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ABSTRACT

BACKGROUND

Cannabis allergy (CA) has mainly been attributed to Can s 3, the nsLTP (non-specific lipid transfer proten) of *Cannabis sativa*. Nevertheless, standardized diagnostic tests are lacking and research on CA is scarce.

OBJECTIVE

To explore the performance of five cannabis diagnostic tests and the phenotypic profile of CA.

METHODS

120 CA patients were included and stratified according to the nature of their cannabis-related symptoms, 62 healthy and 189 atopic controls were included. Specific (s)IgE hemp, sIgE and BAT rCan s 3, BAT with a crude cannabis extract and a skin prick test (SPT) with a nCan s 3-rich cannabis extract were performed. Clinical information was based on patient-history and a standardized questionnaire.

RESULTS

Firstly, up to 72% of CA reporting likely-anaphylaxis (CA-A) are Can s 3 sensitized. Actually, the Can s 3-based diagnostic tests show the best combination of positive and negative predictive values; 80% and 60%, respectively. sIgE hemp displays 82% sensitivity but only 32% specificity. Secondly, Can s 3+CA reported significantly more cofactor mediated reactions and displayed significantly more sensitizations to other nsLTPs than Can s 3-CA. Finally, the highest prevalence of systemic reactions to plant-derived foods was seen in CA-A, namely 72%.

DISCUSSION

The most effective and practical tests to confirm CA are the SPT with a nCan s 3-rich extract and the sIgE rCan s 3. Can s 3 entails a risk of systemic reactions to plant-derived foods and cofactor-mediated reactions. However, as Can s 3 sensitization is not absolute, other cannabis allergens probably play a role.

HIGHLIGHTS BOX

1. What is already known about this topic?

Cannabis allergy, although rare, can manifest with severe and generalized symptoms and has been linked to Can s 3, the nsLTP present in *Cannabis sativa*.

2. What does this article add to our knowledge?

This article is the first to compare the performance of multiple cannabis diagnostic method and explore clinical and in vitro characteristics of cannabis allergy in one of the largest cannabis allergic populations described up till now.

3. How does this study impact current management guidelines?

There are no guidelines available on cannabis allergy diagnosis or management. This article ‘s perspective on diagnostic performances could aid in accurately approximating post-test probabilities and gives insight into the profile of Western-European cannabis allergic patients.

KEYWORDS

cannabis allergy; diagnosis; BAT; specific IgE; skin prick test; Can s 3; nsLTP; cofactor; basophil; anaphylaxis; hemp

ABBREVIATIONS

BAT	basophil activation test
CA	cannabis allergy
CA-A	patients with likely-anaphylaxis to cannabis
CA-C	patients with cutaneous symptoms to cannabis
CA-R	patients with respiratory symptoms to cannabis
CA-RC	patients with localized respiratory and cutaneous symptoms to cannabis
CBA	cytometric bead array
CI	confidence interval
CS	*Cannabis sativa*
CTA	cannabis tolerant but atopic participants with pollen and LTP sensitizations
HC	healthy controls
LHR	likelihood ratio
NPV	negative predictive value
NSAIDs	nonsteroidal anti-inflammatory drugs
nsLTP	nonspecific lipid transfer protein
PPV	positive predictive value
P+LTP-	atopic pollen sensitized participants without an nsLTP sensitization
P+LTP+	atopic pollen and nsLTP sensitized participants
rCan s 3	recombinant Can s 3 protein from *Cannabis sativa*
RuBisCO	Ribulose-1,5-bisphosphate carboxylase/oxygenase
sIgE	specific immunoglobuline E
SPT	skin prick test
TLP	thaumatin-like protein

INTRODUCTION

Cannabis is one of the most consumed drugs worldwide (1). Despite its widespread use, reports on cannabis allergy (CA) remain rare and generally deal with relatively small numbers of cases (2-6). Nevertheless, from these reports evidence is accumulating that CA can manifest with severe and generalized symptoms and a variety of cross-reactive plant-derived food allergies, mainly attributed to a Can s 3 sensitization, the nsLTP (non-specific lipid transfer protein) from *Cannabis sativa*. As a matter of fact, in some European surveys, Can s 3 has been demonstrated to be a major allergen (7-9). NsLTPs are heat stable allergens widely distributed throughout the plant kingdom and showing extensive *in vitro* and *in vivo* cross-reactivity (10). Both the severe phenotype and the extensive cross-reactivity associated with CA can be attributed to the physiochemical properties of Can s 3. Other putative cannabis allergens are RuBisCo, oxygen-evolving enhancer protein 2 and a thaumatin-like protein (2, 4). However, unlike Can s 3 (3), these allergens have not yet been successfully isolated nor expressed as a recombinant protein and are currently unavailable for diagnosis.

So far, in the majority of studies on CA, diagnosis is documented by prick-prick tests with buds or leaves (4-6, 9) and therefore are difficult to standardize, because of the heterogeneous composition of the different source materials. The clinical severity and cross-reactivity of CA together with the unpredictability of the source materials used for skin testing constitute strong incentives for more reliable cannabis diagnostic tests, *in vitro* or *in vivo*.

In two preliminary studies we have standardized and presented initial performance results four different cannabis diagnostic tests namely a basophil activation test (BAT) with rCan s 3, a BAT with a crude CS extract, a skin prick test (SPT) with a nCan s 3-rich extract and finally, a sIgE rCan s 3 assay using a cytometric bead array (CBA) technique. These diagnostic tests were compared to sIgE industrial hemp by FEIA ImmunoCAP. All four of our diagnostic tests have been found reliable in diagnosing CA (7, 8) and revealed Can s 3 sensitization in up to 75% of CA patients with an anaphylaxis-like

phenotype. Alternatively, the sIgE hemp assay showed, albeit an excellent sensitivity, to be poor reliable because of an important proportion of clinically irrelevant positive results in cannabis tolerant individuals sensitized and/or allergic to pollen.

Importantly, for robust validation purposes, our recent study (8) was restricted to patients with an anaphylaxis-like phenotype on cannabis exposure. However, in general practice, physicians might frequently encounter patients with less compelling histories such as isolated respiratory symptoms and in whom sensitization to Can s 3 sensitization seems less predominant (3). Therefore, this study investigates the diagnostic test performances and inter-test differences between these five diagnostic tests in a larger study population expressing distinct clinical phenotypes on cannabis exposure. Secondly, this study explores the clinical phenotype and biological profile of CA; the sensitization profiles, the severity of cross-reactivities with other plant-derived foods and the significance of cofactors, as patients presenting with nsLTP-related allergies have frequently been reported to necessitate a cofactor to become symptomatic (11, 12).

METHODOLOGY

Inclusion

Patients and controls were included through the outpatients' clinic of Allergology at the Antwerp University Hospital and the Dermatology department of the Ghent University Hospital, Belgium. The local ethics committees of both hospitals approved this study (B300201524055) and patients or their representatives signed an informed consent in accordance with the Declaration of Helsinki. Patients with respiratory, gastro-intestinal, cardiovascular and/or cutaneous symptoms on exposure to cannabis were included. Exposure to cannabis was defined as active smoking, ingestion and/or direct cutaneous contact with cannabis. Patients with generalized symptoms in two or more organ systems were categorized as likely-anaphylactic according to the criteria defined by Sampson et al. (13). Furthermore, two distinct control groups were included; firstly, healthy controls without pollen or nsLTP-sensitization, secondly, a so-called atopic control group comprising patients with a documented pollen allergy with (P+LTP+) or without nsLTP (P+LTP-) sensitization. Controls were further stratified according to exposure and tolerance to cannabis, *i.e.* uneventful exposure. Definitions of pollen and nsLTP sensitizations are shown in **the online repository**.

Information on cannabis allergy, cofactor associated reactions[1] and severity of plant-derived food associated reactions was gathered by history taking and a standardized questionnaire. Three cofactors were defined in this study: the use of alcoholic beverages, non-steroidal anti-inflammatory drugs (NSAIDs) and/or the performance of exercise within three hours preceding occurrence of an allergic reaction. A systemic reaction was defined as grade 1 or higher as defined by the WAO criteria of systemic allergic reactions (14). Patients with chronic spontaneous urticaria, uncontrolled asthma, eosinophilic esophagitis/colitis or systemic mastocytosis were excluded.

[1] Reported plant-derived food allergies with a history of of overt or more severe/generalized reactions in the presence of NSAIDs, alcohol or physical exercise than when the reaction occurred in the absence thereof.

166

167 ***Diagnostic tests***

168 *Basophil activation test*

169 Basophil activation tests (BAT) with rCan s 3 and a crude *Cannabis sativa* extract were performed as
170 detailed in the **online repository** and previously validated as described in detail elsewhere (8). Results
171 were expressed as net percentages of $CD63^+$ basophils, calculated by subtraction of the spontaneous
172 expression from the allergen-induced CD63 expression. A result >5% $CD63^+$ basophils was considered
173 positive as defined by previous validation (8).

174

175 *Total and specific IgE*

176 Total and sIgE to industrial hemp, rBet v 1 and rBet v 2 from birch (*Betula verrucosa*), rPhl p 1 and
177 rPhl p 5b from timothy grass (*Phleum pratense*), nArt v 1 and nArt v 3 of mugwort (*Artemisia vulgaris*),
178 rAra h 9 from peanut (*Arachis hypogeae*), rCor a 8 from hazelnut (*Corylus avellana*), rMal d 3 from
179 apple (*Malus domesticus*), rJug r 3 from walnut (Juglans *regia),* rPru p 3 from peach (*Prunus persica*),
180 rPar j 2 from wall pellitory (*Parietaria judaica*) and nAna 2 c (bromelain from *Ananas comosus*), as a
181 marker for sensitization to cross-reactive carbohydrate determinants (CCD), were quantified by FEIA
182 ImmunoCAP technique (ThermoFisher Scientific, Uppsala Sweden) according to the manufacturer's
183 instructions. All sIgE assays are readily available, except for industrial hemp, which is available for
184 research use only and was kindly provided by ThermoFisher Scientific. Specific IgE to rCan s 3 was
185 quantified using a flow cytometric bead array (CBA) technique (BD Biosciences, Franklin Lakes, NJ,
186 USA). The method was validated as previously described (8). Results $\geq$ 0.10 kU_A/L were considered
187 positive.

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Skin prick tests (SPT)

SPT implied a nCan s 3-rich CS extract that was prepared as described elsewhere (7). SPT responses were read after 15 minutes and considered positive when the wheal exceeded 3 mm (largest diameter). A positive control with histamine (10 mg/mL) and a negative saline control without allergen (ALK-Abello Ltd, Berkshire, United Kingdom) were performed to rule out non-responsiveness or dermographism of the skin, respectively.

Statistical analysis

IBM SPSS version 24.0 (IBM, Chicago, Ill., US) software was used for data analysis. Data are expressed as medians and interquartile ranges. Non-parametric tests and $\chi 2$ analysis were used where appropriate. Test performances were compared by using McNemar's test. Where needed, missing values were imputed by using a multiple-imputation model with five imputations based on all available information which were subsequently pooled in SPSS. Significance levels for the pooled imputed data were calculated according to the method described by Schafer et al. (15). A p-value <0.05 was regarded as statistically significant.

RESULTS

Demographics

As shown in figure 1, a total of 371 individuals were included; 120 patients with symptoms on cannabis exposure **(CA)** of which 21% (n=25) were classified as likely-anaphylactic **(CA-A)**, 19% (n=23) presented with mild and localized respiratory and cutaneous symptoms **(CA-RC)**, 51% reported isolated respiratory symptoms **(CA-R)** and 9% report isolated cutaneous symptoms **(CA-C)**. The remaining 251 participants were control individuals, either healthy controls **(HC)** or atopics with a pollen sensitization **(P+LTP+),** with or without nsLTP sensitizations **(P+LTP-)**. As displayed by figure 1, 50-60% of each control group reported regular use of cannabis in the past 12 months without any symptoms apart from the known psychoactive effects, the other half reported no previous contact with cannabis. All CA patients displayed symptoms during active smoking, except for three patients denying any previous direct contact with cannabis (no active smoking, ingestion or cutaneous contact) but who had experienced symptoms on passive exposure to cannabis smoke. Furthermore, in total 34 CA patients reported respiratory and/or cutaneous symptoms on isolated passive exposure to cannabis smoke apart from symptoms on active smoking. Finally, four patients also reported symptoms on ingestion of cannabis processed as space cake, cannabis seeds or oil, resulting in anaphylaxis in two of the cases.

The individual symptoms reported by CA-A are shown in table 1E of **the online repository**. In summary, 23/25 reported respiratory symptoms and/or cutaneous symptoms, four patients also mentioned cardiovascular symptoms comprising palpitations and/or hypotension and finally, five patients additionally reported gastro-intestinal symptoms comprising abdominal pain, nausea and vomiting

Table 1 displays demographic data of the different study groups revealing similar age, sex-ratios and asthma prevalence in all groups. In contrast, atopic dermatitis and elevated total IgE values were significantly more prevalent in the P+LTP+ group than in the CA group and in the P+LTP- group. Total IgE was also significantly higher in the P+LTP- group compared to the CA group. Finally, importantly, 84% of CA patients showed a pollen sensitization and 72% an nsLTP sensitization. It is important to note that pollen sensitization was predominated by Bet v 1; 72% of CA sensitized) and 79% of P+LTP+ exhibited a Bet v 1 sensitization.

Performance of cannabis diagnostic tests.

Figure 2 shows the individual results of five different cannabis diagnostic tests: the sIgE industrial hemp, sIgE rCan s 3 CBA, SPT with a nCan s 3-rich extract and the BAT with both rCan s 3 and a crude cannabis extract. Table 2 compares the test performances. For more details on the difference in test performance for sIgE rCan s 3 and sIgE hemp (considering 0.10 or 0.35 kU_A/L cut-off), the reader is referred to figure 1E and table 2E of **the online repository**)

First of all, test performances showed important variances between the different clinical CA groups. The three Can s 3-based diagnostic methods (BAT, sIgE and SPT) displayed a similar sensitivity; 63-72% in CA-A (45-58% in the total CA group) and a similar specificity (81-87% in the total CA group). However, up to 37% (n=34) of P+LTP+ showed clinically irrelevant Can s 3 sensitizations (measured by BAT, sIgE or SPT): 20/34 reported tolerance to active cannabis use, 14/34 reported no previous cannabis contact. In comparison, the sIgE rCan s 3 and BAT rCan s 3 showed no clinically irrelevant positive results in pollen sensitized individuals without nsLTP sensitizations (P+LTP-).

Secondly, the sIgE industrial hemp displayed a significantly higher sensitivity, up to 82% ($p<0.01$) in the total CA group compared to the Can s 3-based diagnostic tests (45-58%). However, sIgE hemp also demonstrated a significantly higher number of clinically irrelevant positive results in P+LTP- and

P+LTP+ *i.e.* 51-82% respectively compared to 0-25% for the Can s 3 diagnostic tests (all p<0.01). Interestingly, an increase in sensitivity as seen in the sIgE hemp was not found in the BAT with a crude cannabis extract. The latter reached an overall sensitivity of 49% in the total CA group which was not superior to the Can s 3-based assays. Additionally, the BAT with the crude extract was not superior to the Can s 3 diagnostic tests in terms of specificity either, showing 19-38% of clinically irrelevant positive results in P+LTP- and P+LTP+. Collectively, for all diagnostic techniques, the majority of clinically irrelevant results were seen in the P+LTP+ group.

In summary, when all different clinical CA groups are considered (analyses B in table 2), it appears that the three Can s 3-based diagnostic tests did not significantly differ in performance and had the best combined positive and negative predictive values around 80% and 60%, respectively. The sIgE industrial hemp lacked specificity whereas the BAT crude CS extract showed no advantage over the Can s 3-based diagnostic tests.

The clinical phenotype and biological profile of cannabis allergy

Figure 3 compares different clinical and *in vitro* characteristics for the different CA profiles and the control groups. The most prominent differences were found between CA-A and CA-R with significantly higher numbers of Pru p 3, Mal d 3, Cor a 8, Jug r 3, Tri a 14, Art v 3 sensitizations (all p<0.01) in CA-A than in CA-R. Furthermore, CA-A showed a higher prevalence of systemic reactions to plant-derived foods (72% compared to 40%, p=0.02) and cofactor mediated allergic reactions (50% compared to 18%, p=0.01) compared to CA-R. Additionally, CA-C and CA-RC showed a single difference from CA-A, namely a considerably lower prevalence of systemic reactions to plant-derived foods (71% in CA-A compared to 43% in CA-RC (p<0.01) and 18% in CA-C (p=0.08)). It appears that none of the clinical nor *in vitro* parameters displayed significant differences between CA-R, CA-C and CA-RC.

Regarding, the comparison of Can s 3 sensitized and non-sensitized CA (as demonstrated in table 3E in of **the online repository**), it became clear that Can s 3+CA had a significantly higher prevalence of other nsLTP sensitizations (92%) than Can s 3-CA (39%) with higher frequencies of all measured nsLTPs (all $p<0.01$), except for Par j 2. Also, Can s 3+CA displayed higher frequencies of pollen sensitizations than Can s 3-CA (92% compared to 74%) with significant more Bet v 1 sensitizations in the Can s 3 sensitized population. Additionally, Can s 3+ CA showed a considerably higher prevalence of cofactor mediated allergic reactions when compared to Can s 3-CA (41% vs. 12%; $p<0.01$).

In a further analysis, the complete CA group was compared to the P+LTP+ group (as demonstrated in table 4E in of **the online repository**). This exploration revealed a significant ($p<0.01$) higher prevalence of Can s 3 sensitizations in CA (63%) compared to P+LTP+ (35%). Furthermore, a significantly lower prevalence of Pru p 3, Mal d 3, Jug r 3, Par j 2 (all $p<0.01$) but also bromelain ($p=0.02$) and Phl p 1 ($p<0.01$) sensitizations were seen in the CA group compared to P+LTP+. Finally, as already mentioned in the demographic paragraph, significantly ($p<0.01$) more eczema was reported in the P+LTP+ group than the CA group and subsequently total IgE values were also significantly higher in P+LTP+ than in the CA group ($p<0.01$). Although, there was no significant difference between CA and P+LTP+ concerning the frequency of systemic reactions to plant-derived foods ($p=0.11$), CA-A did show double the frequency of systemic reactions to plant-derived foods than P+LTP+ (71% vs. 35%, $p<0.01$).

DISCUSSION

To our best knowledge, this is the largest survey exploring diagnostic performances in different clinical phenotypes of *Cannabis sativa* allergy. Along with the observation that the diagnostic utilities of our tests depend on the clinical presentation, it appears that the cannabis allergy profile in this study population has the following peculiarities:

Primarily, in terms of practicality, efficiency and standardization, the SPT with a nCans 3-rich extract and, the sIgE rCan s 3 are the easiest and fastest tests to confirm a clinical suspicion of CA, both equally reliable. However, due to unavailability, in clinical practice, physicians will need to rely upon other tests to screen patients with a convincing history. As a matter of fact, according to our data it seems that the sIgE hemp assay (available upon request by Thermo Fisher) could serve as a suitable diagnostic in central Europe to exclude cannabis allergy, because a negative test result reduces the risk of CA considerably (only 18% of CA have negative sIgE hemp results). Alternatively, patients with a convincing history together with a positive sIgE hemp should undergo additional testing in order to elucidate the clinical significance of the hemp solid phase assay. In addition, exploration of different cut-offs for the sIgE rCan s 3 and hemp shows that sensitivity of both tests decreases with around 10% in the total CA population. Nevertheless, sensitivity to detect CA-A remains the same for both. Even though specificity of sIgE hemp almost doubles, it still only reaches a maximum of 60%, which is not ideal.

However, none of our diagnostic tests appear absolutely predictive for the clinical outcome. Nevertheless, for the time being, based upon our findings, we propose to perform the SPT with a nCan s 3-rich extract or quantify sIgE rCan s 3 keeping in mind that Can s 3 does not cover the entire IgE sensitization profile, particularly in patients with a less severe/pronounced phenotype. Additionally, it could be questioned whether Can s 3-negative patients, especially if reporting only milder symptoms to cannabis, should effectively be categorized as CA, since their symptoms could result from non-

specific skin or airway irritation. Furthermore, due to ethical and legal limitations, it is impossible to confirm CA by an oral or respiratory challenge. Considering this hypothesis, it follows that the actual test performances are possibly underestimated in this study and that Can s 3 might even play a more prominent role than already suspected.

Furthermore, it is likely that performances of a Can s 3 assay display regional differences due to geographic differences in IgE reactivity profiles. The reason(s) why Can s 3 negative CA patients go undetected in the BAT with the full CS extract remain(s) elusive but could relate to a sensitization to allergens that are poorly present in our crude extract or do not resist our current extraction procedure. Moreover, the low presence and the physicochemical properties of the constituent allergens might also explain the different sensitization profiles in the distinct phenotypes, namely the lower prevalence of nsLTP sensitizations in CA-R compared to CA-A.

Secondly, although historically sensitization to nsLTP has mainly been recognized to occur in the Mediterranean region, characterized by severe reactions and governed by peach (10, 16), more recent data has accumulated showing that sensitization to nsLTP might also occur in other European regions and frequently go asymptomatic with uncertainties about the route(s) of sensitization (17-19). In this survey we confirm that nsLTP sensitization occurs frequently in CA and Can s 3 is a major allergen in CA-A patients but CA also implies a risk of systemic reactions to plant-derived foods and cofactor mediated reactions. Furthermore, Can s 3 sensitization can occur as a result of *in vitro* cross-reactivity to nsLTPs from taxonomically related or more distant sources such as pollen and/or plant-derived foods as suggested by the Can s 3 positive P+LTP+ patients without any previous cannabis contact. On the other hand, it seems that a Can s 3 sensitization in CA patients might also mirror a primary sensitization instead of only *in vitro* cross-reactivity as indicated by the significant higher prevalence of Can 3 and lower prevalence of Pru p 3, Mal d 3, Jug r 3 and Par j 2 sensitizations in CA compared to P+LTP+.

Another important fact to highlight is that, because of the lack of data on the true prevalence of CA, it is likely that the number of patients per study group in this survey do not necessarily reflect the true prevalence of CA. Therefore, the test performances would differ dependent on characteristics of the tested population and the geographic prevalence of CA itself.

Finally, this study was not designed to explore the different individual types of plant-derived food allergies, as symptoms to different plant-derived foods were only assessed by a standardized questionnaire complemented with a history taking without systematic confirmatory testing. However, it would be interesting to further explore the actual differences in individual plant-derived food allergies within CA such as the differences in symptom-severity with and without peel, the types of plant-derived foods eliciting allergic symptoms but also the comparison of these factors between CA and other nsLTP-sensitized individuals.

In conclusion, this study is the largest study exploring diagnostic test performance, clinical phenotypes and biological profiles of CA. It shows that the most effective and practical tests to confirm a clinical suspicion of CA are the the SPT with a nCan s 3-rich extract and sIgE rCan s 3. Both tests display a positive and negative predictive value of about 80% and 60% respectively. However, due to current unavailability, screening with sIgE hemp could be a suitable tool in symptomatic cannabis users, because a negative result considerably reduces the likelihood of CA. Alternatively, we dissuade the general use of sIgE hemp to diagnose CA, mainly because of its limited PPV. Furthermore, we show that Can s 3 is a major allergen in patients with a history of likely-anaphylaxis upon cannabis exposure and, like other nsLTP associated allergies, CA might indicate a risk of systemic reactions to plant-derived foods and cofactor mediated reactions. Because around 30% of CA-A and even higher proportions in other, milder CA groups are not sensitized to Can s 3, it is likely that other cannabis allergens might play a role in CA. Further studies are thus warranted to identify and express other CA allergens which could then be applied to spike natural extracts or to compose mixtures of allergens.

373 Lastly, additional research should further explore the nature of plant-derived food allergies in CA as
374 this study was not designed to evaluate specific plant-derived food allergies in CA.

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FIGURE LEGENDS

Figure 1 Inclusion overview

CA=cannabis allergic patients, HC=healthy controls, P+LTP- pollen sensitized controls without an nsLTP sensitization, P+LTP+=pollen and nsLTP sensitized controls, CS=cannabis sativa

Figure 2 individual test results: legend

***A**. Dotplots showing HC, P+LTP+, P+LTP- and CA. **B.** Dotplots for the different CA groups: CA-A, CA-RC, CA-R, CA-C. percentages reflect the proportion of positive results horizontal lines represent group mean. += patients with ≥15% response to anti-IgE stimulation(=non-responders). 55/371 (15%) were classified as non-responders;15 HC, 12 P+LTP-, 14 P+LTP+ and 14 CA.*

Figure 3 overview of clinical and in vitro parameters: legend

*Color variations represent increasing frequencies of positive results for the shown variable e.g.frequency of asthmatcst (sIgE measurements are shown as percentage "sensitized/not sensitized). * measured by BAT or sIgE rCan s 3. TOL=tolerant, SR=systemic reaction defined by generalized and severe symptoms in at least one organ system (14), OAS= oral allergy syndrome defined as localizedand mild oropharyngeal symptoms without generalization.*

TABLES

Table 1 Demographic data

		HC=62	CA=120	P+LTP-=90	P+LTP+=99
Age (years)	**Median**	28.3	29.2	28.8	29.9
	Q_{25}-Q_{75}	(24.8-36.1)	(25.1-35.2)	(22.9-37.7)	(20.1-37.1)
Sex	**(% male)**	42%	48%	37%	49%
	Eczema[1]	0%	37%	37%	54%
	Asthma[2]	5%	30%	28%	39%
Total IgE (kU/L)	**Median**	16.7	247.4	126.0	424.5
	Q_{25}-Q_{75}	(6.0-46.5)	(83.0-495.0)	(65.0-314.0)	(147.0-1054.0)
	Pollen sensitization[3]	0%	84%	100%	100%
	Ns-LTP sensitization[4]	0%	72%	0%	100%

[1]According to patient recollection and recent use of topical CS. [2]according to patient recollection. [3]At least one of the following sIgE's ≥ 0.1 kU_A/L: rBet v 1, rBet v 2, nArt v 1, rPhl p 1, rPhl 5b. [4]At least one of the following sIgE's ≥0.1 kU_A/L: rPru p 3, rMal d 3, rJug r 3, rAra h 9, rCor a 8, nArt v 3, rPar j 2, rTri a 14.

Table 2: Test performance

A	sIgE hemp	sIgE rCan s 3	BAT rCan s 3	BAT crude CS extract	SPT nCan s 3-rich extract
SENSITIVITY	86% (66-97)	63% (41-81)	71% (48-89)	63% (38-84)	72% (51-89)
SPECIFICITY	32% (20-45)	87% (78-93)	85% (76-92)	67% (55-78)	81% (71-88)
PPV	33% (28-38)	56% (40-70)	54% (39-67)	35% (25-47)	51% (39-63)
NPV	86% (66-95)	90% (84-94)	93% (86-96)	87% (78-92)	91% (84-95)
LHR+	1.3 (1.0-1.6)	4.7 (2.6-8.7)	4.8 (2.7-8.6)	1.9 (1.2-3.1)	3.7 (2.3-6.0)
LHR-	0.4 (0.1-1.3)	0.40 (0.3-0.7)	0.3 (0.2-0.7)	0.6 (0.3-1.0)	0.4 (0.2-0.7)
B	**sIgE hemp**	**sIgE rCan s 3**	**BAT rCan s 3**	**BAT crude CS extract**	**SPT nCan s 3-rich extract**
SENSITIVITY	82% (74-89)	47% (38-56)	45% (35-55)	49% (37-60)	58% (49-67)
SPECIFICITY	32% (20-45)	87% (78-93)	85% (76-92)	67% (55-78)	81% (71-88)
PPV	70% (66-74)	82% (72-89)	78% (67-86)	64% (54-73)	80% (72-86)
NPV	47% (34-61)	56% (51-60)	57% (52-62)	52% (46-59)	58% (53-64)
LHR+	1.2 (1.0-1.5)	3.5 (2.0-6.2)	3.0 (1.8-5.2)	1.5 (1.0-2.2)	3.0 (1.9-4.7)
LHR-	0.6 (0.3-1.0)	0.60 (0.5-0.7)	0.7 (0.5-0.8)	0.8 (0.6-1.0)	0.5 (0.4-0.7)

A: *calculations based upon CA-A group versus cannabis tolerant P+LTP- and P+LTP+.* ***B:*** *calculations based upon the whole CA group (respiratory and/or cutaneous symptoms) versus cannabis tolerant P+LTP- and P+LTP+. Test performance for both BAT's was calculated by considering both responders and non-responders to anti-IgE. PPV and NPV= positive and negative predictive values respectively, LHR+/-= positive and negative likelihood ratio's respectively.*