE, sIgG4 and sIgG4/sIgE, only the sIgG4/sIgE rMal d 3 demonstrated an AUC > 0.5

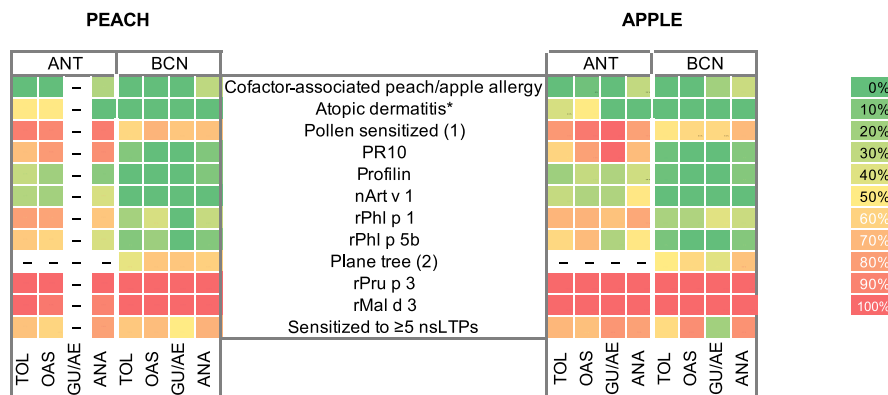


FIGURE 2 Compares the clinical groups between the two regions for different clinical and in vitro characteristics. (1) measured by at least one positive sIgE for the following components: Bet v 1 (PR10), Bet v 2 (PROFILIN), Art v 1, Phl p 1, Phl p 5b, Pla a 1, Pla a 2 or the crude plane tree extract. (2) Measured by a positive sIgE to the crude plane tree extract, Pla a 1 or Pla a 2. - = not performed * atopic dermatitis differed significantly between the whole ANT and BCN nsLTP-sensitized population ($P < .01$)

($P = .04$), corresponding to a sensitivity of 60% and a specificity of 75% (CO = 5%). In ANT, only the sIgE/total IgE rPru p 3 ratio showed an AUC > 0.5 (CO = 0.0236, $P = .04$) with a sensitivity of 60% and a specificity of 83%.

A second analysis comparing asymptomatic patients with all symptomatic patients (OAS GU/AE and ANA) demonstrated consistent results for BCN patients in that only the BAT rMal d 3 (0.001 μ g/mL) and sIgG4/sIgE rMal d 3 demonstrated an AUC > 0.5 ($P < .05$). In ANT, none of these tests reached an AUC > 0.5 for peach or for apple.

In a final analysis (Figure 5), basophil sensitivity is explored using EC50, the concentration generating 50% of the individual maximum of CD63 expression (ie basophil activation). Figure 5 displays the individual EC50 of ANT and BCN patients upon stimulation with rPru p 3 and rMal d 3. In symptomatic BCN patients, basophils are significantly more sensitive to lower doses of allergen (both rPru p 3 and rMal d 3) compared to basophils in symptomatic ANT patients ($P < .01$). However, no significant difference was found between symptomatic and tolerant patients within each region.

4 | DISCUSSION

Current nsLTP-based diagnostic tests such as SPT and sIgE quantification provide evidence of sensitization but not necessarily evidence of clinical reactivity. In clinical practice, the management and guidance of nsLTP-sensitized patients requires differentiation between clinically irrelevant sensitizations from those associated with (severe systemic) symptoms. Consequently, there is urgent need for improved diagnostics.

This study was designed to explore and compare allergy characteristics and several diagnostic tests in nsLTP-sensitized populations from two distinct geographical areas: Antwerp (a typical European birch allergy predominant area) and Barcelona (a typical Mediterranean nsLTP allergy predominant area). Preliminary data

have demonstrated that BAT rPru p 3 could be of additional value¹² in identifying clinically significant sensitization. Here, performance of BAT and those of sIgG4, sIgG4/sIgE, sIgE and sIgE/total IgE for two nsLTP components (Pru p 3 and Mal d 3) were explored in a larger population.

The results of this study show that test performance can differ between regions and might even differ for different nsLTP components. None of the explored diagnostic tests demonstrated the performance hoped for. However, it was shown that the BAT rMal d 3 and sIgG4/sIgE rMal d 3 could be of additional value in differentiating clinically relevant from irrelevant nsLTP sensitization in BCN and that the ratio of sIgE rPru p 3 and total IgE might be of use in the ANT population. Interestingly, by looking in detail to the dose-response curves of individual patients and analyses of the BAT's EC50, it appears that BCN patients are significantly more sensitive to lower allergen concentrations compared to ANT patients. Exploration of smaller allergen concentrations (<0.001 μ g/mL) seems worthwhile to examine in the future.

In Antwerp, as mentioned above, only the total IgE/sIgE rPru p 3 seems to correlate with peach anaphylaxis. This finding most likely relates to the high number of patients with AD in the OAS and TOL groups in this population, as AD is often associated with high total IgE values, which likely explains the significantly higher total IgE in ANT patients compared with BCN patients. We hypothesize that the large majority of the Pru p 3 sensitizations seen in these patients with AD result from the general increase in the number of IgE sensitizations or nonspecific IgE binding in the solid phase assay. It is noteworthy that BAT rPru p 3 almost reached significance ($P = .07$) in the ANT population. One could hypothesize that nsLTP-sensitized patients in ANT present more frequently with AD compared to patients from BCN. However, the difference in AD frequency between both regions warrants careful interpretation as the BCN patients were selected via the Allergology/Pneumology department, whereas the ANT patients were selected via the Immunology/Allergology department, in which a significant number of consultations include dermatological problems.

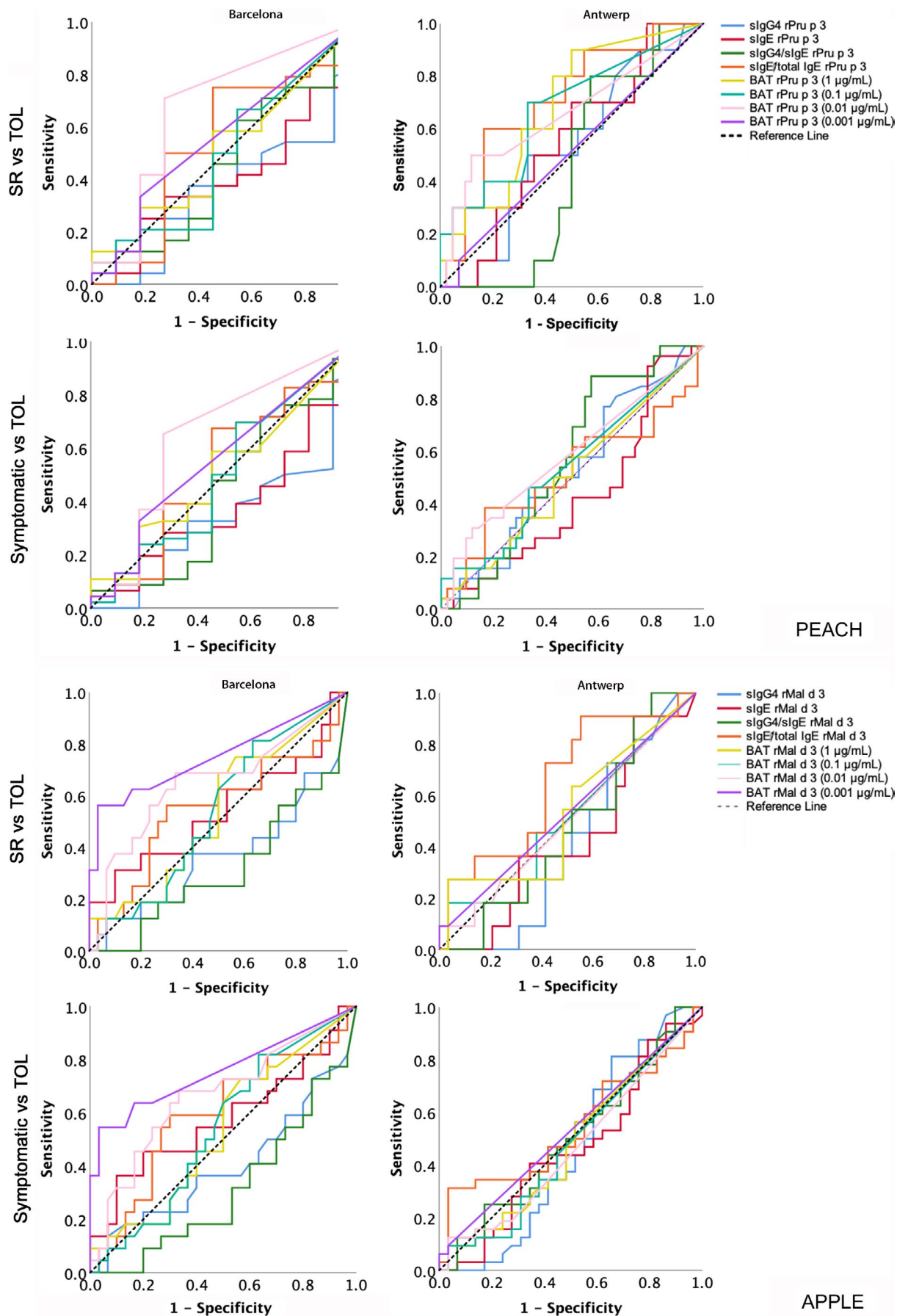


FIGURE 3 Overview of the ROC analyses and AUC for slgG4, slgG4/slGE ratio, slGE, slGE/total IgE ratio and Bat (0.001-0.01-0.1-1 $\mu\text{g}/\text{mL}$) rPru p 3 and rMal d 3. Comparisons were made first between patients with a systemic reaction (SR combining ANA and GU/AE) and the TOL group. A second analysis compared all symptomatic patients (ANA, GU/AE, OAS) with the TOL groups, both for apple (Mal d 3) and for peach (Pru p 3). ANA = anaphylaxis, TOL = tolerant, OAS = oral allergy syndrome, GU/AE = generalized urticaria and/or angioedema

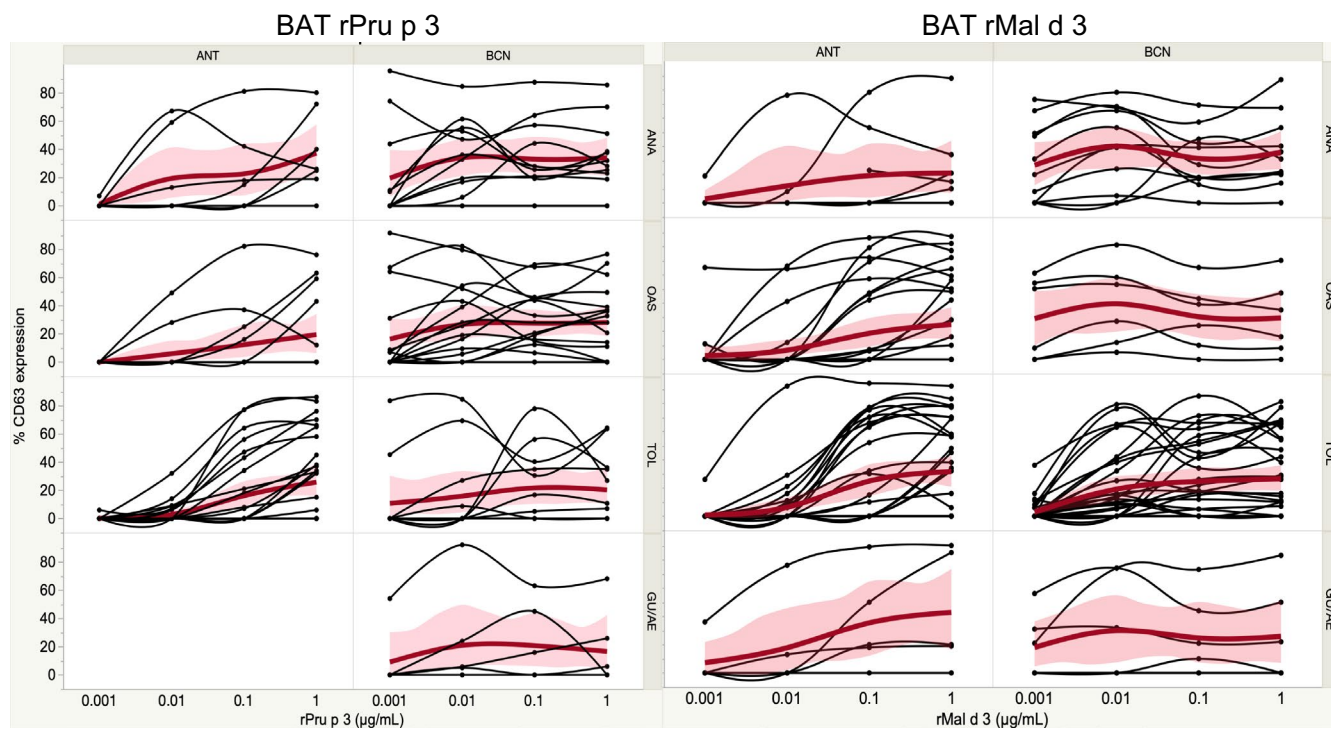


FIGURE 4 The individual dose-response curves of the BAT rPru p 3 (left) and rMal d 3 (right). ANT = Antwerp, BCN = Barcelona. ANA = anaphylaxis, TOL = tolerant, OAS = oral allergy syndrome, GU/AE = generalized urticaria and/or angioedema. BAT = basophil activation test. Red line = mean dose-response curve with standard error

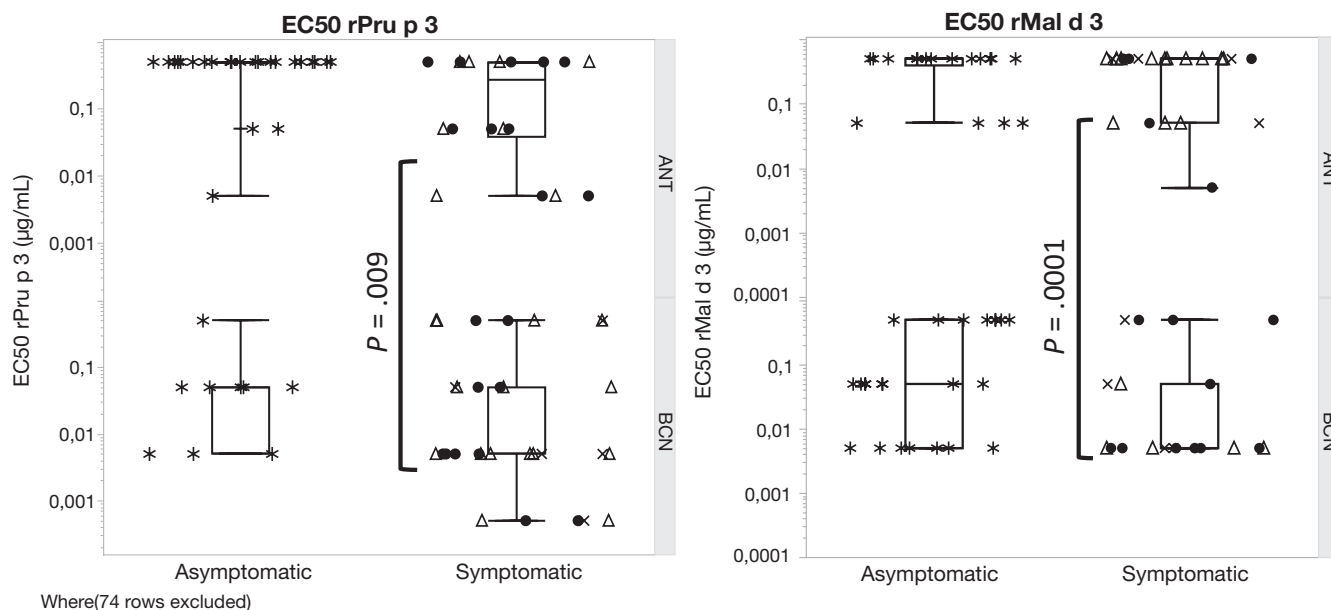


FIGURE 5 The EC50 for rPru p 3 and rMal d 3 in symptomatic (circles = ANA, x = GU/AE, triangles = OAS) and asymptomatic groups (star = TOL). Boxplots showing Q25-50-75. ANA = anaphylaxis, TOL = tolerant, OAS = oral allergy syndrome, GU/AE = generalized urticaria and/or angioedema

In addition, as can be expected, patients from ANT are significantly more often sensitized to birch and grass pollen compared to the BCN patients (who are mostly sensitized to plane tree pollen). However, the frequency of pollen sensitization did not differ between the symptomatic and asymptomatic patients in either region. Likewise, no differences were found in the proportions of patients sensitized to five or more different nsLTPs between the groups within each region. This is an interesting finding as previous research has postulated that both birch pollen sensitization and sensitization to five or more different nsLTPs might modulate symptom severity.^{18,21,22,35}

One possible limitation of this study is the fact that no oral food challenges were performed, as these tests constitute the reference test to confirm the diagnosis of food allergy. However, food challenges by no means identify the causative role of LTPs. In order to evaluate the specific role of LTPs, challenges would require isolated administration of the suspected purified or recombinant LTPs. However, up to now, challenges with specific LTPs have not entered mainstream application mainly for practical reasons because of unavailability of reliable and/or safe source material (purified or recombinant proteins).^{36,37}

In conclusion, this study found that test performance differs not only between regions but seems to be component-specific as well. Consequently, findings concerning nsLTP sensitization and diagnostic performance in a certain area cannot simply be extrapolated to another population without thorough comparative research. In addition, although none of the explored diagnostics demonstrated the performance hoped for, it appears that symptomatic BCN patients are more sensitive to lower allergen concentrations compared to symptomatic ANT patients. This finding indicates that BAT with allergen concentrations lower than 0.001 µg/mL should be explored in the future as a lower threshold of allergen concentration could potentially augment the performance of BAT in the nsLTP-sensitized patients from BCN.

CONFLICTS OF INTEREST

Proposal: Prof De Clerck reports funding from the company Roche, during the conduct of the study; grants from Roche, outside the submitted work. The remaining authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

IID, MP, MF and DGE have made substantial contributions to conception, design, acquisition, analysis and interpretation of data, as well as preparing the manuscript for publication. CHB and LSDC have been especially involved in the study design and analysis of the laboratory results. JB, RMM-C, VS and MMH have made substantial contributions to the recruitment and clinical characterization of patients for the study as well as in the critical revision of the manuscript. GA, ALVG, CM, MT, JR and SB have been involved in the acquisition of clinical data and performance of laboratory tests.

AD-P has provided the allergens required for the BAT experiments. LL has critically reviewed the manuscript as well as carefully revised the English wording as a native speaker. Each author has participated sufficiently in the work and was agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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REPOSITORY FIGURES & TABLES

FIGURE 1E

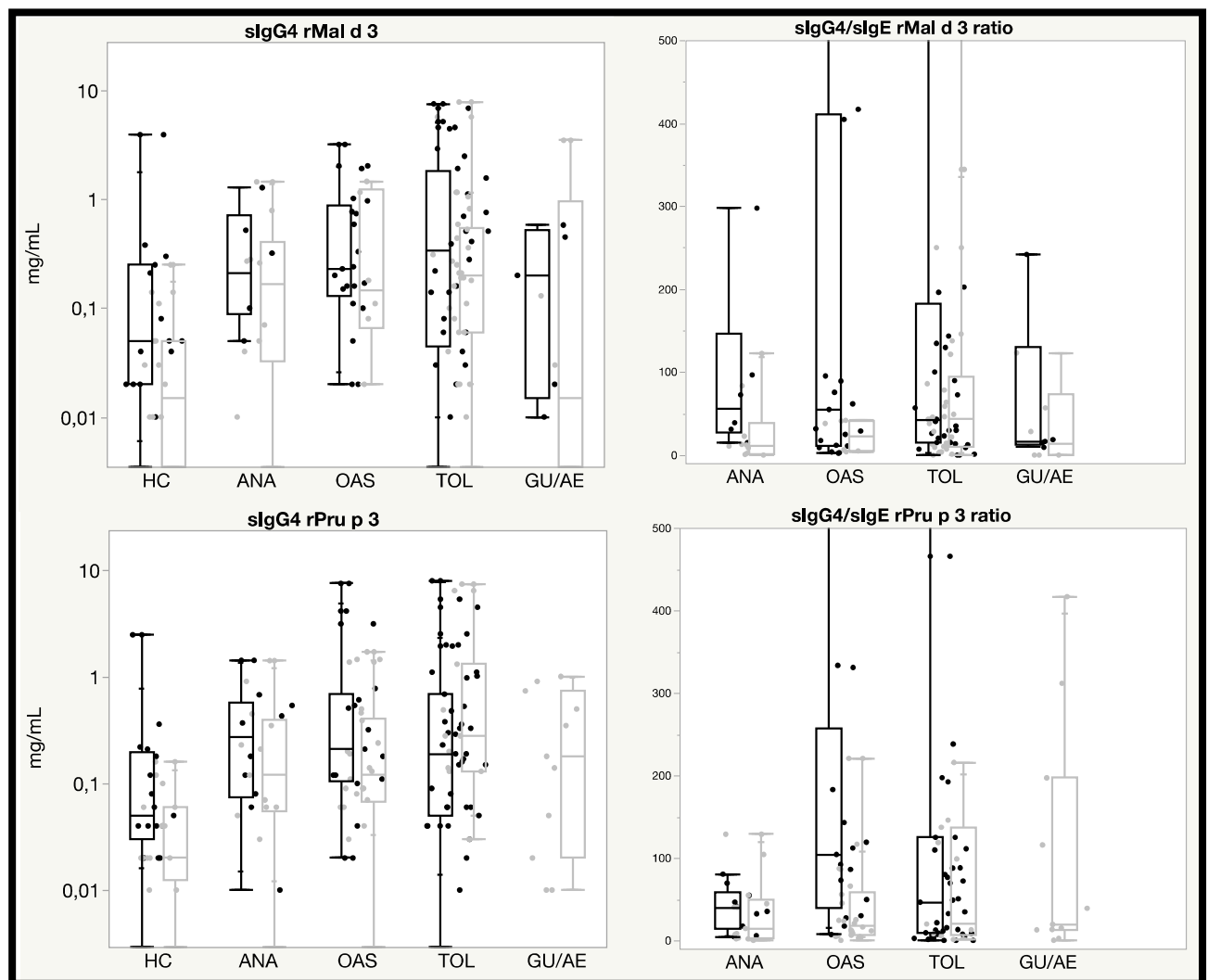


Figure 5 demonstrates the specific IgG4 and slgG4/slE ratios for rPru p 3 and rMal d 3 showing results for ANT (=black) and BCN (=grey). The boxplots represent the Q25-Q50-Q75 with min-max shown by the lines. ANA=anaphylaxis, TOL=tolerant, OAS=oral allergy syndrome, GU/AE=generalized urticaria and/or angioedema.

TABLE 4E

AREA UNDER THE CURVE				
peach systemic reactions versus tolerance				
	Area	Std. Error	p-value	95% CI
ANT				
slgG4 rPru p 3	.526	.092	.798	0.345-0.707
slgG4/slE rPru p 3	.449	.080	.618	0.293-0.605
slgE/total IgE rPru p 3	.710	.088	.041	0.537-0.882
BAT rPru p 3 (1µg/mL)	.686	.086	.070	0.5170-0.855
BAT rPru p 3 (0.1µg/mL)	.673	.099	.092	0.479-0.866
BAT rPru p 3 (0.01µg/mL)	.657	.107	.125	0.447-0.867
BAT rPru p 3 (0.001µg/mL)	.511	.103	.917	0.309-.712
BCN				
slgG4 rPru p 3	.356	.098	.177	0.163-0.549

slgG4/slGE rPru p 3	.451	.108	.644	0.239-0.662
slGE/total IgE rPru p 3	.534	.112	.749	0.314-0.754
BAT rPru p 3 (1µg/mL)	.508	.106	.943	0.301-0.714
BAT rPru p 3 (0.1µg/mL)	.485	.111	.887	0.267-0.702
BAT Pru p 3 (0.01µg/mL)	.674	.108	.102	0.463-0.885
BAT Pru p 3 (0.001µg/mL)	.561	.106	.570	0.353-0.768

ANT=Antwerp, BAT=basophil activation test, BCN= Barcelona, CI= confidence interval, Std. Error= standard error, slGE=specific IgE.

TABLE 5E

AREA UNDER THE CURVE					
apple systemic reactions versus tolerance					
	Area	Std. Error	p-value	95% CI	
ANT	slgG4 rMal d 3	.406	.089	.364	0.2310.581
	slgG4/slge rMal d 3	.470	.095	.774	0.2840.656
	slgE/totIge rMal d 3	.652	.097	.142	0.4620.842
	BAT rMal d 3 (1µg/mL)	.530	.107	.774	0.320-0.739
	BAT rMal d 3 (0.1µg/mL)	.502	.105	.988	0.296-0.707
	BAT rMal d 3 (0.01µg/mL)	.500	.104	1.000	0.297-0.703
	BAT rMal d 3 (0.001µg/mL)	.530	.106	.774	0.322-0.737
BCN	slgG4 rMal d 3	.364	.092	.131	0.183-0.544
	slgG4/slge rMal d 3	.315	.084	.040	0.150-0.479
	slgE/totIge rMal d 3	.550	.096	.580	0.363-0.737
	BAT rMal d 3 (1µg/mL)	.535	.090	.695	0.359-0.712
	BAT rMal d 3 (0.1µg/mL)	.536	.087	.687	0.365-0.708
	BAT rMal d 3 (0.01µg/mL)	.650	.091	.097	0.471-0.829
	BAT rMal d 3 (0.001µg/mL)	.751	.085	.005	0.585-0.918

ANT=Antwerp, BAT=basophil activation test, BCN= Barcelona, CI= confidence interval, Std. Error= standard error, slGE=specific IgE.

AREA UNDER THE CURVE					
peach symptomatic versus tolerance					
		Area	Std. Error	p-value	95% CI
ANT	slgG4 rPru p 3	.531	.071	.672	0.391-0.670
	slgG4/slgE rPru p 3	.571	.069	.328	0.436-0.706
	slgE/total IgE rPru p 3	.518	.077	.801	0.367-0.669
	BAT rPru p 3 (1µg/mL)	.509	.072	.900	0.367-0.651
	BAT rPru p 3 (0.1µg/mL)	.537	.073	.609	0.394-0.680
	BAT rPru p 3 (0.01µg/mL)	.583	.073	.251	0.440-0.727
	BAT rPru p 3 (0.001µg/mL)	.500	.072	.995	0.358-0.642
BCN	slgG4 rPru p 3	.339	.087	.099	0.168-0.510
	slgG4/slgE rPru p 3	.435	.105	.505	0.229-0.640
	slgE/total IgE rPru p 3	.512	.102	.903	0.313-0.711
	BAT rPru p 3 (1µg/mL)	.513	.095	.895	0.327-0.698
	BAT rPru p 3 (0.1µg/mL)	.502	.104	.984	0.299-0.705
	BAT rPru p 3 (0.01µg/mL)	.646	.102	.135	0.445-0.847
	BAT rPru p 3 (0.001µg/mL)	.558	.097	.551	0.369-0.748

AREA UNDER THE CURVE					
apple symptomatic versus tolerance					
		Area	Std. Error	p-value	95% CI
ANT	slgG4 rMal d 3	.417	.093	.361	0.234-0.600
	slgG4/slgE rMal d 3	.479	.092	.814	0.298-0.659
	slgE/totIgE rMal d 3	.560	.094	.514	0.376-0.743
	BAT rMal d 3 (1µg/mL)	.515	.092	.865	0.336-0.695
	BAT rMal d 3 (0.1µg/mL)	.505	.092	.958	0.325-0.684
	BAT rMal d 3 (0.01µg/mL)	.481	.092	.835	0.300-0.661
	BAT rMal d 3 (0.001µg/mL)	.575	.091	.411	0.398-0.752
BCN	slgG4 rMal d 3	.384	.090	.191	0.208-0.559
	slgG4/slgE rMal d 3	.313	.080	.035	0.155-0.470
	slgE/totIgE rMal d 3	.537	.091	.677	0.359-0.715
	BAT rMal d 3 (1µg/mL)	.551	.088	.562	0.378-0.724
	BAT rMal d 3 (0.1µg/mL)	.562	.087	.487	0.392-0.732
	BAT rMal d 3 (0.01µg/mL)	.657	.085	.076	0.490-0.825
	BAT rMal d 3 (0.001µg/mL)	.738	.083	.007	0.575-0.900

Table 1E

	ANTWERP		BARCELONA		p-value*
	HC	nsLTP sensitized	HC	nsLTP sensitized	
age (mean-CI)	<i>matched</i>	30 (18-42)	<i>matched</i>	39 (27-50)	<0.01
sex (male:female)	43%	44%	44%	30%	0.08
eczema	0%	29%	0%	0%	<0.01
pollen sensitization**	0%	87%	0%	54%	<0.01
total IgE (mean-CI)***	54 (0-143)	1076 (0-3321)	161 (0-373)	224 (0-575)	<0.01
slgE rPru p 3 (mean-CI)***	0	6.49 (0.1-18.59)	0	8.37 (0.1-19.29)	0.29
slgE rMal d 3 (mean-CI)***	0	7.49 (0.1-20.78)	0	7.56 (0.1-19.04)	0.97

* Comparison of nsLTP-sensitized patients (Antwerp vs. Barcelona), p-values calculated by chi-square or independent t test where appropriate. CI=95% confidence interval. ** measured by at least one positive slgE for the following components: Bet v 1 (PR10), Bet v 2 (PROFILIN), Art v 1, Phl p 1, Phl p 5b, Pla a 1, Pla a 1 or the crude plane tree extract. *** measured in kU/L for total IgE and kUA/L for slgE.

Table 2E

	BE	SP	P	BE	
	TOL			OAS	
cofactor-associated peach allergy	-	-	0.2	0%	
atopic dermatitis	38%	0%	<0.01	38%	
pollen sensitization**	90%	46%	<0.01	86%	
PR10	58%	8%	<0.01	76%	
PROFILIN	23%	0%	0.06	14%	
nArt v 1	19%	0%	0.10	15%	
rPhl p 1	73%	15%	<0.01	71%	
rPhl p 5b	53%	8%	<0.01	48%	
plane tree***	-	31%	-	-	
Mal d 3	100%	100%	1.00	93%	
sensitized to ≥5 nsLTPs	57%	54%	1.00	48%	
total IgE kU/L (mean-Q25-75)	345 (99-892)	187 (43-506)	0.11	529 (147-1216)	130

*coded as “known ingestion of peel/no known ingestion of peel.” ** measured by at least one positive sIgE to Pru p 3, 5b, Pla a 1 or the crude plane tree extract. *** measured by a positive sIgE to the crude plane tree extract, same clinical group. All comparison made by chi-square e

Table 3E

	BE	SP	P	BE	SP
	TOL			OAS	
cofactor-associated apple allergy	-	-	1.00	4%	0%
atopic dermatitis	30%	0%	<0.01	41%	0%
pollen sensitization**	79%	44%	0.08	93%	50%
PR10	52%	3%	<0.01	75%	0%
PROFILIN	16%	3%	0.20	26%	0%
nArt v 1	26%	0%	<0.01	20%	0%
rPhl p 1	66%	19%	<0.01	67%	20%
rPhl p 5b	50%	6%	<0.01	63%	0%
plane tree***	-	42%	-	-	50%
Pru p 3	100%	100%	1.00	100%	100%
sensitized to ≥5 nsLTPs	65%	48%	0.22	61%	83%
total IgE kU/L (mean-Q25-75)	319 (156-1767)	75 (36-279)	<0.01	356 (122-1395)	117 (65-239)

*coded as “known ingestion of peel/no known ingestion of peel.” ** measured by at least one positive sIgE to Pru p 3, 5b, Pla a 1 or the crude plane tree extract. *** measured by a positive sIgE to the crude plane tree extract, same clinical group. All comparison made by chi-square e