

An Immunodiagnostic Assay for Quantitation of Specific IgE to the Major Pollen Allergen Component, Pas n 1, of the Subtropical Bahia Grass

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Key Words

Allergen · Bahia grass pollen · ImmunoCAP ·
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Abstract

Background: Pollens of the Panicoideae subfamily of grasses including Bahia (*Paspalum notatum*) are important allergen sources in subtropical regions of the world. An assay for specific IgE to the major molecular allergenic component, Pas n 1, of Bahia grass pollen (BaGP) would have immunodiagnostic utility for patients with pollen allergy in these regions. **Methods:** Biotinylated Pas n 1 purified from BaGP was coated onto streptavidin ImmunoCAPs. Subjects were assessed by clinical history of allergic rhinitis and skin prick test (SPT) to aeroallergens. Serum total, BaGP-specific and Pas n 1-specific IgE were measured. **Results:** Pas n 1 IgE concentrations were highly correlated with BaGP SPT ($r = 0.795$, $p < 0.0001$) and BaGP IgE ($r = 0.915$, $p < 0.0001$). At 0.23 kU/l Pas n 1 IgE, the diagnostic sensitivity (92.4%) and specificity (93.1%) for the detection of BaGP allergy was high (area under receiver operator curve 0.960, $p < 0.0001$). The median concentrations of Pas n 1 IgE in non-atopic subjects (0.01 kU/l, $n = 67$) and those with other allergies (0.02 kU/l, $n = 59$)

showed no inter-group difference, whilst grass pollen-allergic patients with allergic rhinitis showed elevated Pas n 1 IgE (6.71 kU/l, $n = 182$, $p < 0.0001$). The inter-assay coefficient of variation for the BaGP-allergic serum pool was 6.92%. **Conclusions:** Pas n 1 IgE appears to account for most of the BaGP-specific IgE. This molecular component immunoassay for Pas n 1 IgE has potential utility to improve the sensitivity and accuracy of diagnosis of BaGP allergy for patients in subtropical regions.

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Introduction

The subtropical Bahia grass, *Paspalum notatum*, of the Panicoideae subfamily of grasses is a clinically important source of pollen allergens. Bahia grass has an extended pollen season and a wide distribution in urban areas of both subtropical and temperate regions [1]. Pollens of subtropical grasses differ significantly from temperate grasses in their allergenic composition, primary sequences of shared allergens and immunological reactivity [2, 3]. We recently showed asymmetric IgE cross-reactivity between Bahia grass pollen (BaGP) and its major allergen

component, Pas n 1, and the temperate Ryegrass pollen and its allergen, Lol p 1 [4]. Furthermore, patients from the subtropical region of Queensland, Australia, showed greater skin prick test (SPT) sensitivity and serum IgE reactivity with BaGP than Ryegrass pollen [5]. Subfamily-specific and, in some cases, species-specific IgE reactivity with BaGP was evident in sera of grass pollen (GP)-allergic patients from a subtropical region [5]. Importantly, Pas n 1 also shows unique and non-cross-reactive T cell epitopes in the carboxyl terminal domain [6]. The targeting of specific allergen immunotherapy to the most clinically relevant source of sensitizing allergens could increase the efficacy of desensitization. Patients who are primarily sensitized to allergen components of subtropical BaGP may benefit from treatment with BaGP or its allergen components [7]. However, a prerequisite for optimal BaGP allergen-specific immunotherapy is accurate diagnosis of the sensitizing pollen and knowledge of its allergenic components.

Specific IgE tests for molecular components of Timothy GP (e.g. Phl p 1, Phl p 2, Phl p 4 and Phl p 5) and the Chloridoideae Bermuda GP (Cyn d 1) are available [8]. However, the current range of immunodiagnostic tests does not contain any allergen components of pollen from the subtropical Panicoideae subfamily. Although immunoassays based on whole crude extract of BaGP are available for skin prick and serum IgE determination, these have not been standardized for allergen content. Two dominant allergen components of BaGP have been characterized – Pas n 1 and Pas n 13 – with Pas n 1 being the only major allergen [9, 10]. A new diagnostic test based on the major allergenic component, Pas n 1, would have potential advantages of increased sensitivity, specificity and standardization over crude pollen extract. Here we evaluate the potential of measuring Pas n 1-specific IgE concentrations as a tool for the diagnosis of BaGP allergy.

Materials and Methods

Bio-Pas n 1 Coating of Streptavidin ImmunoCAPs

Pas n 1 of BaGP was purified by a 4-step process from an aqueous pollen extract as described [11] and biotinylated according to the manufacturer's instructions at a 10:1 molar ratio (Roche Diagnostics, Mannheim, Germany). Bio-Pas n 1 was examined for purity and function by sodium dodecyl sulphate gel electrophoresis, Coomassie Brilliant Blue staining, immunoblotting with monoclonal antibody FMC-A1 [12] that recognises Pas n 1, and streptavidin labelling with horseradish peroxidase. Biotinylated and unlabelled Pas n 1 were assessed for serum IgE reactivity by ELISA with a set of sera from subjects with and without BaGP allergy. Bio-Pas n 1 was then coupled to streptavidin-coated ImmunoCAP cellu-

lose discs (SA-ImmunoCAP) [13] using an ImmunoCAP 100 Instrument (Thermo Fisher Scientific, Uppsala, Sweden). Serum IgE reactivity with bio-Pas n 1-coated SA-ImmunoCAP was tested according to standard procedures and the concentration of specific IgE was determined from low- and high-range IgE calibration curve controls. Included in each assay were pools of sera from 3 GP-allergic subjects (GP group), 4 non-atopic subjects (NA group) and 4 subjects with allergies to allergens other than GP (OA group). A sample with 6% weight/volume bovine serum albumin was used as a no serum substitute (designated none).

Patients

A total of 238 adult subjects were recruited with ethical approval and informed consent from 12 allergy and immunology clinics across Australia as part of a multicentre cross-sectional Grass Pollen Allergy Survey (GPAS; HREC/2009/QPAH/296). GP-allergic patients (n = 159) were included if they presented with clinical symptoms of allergic rhinitis consistent with GP exposure and SPT response to Bahia and Ryegrass pollen extracts (Hollister-Stier, Spokane, Wash., USA) according to the Australasian Society of Clinical Immunology and Allergy guidelines [14]. Additionally, non-atopic subjects (NA group) with no clinical history of any allergic disease and no SPT response to a panel of 10 environmental aeroallergens were recruited from the sites at Princess Alexandra Hospital, Brisbane, Qld., and Royal North Shore Hospital, Sydney, N.S.W. (n = 29). Patients presenting with other allergic sensitizations (OA group), frequently with perennial rhinitis without seasonal exacerbation and positive SPT to house dust mite or cat dander, were recruited from these same 2 sites as control subjects (n = 50). Blood was collected by venepuncture and serum was stored and transported to Sullivan Nicolaides Pathology, Brisbane, Qld., for measurement in the same laboratory of total and specific IgE concentrations to each of Bahia (g17) and Ryegrass (g5) pollen extract by ImmunoCAP on an ImmunoCAP 250 instrument as part of a routine, NATA-certified commercial immunopathology service. An additional set of de-identified serum samples (n = 70) obtained from Sullivan Nicolaides Pathology immunology laboratory that had been tested for total and specific IgE to Bahia, Bermuda, Johnson and Ryegrass GPs were also tested for serum IgE reactivity with Pas n 1. Of these, there were 38 with no GP-specific IgE and low total IgE (median 46, interquartile range, IQR 21–91) assigned to the NA group, 23 with GP-specific IgE and high total IgE (median 282.5, IQR 117–1,121) assigned to the GP group and 9 with no GP-specific IgE with high total IgE (median 448, IQR 162–823) assigned to the OA group.

Data Analysis

Inter-assay variation in the two curve controls and the GP, OA and NA serum pools and no serum (none) were calculated as the standard deviation divided by the mean. The data on the case sera were assessed for normality of distribution by the Kolmogorov-Smirnoff test. The values obtained for the 6 controls within each assay were examined for bias due to the instruments (n = 2), operator (n = 2) and assay number (n = 10) by the Mann-Whitney U test for difference in median values between two groups (instrument and operator) and the Kruskal-Wallis test for differences in median values between multiple groups. Receiver operator characteristic (ROC) curve analysis [15] was performed with the complete data set and without the additional de-identified serum set.

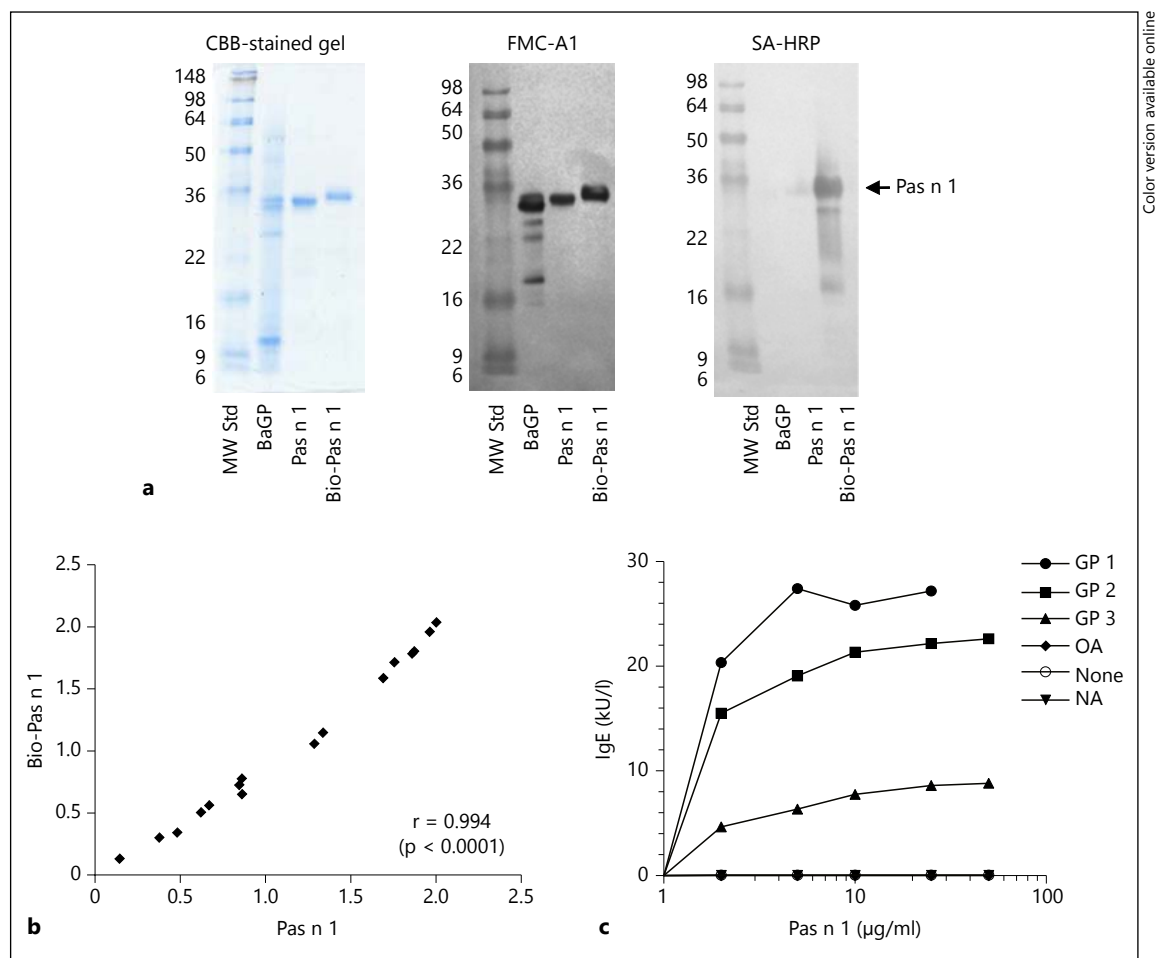


Fig. 1. Preparation of bio-Pas n 1-coated SA-ImmunoCAPs. **a** SDS-PAGE gels of *P. notatum* pollen extract (BaGP), purified Pas n 1 and bio-Pas n 1 stained with Coomassie Blue (left panel), or immunoblotted with mAb FMC-A1 reactive with Pas n 1 (middle panel) and with SA-HRP (right panel). Sizes of molecular weight standards are shown in kilodaltons. MW Std = Molecular weight standards. **b** Correlation of serum IgE of GP-allergic subjects with

bio-Pas n 1 and unlabelled Pas n 1 by ELISA. **c** IgE ImmunoCAP assay with titration of bio-Pas n 1 concentration tested with GP-allergic sera (GP 1, SPT 10 mm in diameter, 31 kU/l BaGP IgE; GP 2, SPT 10 mm in diameter, 15.7 kU/l BaGP IgE, and GP 3, SPT 8 mm in diameter, 6.41 kU/l BaGP IgE), a non-atopic serum (NA), a serum control for a patient with house dust mite and other than GP allergy (OA) and no serum (none).

Results

Preparation of Bio-Pas n 1-Coated SA-ImmunoCAP

Natural Pas n 1 purified from BaGP extract retained its immunoreactivity upon biotinylation, as assessed with a group 1 allergen-specific mAb (fig. 1a). Bio-Pas n 1 bound to streptavidin showed a slightly higher molecular weight than unlabelled Pas n 1 consistent with the addition of the biotin ligand. Bio-Pas n 1 retained serum IgE reactivity in comparison with unlabelled Pas n 1 ($r = 0.994$, $p < 0.001$) when tested in a panel of GP-allergic patients (fig. 1b). The optimal coating concentration of SA-ImmunoCAP was found to be 25 μg/ml of bio-Pas n 1 (fig. 1c) based on

IgE reactivity of three BaGP-allergic sera in comparison with a no serum control (none), a non-atopic serum (NA) and a serum from a patient with house dust mite and other than GP allergy (OA).

Demographics and Allergy Status of Subjects

Subjects recruited sequentially from allergy clinics as part of the GPAS study were assigned to one of three groups based on a clinical history of allergic rhinitis consistent with GP allergy and SPT to BaGP (table 1). The ages and genders of the subjects did not differ between groups. Of the patients with allergic rhinitis assigned to the GP allergy group, 60.3% had seasonal rhinitis whilst

Table 1. Demographics and allergy status of participants

	NA group	GP group	OA group	Statistical differences
Number	29	159	50	
Gender (female), %	65.2	66.9	63.3	n.s. ¹
Median age	32 (25–42)	39 (28–50)	39 (27–50)	n.s. ¹
Allergic rhinitis, %	0	100	61.7	GP > NA (p < 0.0001) ² GP > OA (p = 0.0001) ²
Seasonal rhinitis, %	0	59.7	30.0	GP > OA (p = 0.0091) ²
Taking medication, %	3.7	57.1	33.3	GP > OA (p = 0.0050) ²
Diagnosed asthma, %	3.7	43.7	48.9	GP > NA (p = 0.0004) ² GP versus OA (n.s.)
SPT to BaGP, mm diameter	0 (0–0)	6 (4–8)	0 (0–0)	GP > OA and NA (****) ¹
Median IgE to BaGP, kU/l	0.02 (0.00–0.04)	6.6 (1.35–16.3)	0.02 (0.00–0.13)	GP > OA and NA (****) ¹
Median total IgE, kU/l	34 (14–85)	128 (53–282)	160 (56–353)	GP and OA > NA (****) ¹

Values in parentheses are IQR. OA group: subjects showed SPT response to other allergens (73% were positive to house dust mite extract). Taking medication: medications included anti-histamines and/or nasal corticosteroids. n.s. = Not significant.

¹ Kruskal-Wallis test for difference between groups with Dunn's pairwise comparisons. **** p < 0.0001.

² Fisher's exact test for difference in frequency.

only 30% of patients with allergic rhinitis assigned to the OA group presented with seasonal rhinitis. The GP and OA groups both showed a significantly higher frequency of asthma than the NA group. A total of 57% of the GP-allergic group were taking medication for symptom relief, including anti-histamines and nasal corticosteroids. As expected, both BaGP SPT and BaGP IgE were significantly elevated in the GP-allergic group over both non-atopic subjects and patients with other allergies. Whilst both patient groups had significantly higher total serum IgE than the non-atopic subjects there was no difference in total serum IgE between the two patient groups.

Assay Reproducibility of Bio-Pas n 1 SA-ImmunoCAP for Measurement of Specific IgE

Pools of sera from three GP-allergic donors, non-atopic donors and donors with allergies other than GP and a no serum control (none) were tested in each of the Pas n 1 IgE ImmunoCAP assays (fig. 2a). In addition, separate ImmunoCAPs bound with low and high amounts of IgE served as curve controls for the detection of IgE. The inter-assay coefficients of variation for the low (average 0.775 kU/l) and high (average 19.42 kU/l) IgE curve controls were 5.14 and 13.3%, respectively. The coefficient of variation for the GP-allergic serum pool (average 9.20 kU/l IgE) was 6.92%. The levels of specific IgE without serum (none), or the NA and OA serum pool controls, were below the limit of

detection and variability between assays and could not be assessed. There was no difference between assays for values acquired across 10 assays for the control samples (low and high IgE curve controls), the GP, NA or OA serum pools or no serum (none), between the two ImmunoCAP instruments used throughout the study or the two assay operators (Mann-Whitney U tests). There was also no inter-assay difference in values for these controls across data from each of the 10 assays (Kruskal-Wallis test). The concentration of Pas n 1 IgE determined for a set of 50 serum samples, including subjects with and without GP allergy whose serum was measured on two separate occasions, did not differ between assays (fig. 2b) but was highly correlated between repeat assays (fig. 2c).

Performance of Pas n 1-Specific IgE Assay

Pas n 1 IgE was highly correlated with allergic sensitivity assessed by the SPT response to BaGP (Spearman's r = 0.795; fig. 3a) and BaGP IgE concentrations (r = 0.951; fig. 3b). The correlation between BaGP IgE and BaGP SPT in this cohort was r = 0.778 (95% CI 0.716–0.827, p < 0.0001; data not shown). There was a moderate positive correlation between Pas n 1 IgE and total serum IgE concentration (r = 0.409; table 2). The proportion of Pas n 1 IgE out of the BaGP IgE showed a median value of 100% (IQR 73–130%) of BaGP IgE. The detected Pas n 1 IgE concentrations were higher than the BaGP IgE concentration in 52.6% of GP-allergic cases.

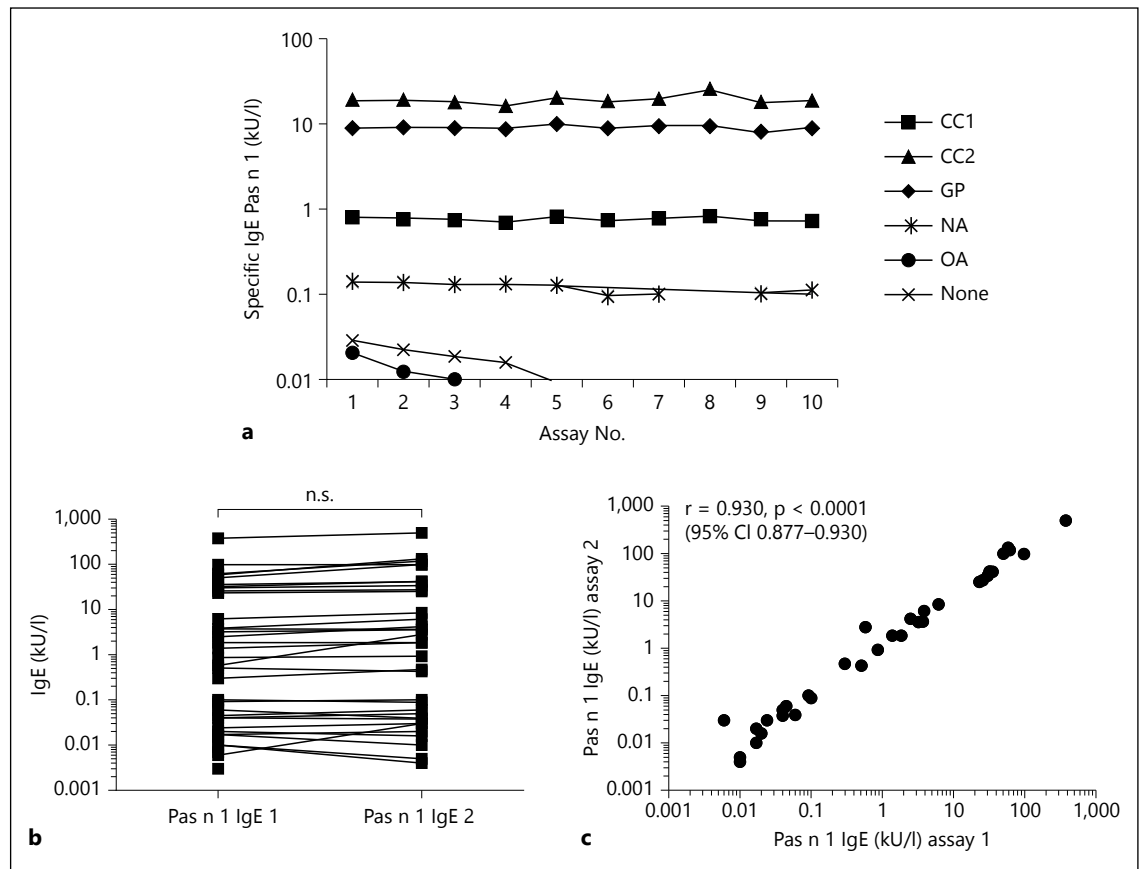


Fig. 2. Reproducibility of Pas n 1 IgE testing. n.s. = Not significant. **a** Values for assay controls; CC1: low IgE curve control; CC2: high IgE curve control; GP: BaGP-allergic serum pool; NA: non-atopic serum pool; OA: pool of sera from patients with allergies other than GP, and none: no serum control. Values below 0.01 are below

the instrument detection limits and could not be determined reliably. **b** Comparison of Pas n 1 IgE measured in 2 separate assays (Wilcoxon test for paired data). **c** Correlation of Pas n 1 IgE measured in repeat assays.

The distribution of BaGP SPT responses in each subject group is shown in figure 3c. The median levels of Pas n 1-specific IgE in non-atopic controls (0.01, IQR 0–0.02) and patients with allergies other than GP (0.02, IQR 0–0.07) did not differ between groups, whilst in allergic rhinitis patients with GP allergy the concentration of Pas n 1 IgE was significantly higher at 6.71 kU/l (IQR 1.60–21.3, $p < 0.0001$, Kruskal-Wallis test with Dunn's pairwise comparisons; fig. 3d). The area under the curve (AUC) for the ROC comparing the NA group with the GP group was 0.960 (fig. 3e; $p < 0.0001$). At a cut-off of 0.225 kU/l Pas n 1-specific IgE, the sensitivity of 92.4% (CI 87.0–96.0%) and specificity of 93.1% (CI 77.2–99.2%) of the detection of BaGP allergy was very high. Since there was no significant difference in Pas n 1 IgE concentrations between the NA and OA groups, ROC analysis was also performed with the NA and OA groups com-

bined. The AUC in this analysis was 0.925 ($p < 0.0001$) and at a cut-off of 0.35 kU/l Pas n 1 IgE the sensitivity (89.9%, CI 84.1–94.1%) and specificity (98.4%, CI 81.2–96.1%) were high.

Validation of Performance with an Additional Serum Set

An additional set of de-identified sera, which were assigned to the three groups on the basis of GP IgE and total serum IgE, were also tested and found to have a near perfect AUC of 0.999 (CI 0.995–1.00), demonstrating the close relationship between concentrations of Pas n 1 IgE and BaGP IgE. If both data sets were combined, then at the cut-off value 0.35 kU/l Pas n 1 IgE, 1 of 67 non-atopic subjects (1.5%), 166 of 182 (91.2%) GP-allergic subjects and 6 of 59 (10.1%) subjects with allergies other than GP would have tested positive for BaGP allergy (table 3;

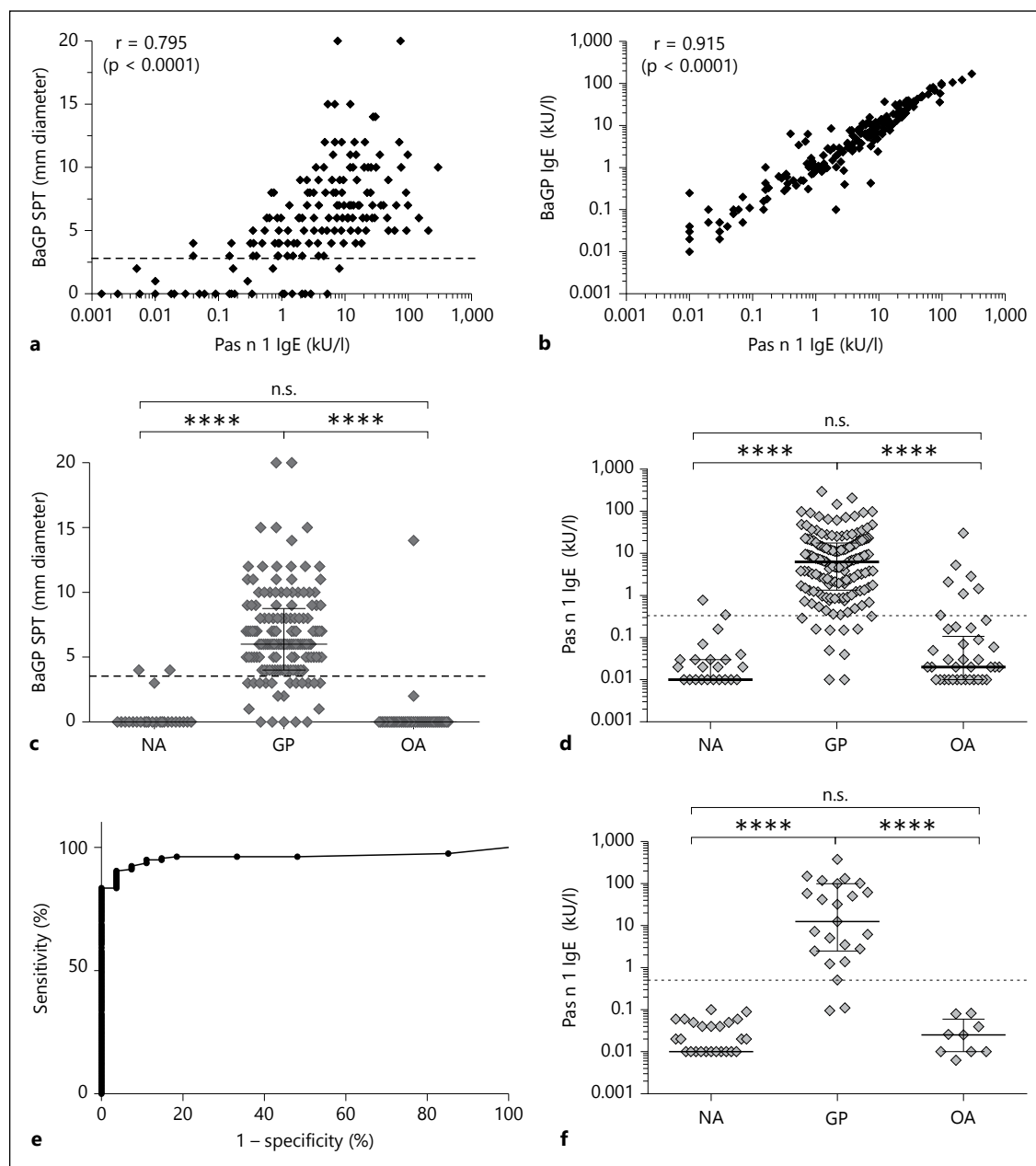


Fig. 3. Overall performance of Pas n 1 IgE assay. n.s. = Not significant. Correlation between Pas n 1 IgE and BaGP SPT (218 data points; **a**) and BaGP IgE (232 data points; **b**). Distribution of Pas n 1 SPT (**c**) and BaGP IgE concentrations (**d**) in the non-atopic (NA), BaGP-allergic group (GP) and subjects with allergies other than GP (OA) (Kruskal-Wallis test with Dunn's pairwise comparison test,

**** $p < 0.0001$). **e** ROC curve for diagnostic utility of Pas n 1 IgE for NA compared with GP. **f** Distribution of Pas n 1 IgE concentrations of de-identified serum set allocated to NA, OA or GP groups on the basis of serum IgE to GP and total IgE. Cut-off for SPT at 3 mm diameter is shown in **a** and **c** and for Pas n 1 IgE at 0.35 kU/l in **d** and **f**. Values below limit of detection are not shown.

fig. 3d, f). The numbers of subjects who would be diagnosed with BaGP allergy using BaGP-specific IgE at the standard 0.35 kU/l cut-off (table 4) or BaGP SPT at 3 mm in diameter (table 5; fig. 3c) were calculated for the NA, GP and OA groups.

Discussion

The diagnosis of allergy to a given allergen source is usually based on the clinical history of symptoms upon exposure to the source in conjunction with SPT or the

measurement of serum-specific IgE to a whole allergen extract. However, pollen extracts of the subtropical Panicoideae grasses currently used for SPT or serum IgE tests have not been standardized for allergen content. A molecule-based immunodiagnostic assay would improve batch consistency and quality control over whole pollen extracts that are composed of variable amounts of allergen and irrelevant non-allergenic material [16, 17]. Molecular component diagnosis may allow for future personalized allergen immunotherapy treatment targeting the allergen components of a given source to which a given patient is sensitized [18]. Here we show that an IgE Immunoassay based on the major allergen component of BaGP, Pas n 1, was highly sensitive and specific for the diagnosis of BaGP allergy.

IgE reactivity with Bio-Pas n 1 showed an excellent correlation with IgE reactivity with unlabelled Pas n 1 assessed by ELISA, indicating that the purified allergen component coupled to biotin essentially retained all its IgE reactivity in the sera of clinically relevant patients. The measurement of the concentration of Pas n 1 IgE by ImmunoCAP assay showed excellent reproducibility across assays.

The ROC curve generated for comparison of Pas n 1 IgE between the non-atopic and symptomatic BaGP-allergic group was remarkably high. Given that the assay is based on a technique already known for its excellent sensitivity and specificity, the high performance was anticipated. The utility of this assay to measure serum IgE to Pas n 1 was demonstrated by the near perfect AUC for the validation serum set that was allocated to group solely on the basis of GP-specific and total serum IgE. However, we observed, not unexpectedly, that 6 of the OA group tested positive for Pas n 1 IgE (10.1%) at the cut-off of 0.35 kU/l. Notably, 4 of the 6 subjects with allergies other than GP who showed IgE to Pas n 1 above 0.35 kU/l also showed BaGP IgE; 2 of these subjects were highly sensitized to the temperate Ryegrass pollen (SPT responses 14 and 15 mm in diameter). However, these 2 patients reported no pollen allergy symptoms, presumably due to the lack of exposure to temperate grasses in their current Queensland environment where subtropical GPs predominate. It is possible that shared cross-reactive carbohydrate determinants (CCD) present on natural Pas n 1, which is glycosylated [9], account for some false-positive Pas n 1 IgE detections in patients with other allergies. This would be expected based on other reports of up to 20% false-positive IgE tests due to CCD present on many natural allergens [19]. The addition of CCD blockers has recently been found to minimize the confounding influence of

Table 2. Correlation between Pas n 1 IgE and other measures

	BaGP ImmunoCAP	BaGP SPT	Tot IgE
Spearman's r	0.915	0.795	0.409
95% CI	0.890–0.934	0.738–0.841	0.291–0.515
p value (2-tailed)	<0.0001	<0.0001	<0.0001
XY pairs, n	232	228	218

Table 3. Frequency of detection of BaGP allergy based on Pas n 1 IgE at a cut-off of 0.35 kU/l

	NA	OA	GP
Number	67	59	182
Number positive	1	6	166
Percentage	1.5	10.1	91.2

OA: those OA subjects who were positive to Pas n 1 IgE included a subject from New Zealand with SPT to Ryegrass pollen (15 mm in diameter), but with no reported symptoms whilst living in a subtropical region of Brisbane, and subjects with high IgE (722–2,379 kU/l). Data include the GPAS study cohort and additional de-identified subjects for whom specific IgE data were available.

Table 4. Frequency of detection of BaGP allergy based on BaGP IgE at a cut-off of 0.35 kU/l

	NA	OA	GP
Number	66	54	177
Number positive	1	6	163
Percentage	1.5	11.1	92.1

Data include the GPAS study cohort and additional de-identified subjects for whom specific IgE data were available.

Table 5. Frequency of detection of BaGP allergy based on BaGP SPT (>3 mm in diameter)

	NA	OA	GP
Number	25	43	145
Number positive	2	1	139
Percentage	8.0	2.0	95.9

Data include the GPAS study cohort for whom SPT data were available.

clinically irrelevant IgE binding to CCD in in vitro diagnostic assays [20]. In future studies, the preparation of a new recombinant (unglycosylated) version of Pas n 1 may be advantageous over purified natural Pas n 1 for commercial diagnostic testing and may increase the specificity of a Pas n 1 IgE test. However, recombinant Pas n 1 expressed from its native sequence is not soluble in aqueous buffer [9], which prevented its use in this study. It may be feasible by site-directed mutagenesis to manipulate the solubility of recombinant Pas n 1 whilst retaining its IgE reactivity for use as a molecular component test. This approach was employed for the generation of recombinant Phl p 1 of Timothy GP [8].

A key outcome of this current study was the very high correlation between Pas n 1 IgE and BaGP IgE. The measured concentrations of Pas n 1 IgE were higher than BaGP IgE in half of the GP-allergic patients. This outcome is consistent with the higher capacity to coat the active determinants onto the test matrix with a purified allergen component compared with a complex pollen extract. Notably in this study, an indirect coupling of biotinylated Pas n 1 bound the allergen component to the test matrix via interaction with streptavidin. Direct coupling chemistry may further increase the sensitivity of detection of Pas n 1 IgE and the overall performance of the test. Whilst Pas n 1 IgE appeared to account for all of the BaGP IgE in half of the cases in this patient cohort, those sera in the lower quartile showed that Pas n 1 IgE accounted for less than 73% of BaGP IgE. Future inclusion of other important allergen components of subtropical GPs (e.g. Pas n 13 of BaGP [10]) in molecular diagnostics may allow for the assessment of the importance of other allergens to the total BaGP-specific IgE response and may enhance the capacity for accurate differential diagnosis between subtropical GP allergy and allergy to temperate grasses. We have demonstrated that the measurement of serum IgE to Pas n 1 performs as well as current tests when the data are analysed based on the clinical presentation of allergic rhinitis as the primary selection criterion with an AUC of 0.956. In clinical practice, the capacity to measure allergen-specific serum IgE must be utilized in the context of symptoms and patient exposure to the given allergen source [21, 22].

These data indicate that Pas n 1 would be suitable in replacing unstandardized whole BaGP extracts in future assays for the diagnosis of BaGP allergy. The data herein provide strong evidence for the utility of the further development of a Pas n 1 IgE ImmunoCAP based on the following: (1) optimized recombinant production of soluble Pas n 1 and (2) direct coupling to the ImmunoCAP

surface. Whilst we have utilized the ImmunoCAP assay format in this study, the indirect coupling method mediated by the biotin-streptavidin interaction means that the measurement of Pas n 1 IgE could be transferrable to a number of alternative assay formats, including the IMMULITE system [23], solid-phase multi-allergen arrays (e.g. ISAC [24]), multi-allergen bead arrays [25], or point-of-care devices [26, 27].

The benefits of quantitative molecular component immunoassays go beyond the capacity for better standardization, batch control of the test and meeting regulatory guidelines on the preparation of allergen diagnostics (EMA/PDCO/737605/2009, http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2009/11/WC500015814.pdf [28]). The measurement of allergen-specific serum IgE concentration has the advantage of precise quantitation and the lack of interference of anti-histamine drugs that can occur with SPT [29]. Moreover, the future use of allergen-specific to total IgE ratio may allow a more tailored use of allergen-specific immunotherapy by identifying those most likely to respond [30]. The ratio of allergen-specific to total IgE could assist with the clinical management of patients [31]. The levels of specific IgE in serum to a given allergen are related to in vitro measures of basophil activation, a surrogate for in vivo allergic sensitivity [32]. Indeed Pas n 1 performs well in basophil activation studies [4, 9]. The quantitation of allergen-specific IgE has the potential to provide increased confidence in the prediction of the risk of an allergic reaction of a patient to subsequent allergen exposure compared with dichotomous positive/negative results frequently used in clinical diagnosis [15, 33]. Qualitatively, specific IgE to certain allergens (Cor a 9 and Cor a 14) in hazelnut-allergic patients was associated with the risk of severe reactions [34], whereas the presence of low Phl p 5 IgE in patients with GP allergy was associated with the absence of asthma [35], further demonstrating the potential clinical value for allergen component tests. Allergen component-resolved diagnosis has the potential to improve the differential diagnosis of pollen allergy (i.e. birch vs. grass vs. olive) and the optimal prescription of allergen-specific immunotherapy [36].

Given the high proportion of IgE directed to Pas n 1 in most BaGP-allergic subjects, Pas n 1 appears to be the initiator allergen [37, 38] for the induction of sensitization to BaGP in these patients from a subtropical region. It will be informative in future to conduct longitudinal studies of IgE reactivity with Pas n 1 in relevant patient populations to track the emergence of IgE to Pas n 1 as

well as an array of pollen allergen components of Bahia (e.g. Pas n 13) and other grasses. Bahia grass is present within parts of southern Europe (Portugal, Spain and Greece) and southern states of the USA (<http://www.discoverlife.org/mp/20m?kind=Paspalum+notatum>). Indeed subtropical GPs including BaGP may have increasing importance for allergic rhinitis with the changing biogeographical distributions of grasses due to global warming [39, 40]. Thus, future component-resolved diagnosis for Australia and elsewhere, including southern Europe and southern USA, should incorporate the measurement of specific IgE to Pas n 1 as well as the currently included Cyn d 1 and Phl p allergens for optimal pollen allergy diagnosis [41].

In summary, we report the first molecular component immunoassay for IgE to an allergen of the Panicoideae subtropical BaGP. The Pas n 1 IgE ImmunoCAP assay

shows very high sensitivity and specificity and is well correlated with IgE to whole BaGP. Such an assay may open the way for a more accurate and specific diagnosis of subtropical GP allergy.

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