

Exploring the Diagnosis and Profile of Cannabis Allergy



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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 nonspecific lipid transfer protein present in *Cannabis sativa*.

What does this article add to our knowledge? This article compares the performance of multiple cannabis diagnostic methods and explores clinical and *in vitro* characteristics of cannabis allergy in one of the largest cannabis allergic populations described up until now.

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.

BACKGROUND: Cannabis allergy (CA) has mainly been attributed to Can s 3, the nonspecific lipid transfer protein (nsLTP) of *Cannabis sativa*. Nevertheless, standardized diagnostic tests are lacking and research on CA is scarce. **OBJECTIVE:** To explore the performance of 5 cannabis diagnostic tests and the phenotypic profile of CA. **METHODS:** A total of 120 patients with CA were included and stratified according to the nature of their cannabis-related symptoms; 62 healthy and 189 atopic controls were included.

Specific IgE (sIgE) hemp, sIgE and basophil activation test (BAT) with a recombinant Can s 3 protein from *Cannabis sativa* (rCan s 3), BAT with a crude cannabis extract, and a skin prick test (SPT) with an nCan s 3-rich cannabis extract were performed. Clinical information was based on patient history and a standardized questionnaire. RESULTS: First, 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%.

CONCLUSIONS: The most effective and practical tests to confirm CA are the SPT with an nCan s 3-rich extract and the sIgE rCan s 3. Can s 3 sensitization 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. © 2018 Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2019;7:983-9)

Key words: Cannabis allergy; Diagnosis; BAT; Specific IgE; Skin prick test; Can s 3; nsLTP; Cofactor; Basophil; Anaphylaxis; Hemp

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This work was supported by the Agency for Innovation by Science and Technology (grant number 140185). Furthermore, V. Sabato is a senior clinical researcher of the Research Foundation Flanders/Fonds Wetenschappelijk Onderzoek (FWO: 1804518N). D. G. Ebo is a senior clinical researcher of the Research Foundation Flanders/Fonds Wetenschappelijk Onderzoek (FWO: 1800614N). A. L. Van Gasse is a fellow of the Research Foundation Flanders/Fonds Wetenschappelijk Onderzoek (FWO: 1113617N).

Conflicts of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication July 17, 2018; revised August 17, 2018; accepted for publication September 15, 2018.

Available online September 29, 2018.

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2213-2198

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<https://doi.org/10.1016/j.jaip.2018.09.017>

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

Abbreviations used

BAT-	Basophil activation test
CA-	Cannabis allergy
CA-A-	Likely-anaphylaxis to cannabis
CA-C-	Cutaneous symptoms to cannabis
CA-R-	Respiratory symptoms to cannabis
CA-RC-	Localized respiratory and cutaneous symptoms to cannabis
CBA-	Cytometric bead array
CS-	Cannabis sativa
HC-	Healthy controls
NPV-	Negative predictive value
nsLTP-	Nonspecific lipid transfer protein
P+LTP--	Atopic pollen-sensitized participants without an nsLTP sensitization
P+LTP+-	Atopic pollen and nsLTP-sensitized participants
PPV-	Positive predictive value
rCan s 3-	Recombinant Can s 3 protein from CS
sIgE-	Specific IgE
SPT-	Skin prick test

symptoms and a variety of cross-reactive plant-derived food allergies, mainly attributed to a Can s 3 sensitization, the nonspecific lipid transfer protein (nsLTP) from *Cannabis sativa* (CS). 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 ribulose-1,5-bisphosphate carboxylase/oxygenase, 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 2 preliminary studies, we have standardized and presented initial performance results using 4 different cannabis diagnostic tests, namely, a basophil activation test (BAT) with recombinant Can s 3 protein from CS (rCan s 3), a BAT with a crude CS extract, a skin prick test (SPT) with an nCan s 3-rich extract, and finally, a specific IgE (sIgE) rCan s 3 assay using a cytometric bead array (CBA) technique. These diagnostic tests were compared with sIgE industrial hemp by fluorescence enzyme immunoassay (FEIA) ImmunoCAP. All 4 of our diagnostic tests have been found reliable in diagnosing CA^{7,8} and revealed Can s 3 sensitization in up to 75% of patients with CA with an anaphylaxis-like phenotype. Alternatively, the sIgE hemp assay, albeit displaying an excellent sensitivity, was shown to be poorly reliable because of an important proportion of clinically irrelevant positive results in cannabis-tolerant individuals sensitized to pollen and/or nsLTPs.

Importantly, for robust validation purposes, our recent study⁸ 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 Can s 3 sensitization seems less predominant.³ Therefore, this study investigates the diagnostic test performances and intertest differences between these 5 diagnostic tests in a larger study population expressing distinct clinical phenotypes on cannabis exposure. Secondly, this study explores the clinical and molecular characteristics 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}

METHODS**Inclusion**

Patients and controls were included through the outpatients' clinic of Allergy 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, gastrointestinal, 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 2 or more organ systems were categorized as likely-anaphylactic according to the criteria defined by Sampson et al.¹³ Furthermore, 2 distinct control groups were included: first, healthy controls (HC) 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, that is, uneventful exposure. Definitions of pollen and nsLTP sensitizations are shown in this article's Online Repository at www.jaci-inpractice.org.

Information on CA, cofactor-associated reactions (reported plant-derived food allergies with a history of overt or more severe/generalized reactions in the presence of nonsteroidal anti-inflammatory drugs, alcohol, or physical exercise than when the reaction occurred in the absence thereof), 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, nonsteroidal anti-inflammatory drugs, and/or the performance of exercise within 3 hours preceding occurrence of an allergic reaction. A systemic reaction was defined as grade 1 or higher as defined by the World Allergy Organization criteria of systemic allergic reactions.¹⁴ Patients with chronic spontaneous urticaria, uncontrolled asthma, eosinophilic esophagitis/colitis, or systemic mastocytosis were excluded.

Diagnostic tests

Basophil activation test. BAT with rCan s 3 and a crude CS extract were performed, as detailed in this article's Online Repository at www.jaci-inpractice.org and previously validated as described in detail elsewhere.⁸ Results were expressed as net percentages of CD63⁺ basophils, calculated by subtraction of the spontaneous expression from the allergen-induced CD63 expression. A result

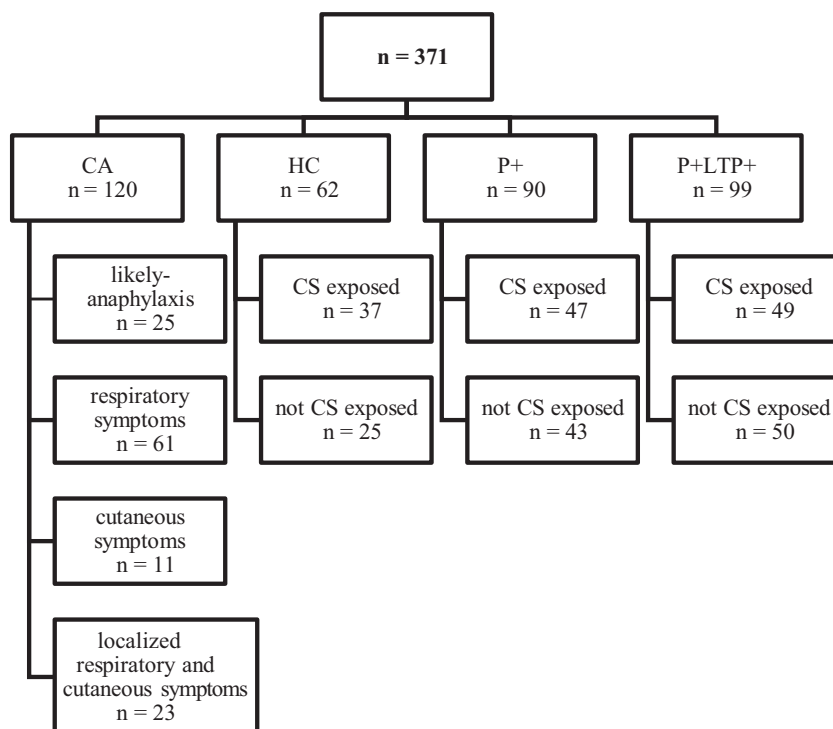


FIGURE 1. Inclusion overview. CA, Cannabis allergic patients; CS, *Cannabis sativa*; HC, healthy controls; P+LTP−, pollen-sensitized controls without an nsLTP sensitization; P+LTP+, pollen and nsLTP-sensitized controls.

>5% CD63⁺ basophils was considered positive as defined by previous validation.⁸

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

Skin prick tests. SPT implied an 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) software was used for data analysis. Data are expressed as medians and interquartile ranges.

Nonparametric tests and χ^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 5 imputations based on all available information that 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 of <.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 HC or atopics with a pollen sensitization (P+LTP+), with or without nsLTP sensitizations (P+LTP−). As displayed in Figure 1, 50% to 60% of each control group reported the 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 patients with CA displayed symptoms during active smoking, except for 3 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 patients with CA reported respiratory and/or cutaneous symptoms on

TABLE I. Demographic data

	HC = 62	CA = 120	P+LTP- = 90	P+LTP+ = 99
Age (y), median (Q ₂₅ -Q ₇₅)	28.3 (24.8-36.1)	29.2 (25.1-35.2)	28.8 (22.9-37.7)	29.9 (20.1-37.1)
Sex				
(% male)	42%	48%	37%	49%
Eczema*	0%	37%	37%	54%
Asthma†	5%	30%	28%	39%
Total IgE (kU/L), median (Q ₂₅ -Q ₇₅)	16.7 (6.0-46.5)	247.4 (83.0-495.0)	126.0 (65.0-314.0)	424.5 (147.0-1054.0)
Pollen sensitization‡	0%	84%	100%	100%
NsLTP sensitization§	0%	72%	0%	100%

CA, Cannabis allergy; CS, *Cannabis sativa*; HC, healthy controls; P+LTP+, pollen and nsLTP-sensitized controls; P+LTP-, pollen-sensitized controls without an nsLTP sensitization; nsLTP, nonspecific lipid transfer protein.

*According to patient recollection and recent use of topical CS.

†According to patient recollection.

‡At least one of the following sIgEs ≥ 0.1 kU_A/L: rBet v 1, rBet v 2, nArt v 1, rPhl p 1, rPhl 5b.

§At least one of the following sIgEs ≥ 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.

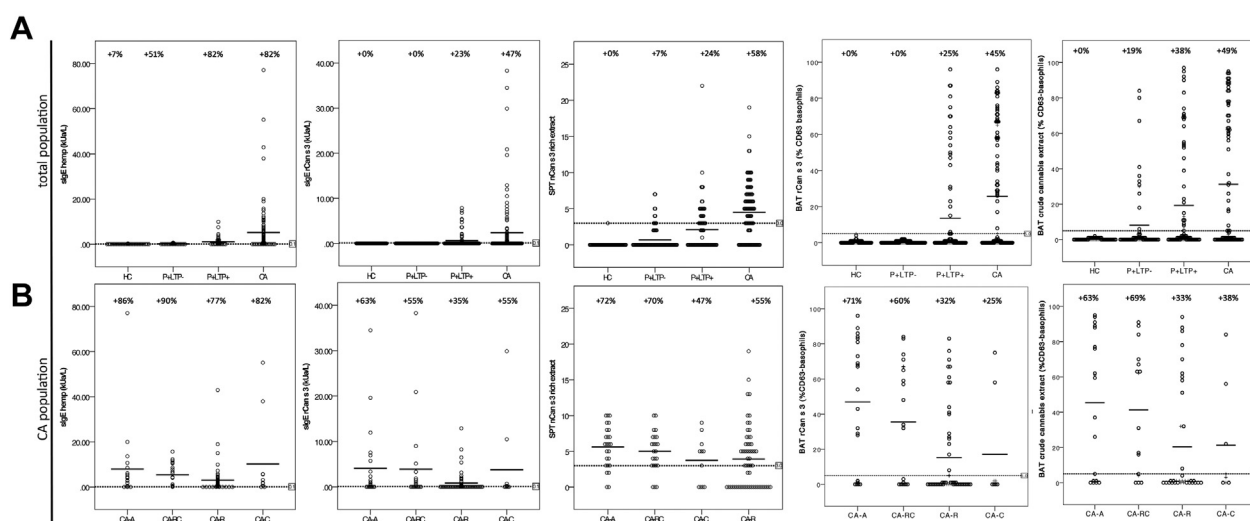


FIGURE 2. Individual test results. **A**, Dot plots showing healthy controls (HC), pollen and nsLTP-sensitized controls (P+LTP+), pollen-sensitized controls without an nsLTP sensitization (P+LTP-), and cannabis allergy (CA). **B**, Dot plots for the different CA groups: CA-A, CA-RC, CA-R, and CA-C. Percentages reflect the proportion of positive results and horizontal lines represent group mean. + indicates patients with <15% response to anti-IgE stimulation (nonresponders). Of 371, 55 (15%) were classified as nonresponders: 15 HC, 12 P+LTP-, 14 P+LTP+, and 14 CA. BAT, Basophil activation test; CA-A, likely-anaphylaxis to cannabis; CA-C, cutaneous symptoms to cannabis; CA-R, respiratory symptoms to cannabis; CA-RC, localized respiratory and cutaneous symptoms to cannabis; slgE, specific IgE; SPT, skin prick test.

isolated passive exposure to cannabis smoke apart from symptoms on active smoking. Finally, 4 patients also reported symptoms on ingestion of cannabis processed as space cake, cannabis seeds or oil, resulting in anaphylaxis in 2 of the cases.

The individual symptoms reported by CA-A are shown in Table E1 in this article's Online Repository at www.jaci-inpractice.org. In summary, 23 of 25 reported respiratory symptoms and/or cutaneous symptoms, 4 patients also mentioned cardiovascular symptoms comprising palpitations and/or hypotension, and finally, 5 patients additionally reported gastrointestinal symptoms comprising abdominal pain, nausea, and vomiting.

Table I 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 with the CA group. Finally, importantly, 84% of patients with CA 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 5 different cannabis diagnostic tests: the slgE industrial hemp, slgE rCan s 3 CBA, SPT with an nCan s 3-rich extract, and the BAT with both rCan

TABLE II. Test performance

	sIgE hemp	sIgE rCan s 3	BAT rCan s 3	BAT crude CS extract	SPT nCan s 3-rich extract
A					
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					
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 on CA-A group vs cannabis-tolerant P+LTP– and P+LTP+.

B: calculations based on the whole CA group (respiratory and/or cutaneous symptoms) vs cannabis-tolerant P+LTP– and P+LTP+. Test performance for both BATs was calculated by considering both responders and nonresponders to anti-IgE.

BAT, Basophil activation test; CA-A, likely-anaphylaxis to cannabis; CS, *Cannabis sativa*; NPV, negative predictive value; PPV, positive predictive value; LHR+, positive likelihood ratio; LHR–, negative likelihood ratio; P+LTP+, pollen and nsLTP-sensitized controls; P+LTP–, pollen-sensitized controls without an nsLTP sensitization; sIgE, specific IgE; SPT, skin prick test.

s 3 and a crude cannabis extract. Table II 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 cutoff), the reader is referred to Figure E1 and Table E2 in this article's Online Repository at www.jaci-inpractice.org.

First of all, test performances showed important variances between the different clinical CA groups. The 3 Can s 3-based diagnostic methods (BAT, sIgE, and SPT) displayed a similar sensitivity, 63% to 72% in CA-A (45% to 58% in the total CA group), and a similar specificity (81% to 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 of 34 reported tolerance to active cannabis use and 14 of 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% ($P < .01$) in the total CA group compared with the Can s 3-based diagnostic tests (45% to 58%). However, sIgE hemp also demonstrated a significantly higher number of clinically irrelevant positive results in P+LTP– and P+LTP+, that is, 51% to 82% respectively compared with 0% to 25% for the Can s 3 diagnostic tests (all $P < .01$). 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 that was not superior to the Can s 3-based assays. In addition, the BAT with the crude extract was not superior to the Can s 3 diagnostic tests in terms of specificity either, showing 19% to 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 (analysis B in Table II), it appears that the 3 Can s

3-based diagnostic tests did not significantly differ in performance and had the best combined positive predictive value (PPV) and negative predictive value (NPV) of 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 and molecular characteristics of

CA. 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 $P < .01$) in CA-A than in CA-R. Furthermore, CA-A showed a higher prevalence of systemic reactions to plant-derived foods (72% compared with 40%, $P = .02$) and cofactor-mediated allergic reactions (50% compared with 18%, $P = .01$) compared with CA-R. In addition, 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 with 43% in CA-RC [$P < .01$] and 18% in CA-C [$P = .08$]). 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 E3 in this article's Online Repository at www.jaci-inpractice.org), 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 $P < .01$), except for Par j 2. Also, Can s 3+CA displayed higher frequencies of pollen sensitizations than Can s 3-CA (92% compared with 74%) with significantly more Bet v 1 sensitizations in the Can s 3-sensitized population. In addition, Can s 3+CA showed a considerably higher prevalence of cofactor-mediated allergic reactions when compared with Can s 3-CA (41% vs 12%; $P < .01$).

In a further analysis, the complete CA group was compared with the P+LTP+ group (as demonstrated in Table E4 in this

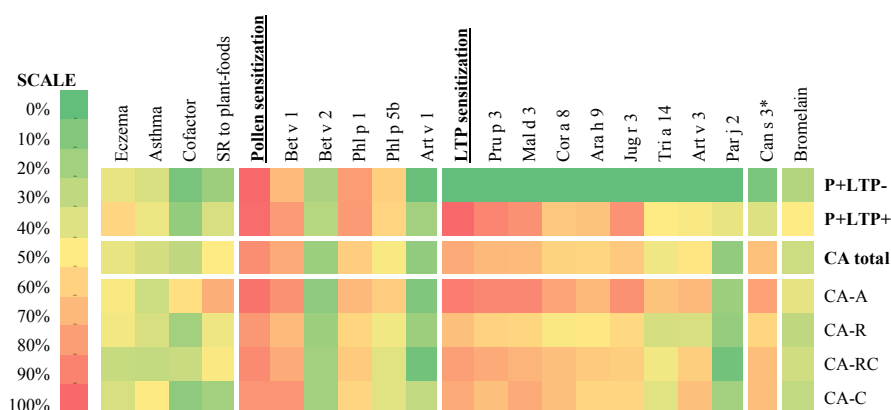


FIGURE 3. Overview of clinical and *in vitro* parameters. Color variations represent increasing frequencies of positive results for the shown variable. For example, frequency of asthmatics (specific IgE [sIgE] measurements are shown as percentage “sensitized/not sensitized”). *Measured by BAT or sIgE rCan s 3.BAT, Basophil activation test; CA-A, likely-anaphylaxis to cannabis; CA-C, cutaneous symptoms to cannabis; CA-R, respiratory symptoms to cannabis; CA-RC, localized respiratory and cutaneous symptoms to cannabis; LTP, lipid transfer protein; OAS, oral allergy syndrome defined as localized and mild oropharyngeal symptoms without generalization; P+LTP-, pollen-sensitized controls without an nsLTP sensitization; P+LTP+, pollen and nsLTP-sensitized controls; SR, systemic reaction defined by generalized and severe symptoms in at least 1 organ system¹⁴; TOL, tolerant.

article’s Online Repository at www.jaci-inpractice.org). This exploration revealed a significantly ($P < .01$) higher prevalence of Can s 3 sensitizations in CA (63%) compared with P+LTP+ (35%). Furthermore, a significantly lower prevalence of Pru p 3, Mal d 3, Jug r 3, Par j 2 (all $P < .01$) but also bromelain ($P = .02$) and Phl p 1 ($P < .01$) sensitizations was seen in the CA group compared with P+LTP+. Finally, as already mentioned in the demographic paragraph, significantly ($P < .01$) 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 ($P < .01$). Although there was no significant difference between CA and P+LTP+ concerning the frequency of systemic reactions to plant-derived foods ($P = .11$), CA-A did show double the frequency of systemic reactions to plant-derived foods than P+LTP+ (71% vs 35%, $P < .01$).

DISCUSSION

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

Primarily, in terms of practicality, efficiency, and standardization, the SPT with an nCan s 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, because of unavailability, in clinical practice, physicians will need to rely on 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 on request from ThermoFisher) could serve as a suitable diagnostic in central Europe to exclude CA, 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 to elucidate the clinical significance of the hemp solid phase assay. In addition, exploration of different cutoffs for the sIgE rCan s 3 and hemp shows that, when considering the 0.35 kUa/L cutoff, sensitivity of both tests decreases by around 10% in the total CA population. Nevertheless, sensitivity to detect CA-A remains the same for both. Even though the 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, on the basis of our findings, we propose to perform the SPT with an 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. In addition, it could be questioned whether Can s 3-negative patients, especially if reporting only milder symptoms to cannabis, should effectively be categorized as CA, because their symptoms could result from nonspecific skin or airway irritation. Furthermore, because of 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 with 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 have accumulated showing that sensitization to nsLTP might also occur in other European regions and frequently remain 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 patients with CA-A, 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 patients with CA might also mirror a primary sensitization instead of only *in vitro* cross-reactivity as indicated by the significantly 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 with 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 depending on the 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 SPT with an nCan s 3-rich extract and sIgE rCan s 3. Both tests display a PPV and NPV of approximately 80% and 60%, respectively. However, because of 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 on 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 that could then be applied to spike natural extracts or to compose mixtures of allergens. Lastly, additional research should further explore the nature of plant-derived food allergies in CA as this study was not designed to evaluate specific plant-derived food allergies in CA.

Acknowledgments

We thank B. Van Camp, head of the Unit “drug production,” Central Drug Department, Directorate of organized crime, Belgian Federal Judicial police for his help with providing the necessary plant materials. We would also like to thank Mrs. K. Vandebos and N. Maes, our study nurses, for their help in the performance of the skin prick tests and blood sampling. Finally, our gratitude goes out to Mrs. K. Wouters, for her help and statistical advice.

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METHODS

Patient stratification. Pollen sensitization was defined by at least 1 positive specific IgE (sIgE) result to the following recombinant (r) or native (n) allergens: rBet v 1 and rBet v 2 from birch (*Betula verrucosa*), rPhl p 1 and rPhl p 5b from timothy grass (*Phleum pratense*), and/or nArt v 1 of mugwort (*Artemisia vulgaris*). Nonspecific lipid transfer protein (nsLTP) sensitization was documented by at least 1 positive sIgE result to the following nsLTP allergens: rAra h 9 from peanut (*Arachis hypogaea*), rCor a 8 from hazelnut (*Corylus avellana*), rMal d 3 from apple (*Malus domestica*), rJug r 3 from walnut (*Juglans regia*), rPru p 3 from peach (*Prunus persica*), nArt v 3 from mugwort (*Artemisia vulgaris*), and rPar j 2 from wall pellitory (*Parietaria judaica*).

Diagnostic methods. Briefly, prewarmed heparinized blood samples were stimulated with rCan s 3 (expressed in *Escherichia coli*^{E1}) and a crude *Cannabis sativa* extract (extraction method as described elsewhere,^{E2} obtained by solvent precipitation in the presence of enzyme inhibitors). Antihuman IgE served as a positive control (10 µg/mL, clone G7-18, BD Biosciences, Erembodegem, Belgium) and stimulation buffer was used to measure spontaneous CD63 expression by quiescent cells.

Basophil activation test was performed using side scatter, anti-IgE, and anti-CD203c (clone NP4D6, BD Biosciences) to characterize the basophils. Subsequently, within this gate, basophil activation was quantified as the percentage CD203c++CD63+ expressing basophils using anti-CD63 (clone H5C6, BD Biosciences). Results were expressed as net percentages of CD63+ basophils, calculated by subtraction of the spontaneous expression from the allergen-induced CD63 expression.

Results

Cannabis allergy—related symptoms

Performance of cannabis diagnostic tests. As shown in Figure E1 and Table E2, sIgE rCan s 3's specificity does not change when the cutoff is increased from 0.10 to 0.35 kU_A/L. Specificity of sIgE hemp rises almost 2-fold (32% to 60%) when a cutoff of 0.35 kU_A/L is considered.

When only considering CA reporting likely-anaphylaxis (CA-A), there is no difference in sensitivity between a cutoff of 0.10 vs 0.35 kU_A/L for either sIgE rCan s 3 or sIgE hemp. In other words, all sensitized CA-A have an sIgE result ≥0.35 kU_A/L for both tests. When the complete CA group is considered, sensitivity decreases around 10% for both tests: sIgE hemp (82% to 72%; $P < .001$), sIgE rCan s 3 (47% to 38%; $P = .002$).

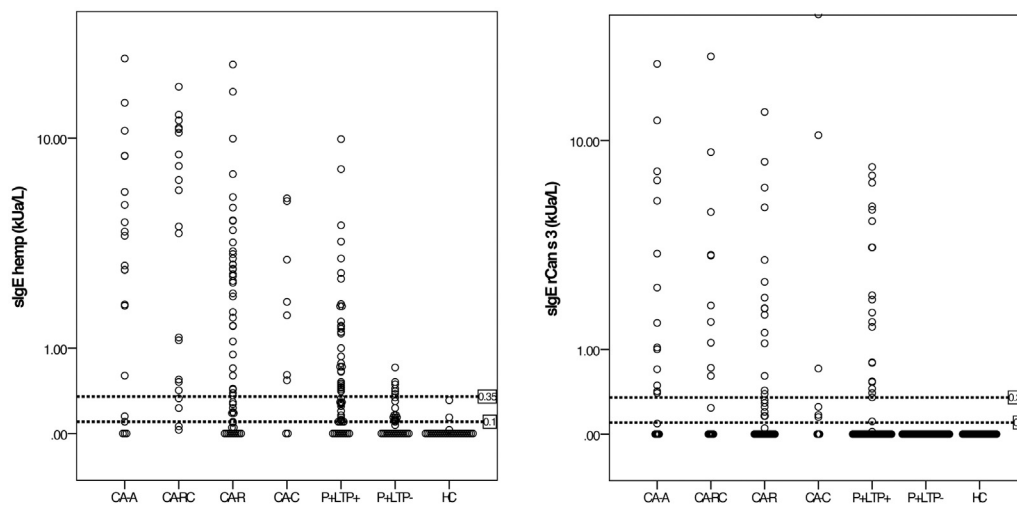


FIGURE E1. sIgE results for sIgE hemp and sIgE rCan s 3 showing both cutoff 0.10 kU_A/L and 0.35 10 kU_A/L. *CA-A*, CA reporting likely-anaphylaxis; *CA-C*, cutaneous symptoms to cannabis; *CA-R*, respiratory symptoms to cannabis; *CA-RC*, localized respiratory and cutaneous symptoms to cannabis; *HC*, healthy controls; *P+LTP-*, pollen-sensitized controls without an nsLTP sensitization; *P+LTP+*, pollen and nsLTP-sensitized controls; *sIgE*, specific IgE.

TABLE E1. Symptoms reported on cannabis exposure by CA-A

Patient	Symptoms on cannabis exposure
PT1	AE, D, RC
PT2	AE, AP, C, U, W
PT3	AE, D, P
PT4	D, P, RC, U
PT5	AE, D, RC, U
PT6	AD, D, P, RC, U
PT7	AE, D, P, U, W
PT8	AE, C, D, RC
PT9	BP, OAS, RC, U
PT10	D, RC, U
PT11	AE, D, N, V
PT12	D, RC, U
PT13	D, RC, U
PT14	D, OAS, RC, U
PT15	D, RC, U
PT16	AE, D, P, PL, U
PT17	AE, D, OAS
PT18	AE, D, U
PT19	D, RC, U
PT20	OAS, V, U
PT21	D, N, PL, W
PT22	D, P, RC, U
PT23	N, U, V
PT24	AE, D, P, PL, RC, U
PT25	D, RC, P, U

AD, Atopic dermatitis flare; AE, angioedema; AP, abdominal pain; BP, blood pressure drop; C, cough; CA-A, CA reporting likely-anaphylaxis; D, dyspnea; N, nausea; OAS, oral allergy syndrome; P, pruritus; PL, palpitations; RC, rhinoconjunctivitis; U, urticaria; V, vomiting; W, wheezing.

TABLE E2. Differences in sIgE results between 0.1 and 0.35 kU_A/L cutoffs

Cutoff	sIgE hemp		P value	sIgE rCan s 3		P value
	0.10 kU _A /L	0.35 kU _A /L		0.10 kU _A /L	0.35 kU _A /L	
Sensitivity* CA-A	86 (66-97)	77 (55-92)	.5	63 (41-81)	63 (41-81)	1
Sensitivity CA†	82 (74-89)	72 (63-80)	.001	47 (38-56)	38 (29-48)	.002
Specificity‡	32 (20-45)	60 (49-70)	<.001	87 (78-93)	89 (83-93)	1

CA, Cannabis allergy; CA-A, CA reporting likely-anaphylaxis; *P+LTP*−, pollen-sensitized controls without an nsLTP sensitization; *P+LTP*+, pollen and nsLTP-sensitized controls; *sIgE*, specific IgE.

*Calculations based on the CA-A group vs cannabis-tolerant *P+LTP*− and *P+LTP*+

†Calculations based on the whole CA group (respiratory and/or cutaneous symptoms) vs cannabis-tolerant *P+LTP*− and *P+LTP*+

‡Calculations based on the *P+LTP*− and *P+LTP*− groups. *P* values calculated by McNemar analyses.

TABLE E3. Comparison of Can s 3-sensitized and nonsensitized CA patients

	Can s 3+ CA	Can s 3− CA	P value
Eczema	31%	47%	.08
Asthma	30%	35%	.42
Cofactor*	42%	15%	<.01
Systemic reaction to plant foods†	52%	35%	.14
Pollen-sensitized individuals*	92%	74%	.03
Bet v 1*	82%	56%	<.01
Bet v 2	14%	20%	.46
Phl p 1	54%	62%	.45
Phl p 5b	43%	43%	1
Art v 1	17%	8%	.15
nsLTP-sensitized individuals*	92%	39%	<.01
Pru p 3*	86%	35%	<.01
Mal d 3*	87%	30%	<.01
Cor a 8*	80%	16%	<.01
Ara h 9*	76%	18%	<.01
Jug r 3*	80%	28%	<.01
Tri a 14*	55%	19%	<.01
Art v 3*	70%	10%	<.01
Par j 2	9%	20%	.11
Bromelain	37%	20%	.07

CA, Cannabis allergy; *nsLTP*, nonspecific lipid transfer protein; *WAO*, World Allergy Organization.

*Significant differences as measured by *P* < .05.

†Defined as grade 1 or higher as described by the *WAO* criteria of systemic allergic reactions.^{E3}

TABLE E4. Comparison of CA and *P+LTP*− patients

	CA	<i>P+LTP</i> −	P value
Eczema*	37%	54%	.01
Asthma	30%	39%	.14
Cofactor*	31%	19%	.05
Systemic reaction to plant foods†	45%	33%	.11
Total IgE (mean)*	247.4	424.5	<.01
Pollen-sensitized individuals*	84%	100%	<.01
Bet v 1	72%	79%	.27
Bet v 2	16%	23%	.17
Phl p 1*	57%	79%	<.01
Phl p 5b	43%	54%	.09
Art v 1	14%	19%	.32
nsLTP-sensitized individuals*	72%	100%	<.01
Pru p 3*	66%	88%	<.01
Mal d 3*	65%	82%	<.01
Cor a 8	56%	61%	.50
Ara h 9	54%	62%	.23
Jug r 3*	60%	79%	<.01
Tri a 14	41%	45%	.54
Art v 3	47%	42%	.45
Par j 2*	14%	38%	<.01
Can s 3*‡	63%	35%	<.01
Bromelain*	30%	45%	.02

BAT, Basophil activation test; CA, cannabis allergy; *nsLTP*, nonspecific lipid transfer protein; *P+LTP*−, pollen and nsLTP-sensitized controls; *sIgE*, specific IgE; *SPT*, skin prick test; *WAO*, World Allergy Organization.

*Significant differences as measured by *P* < .05.

†Defined as grade 1 or higher as described by the *WAO* criteria of systemic allergic reactions.^{E3}

‡Measured by *sIgE*, *BAT*, or *SPT*.

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