Cannabis allergy : a diagnostic challenge

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Cannabis allergy: a diagnostic challenge

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Abbreviations

BAT	basophil activation test
CA	cannabis allergic participants
CBA	cytometric bead array
CI	confidence interval
CS	*Cannabis sativa* ~~*Can s*~~
CTA	cannabis tolerant but atopic participants with pollen and LTP sensitizations
HC	healthy controls
NPV	negative predictive value
nsLTP	non-specific lipid transfer protein
PPV	positive predictive value

rCan s 3 recombinant Can s 3 protein from *Cannabis sativa*

sIgE specific immunoglobulin E

To the editor,

In the absence of a validated confirmatory test to document cannabis allergy, physicians use skin prick-prick testing with cannabis buds or leaves to confirm their clinical suspicion of a cannabis allergy (1-4). Obviously, this approach is difficult to standardize as results are dependent on the source material. Our earlier study (5) revealed that a basophil activation test (BAT) with an nsLTP rich cannabis extract could reliably diagnose cannabis allergy but leaves room for improvement. Here, we take advantage of the newly expressed Can s 3 protein (6) to develop three flow cytometric diagnostics for cannabis allergy; a BAT with rCan s 3, a BAT with a crude CS extract and a bead assay (CBA) quantifying sIgE to rCan s 3.

Twelve patients reporting immediate respiratory and cutaneous symptoms (as detailed in table 2 of the online supplementary information) on exposure to cannabis were selected (CA). Exposure to cannabis implied smoking or ingestion of cannabis, except one patient who experienced repetitive generalized respiratory and cutaneous symptoms upon passive cannabis smoke exposure (7). Fifteen cannabis tolerant healthy controls (HC) and 14 cannabis tolerant atopic (CTA) patients, all demonstrating pollen and nsLTP sensitization were also enrolled. All control subjects reported repeated smoking of cannabis in the last year without symptoms apart from its psychoactive effects. These study groups were included because the evaluation of diagnostics cannot be considered complete when it fails to identify conditions that might affect the outcome e.g. multiple allergic sensitizations. Definitions of pollen and nsLTP sensitizations can be found in the online supplementary information. BAT's were performed with 0.01, 0.1, 1, and 10 µg/mL of rCan s 3 (expressed as previously described (6)) and a crude cannabis extract (extraction method see Rudeschko (8)). Specific IgE to rCan s 3 was quantified using a CBA (BD Biosciences, Franklin Lakes, NJ, USA). Details of the methods are provided in the online supplementary information.

Ten out of twelve CA patients have a pollen and nsLTP sensitization, one patient demonstrates a pollen but no nsLTP sensitization and one patient is nsLTP-sensitized without a pollen sensitization. Demographic data, sensitization profiles and history are summarized in table 2 and 3 of the online supplementary information.

Figure 1A displays a dose-response curve for the BAT with rCan s 3 and the cannabis extract and corresponds with the mathematical comparison of the areas under de curve (ROC-analyses) showing that the three highest concentrations in both BATs are equally performant to discriminate HC from CA. The lowest allergen concentration showing optimal activation potential in the CA group and fewest clinically irrelevant results in the CTA group was chosen resulting in 0.1 µg/mL crude cannabis extract and 1 µg/mL rCan s 3 with a cutoff of >5% CD63+ basophils, corresponding to the highest activation percentage seen in HC and the test variability (4.5% for 500 gated basophils). Both BAT's show an absolute specificity and a similar sensitivity (table 1). The individual results for both BATs with the chosen concentrations are shown in figure 1B. This figure also shows the individual results for the sIgE measurement of rCan s 3. ROC-analysis shows a specificity of 100% and a sensitivity of 75% for a cutoff value of 0.10 kU_A/L (technical limit of detection for this technique).

These results show an absolute specificity and a sensitivity between 60-75% for all three tests, comparing HC and CA. Notably, the inter-test differences for the sensitivity and specificity are not significant. When these results are compared to the initial exploration of the rCan s 3 allergen (6) which observed 30% sensitivity applying a streptavidine-ImmunoCAP method in patients mainly exhibiting respiratory symptoms, it appears test sensitivity and sensitization patterns depend on the clinical phenotype, as our participants with CA had both respiratory and cutaneous reactions Obviously, differences in test performance and sensitization profiles might also result from the use of different techniques.

The validation of novel diagnostics cannot be considered complete when it fails to identify confounders that could affect the outcome of the evaluation. Therefore, the diagnostic test performance was additionally investigated in a group of individuals tolerant to cannabis but showing a pollen and nsLTP sensitization profile similar to the majority of our CA patients, the CTA group. From this "worst case scenario" it appears both BATs and the sIgE assay are reliable with only limited clinically irrelevant results in CTA. It is likely, but currently unproven that these low numbers of

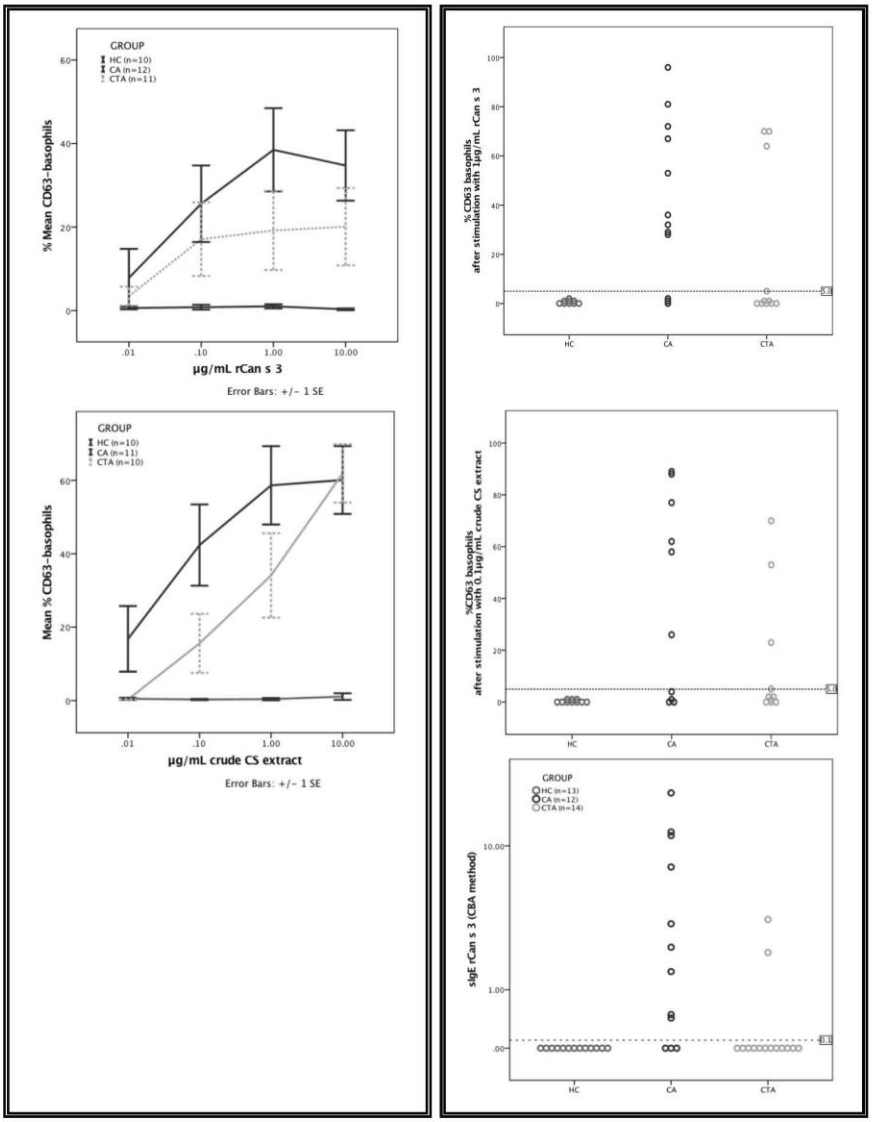
clinically irrelevant Can s 3 results in the ns-LTP sensitized CTA group reflect genuine CA to result from Can s 3 specific epitopes that go undetected in more traditional nsLTP tests, mainly the rPru p 3 assay.

As displayed in figure 3 (supplementary information), three CA patients show triple negative results. One possible explanation is the potential shortcoming of our crude cannabis extract that could lack sufficient amounts of other relevant allergens to trigger basophil responsiveness and sIgE binding. Therefore, further studies are warranted to identify and produce other allergens, as these could be applied to spike natural extracts or to compose mixtures of allergens. A second possibility is that some patients might experience symptoms to contaminants such as molds that have been found in illicit cannabis plantations (9). A third hypothesis is that the reported symptoms could be caused by non-IgE mediated reactions on exposure to cannabis which cannot be demonstrated by our tests. This theory is fostered by the finding that both this study and Rihs et al. (6) show patients with clear symptoms on cannabis exposure but no response in BAT or sIgE to rCan s 3 nor a crude extract.

In summary, BAT with a crude cannabis extract, rCan s 3 and sIgE (CBA) with rCan s 3 might be reliable diagnostics identifying two-thirds of our patients. However, different questions remain to be resolved including the identification and expression of novel allergens is warranted, as these could benefit the tests. Furthermore, larger studies are mandatory to confirm our observations including the fact that sensitization profile and test performance might depend on the clinical phenotype.

FIGURES & TABLES

Figure 1A 1B



1A *Dose-response curve showing basophil activation after 10-1-0.1-0.01 µg/mL rCan s 3 and a crude cannabis extract.* ***1B*** *Basophil activation after 1 µg/mL rCan s 3 and 0.1 µg/mL crude cannabis extract and sIgE measurement for rCan s 3 expressed in kU_A/L. Basophil activation is expressed as net percentage of CD63 expressing basophils. HC=healthy controls, CA=cannabis allergic patients, CTA= cannabis tolerant but atopic patients (with pollen and nsLTP sensitisations). SE= standard error of the mean*

TABLE 1

		Healthy Controls (HC) vs. Cannabis Allergics (CA)	Cannabis Tolerant Atopics (CTA) vs. Cannabis Allergics (CA)
BAT rCan s 3	SENSITIVITY (95% CI)	66.7% (34.9-90.1)	-
	SPECIFICITY (95% CI)	100.0% (69.2-100.0)	-
	PPV (95% CI)	100.0% (69.2-100.0)	72.7% (48.4-88.3)
	NPV (95% CI)	71.4% (52.9-84.8)	66.7% (45.4-82.8)
BAT crude CS extract	SENSITIVITY (95% CI)	60.0% (26.2-87.8)	-
	SPECIFICITY (95% CI)	100.0% (69.2-100.0)	-
	PPV (95% CI)	100.0% (69.2-100.0)	66.7% (40.2-85.6)
	NPV (95% CI)	71.4% (53.9-84.2)	66.7% (38.5-86.5)
sIgE rCan s 3	SENSITIVITY (95% CI)	75.0% (42.8-94.5)	-
	SPECIFICITY (95% CI)	100.0% (75.3-100.0)	-
	PPV (95% CI)	100.0% (75.3-100.0)	81.8% (54.5-94.4)
	NPV (95% CI)	81.3% (61.9-92.0)	80.0% (59.5-91.6)

PPV= positive predictive value, NPV= negative predictive value, CI= confidence interval

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