

Original Article

IgE to Poppy Seed and Morphine Are Not Useful Tools to Diagnose Opiate Allergy

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What is already known about this topic? Recently, it has been suggested that specific immunoglobulin E (sIgE) to poppy seed (*Papaver somniferum*) extract and morphine would be reliable in the diagnosis of opiate allergy.

What does this article add to our knowledge? A positive sIgE to poppy seed or morphine is not per se predictive for genuine opiate allergy and should not be used in isolation to diagnose morphine or codeine allergy.

How does this study impact current management guidelines? An erroneous opiate allergy diagnosis might not only entail unnecessary avoidance measures but also, most importantly, ultimately put patients at risk by overlooking alternative diagnoses.

BACKGROUND: Correct diagnosis of genuine IgE-mediated opiate allergy poses a significant challenge, mainly because of uncertainties associated with opiate skin testing and the unavailability of drug-specific IgE (sIgE) assays. Recently, it has been suggested that sIgE to poppy seed extract and morphine would be reliable in the diagnosis of opiate allergy. However, given the high prevalence of sIgE antibodies to these compounds in an allergic population, the predictive value of these tests leaves significant doubts.

OBJECTIVE: This study aims at verifying the predictive value of positive poppy seed and morphine sIgE assays results.

METHODS: A total of 22 individuals with a positive sIgE to poppy seed or morphine were selected. All had controlled drug challenges with increasing doses of morphine and/or codeine. Of these, 18 patients had an additional basophil activation test (BAT) with morphine and codeine.

RESULTS: None of the 22 patients demonstrated objective or subjective symptoms on provocation with morphine and/or codeine. Regarding BAT with morphine and codeine, expression of CD63 on basophils from 14 opiate tolerant individuals remained comparable to spontaneous expression by resting cells. The remaining 4 patients were classified as nonresponders.

CONCLUSION: Positive sIgE results to poppy seed and morphine are not per se predictive for genuine opiate allergy and should not be used in isolation to diagnose morphine or codeine allergy. To avoid overdiagnosis, for the time being, we propose to supplement serological diagnosis with an oral provocation test. Whether BAT might help to discriminate between clinical reactivity and sensitization remains to be confirmed in larger collaborative studies. © 2015 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2015;■:■-■)

Key words: Allergy; Basophil activation; Codeine; IgE; Morphine; Opiate; Poppy seed

Correct diagnosis of genuine IgE-mediated allergy to opiates is not straightforward, mostly due to the unavailability of opiate drug-specific IgE (sIgE) antibody assays and uncertainties associated with skin testing with potent nonspecific histamine releasers from cutaneous mast cells.^{1,2} Recently, a morphine and pholcodine solid-phase sIgE assay has become available from Thermo Fisher Scientific (Uppsala, Sweden). However, almost no attempts have currently been made to clinically validate these tests in opiate allergy, mainly because genuine IgE-mediated opiate allergy is exceedingly rare.² In 2 studies from the same Spanish group, the authors claim sIgE to whole poppy seed (*Papaver somniferum*) extract, and to a lesser extent also sIgE to morphine, to be “easy, cheap, simple and useful in the prevention of intraoperative anaphylaxis and hypersensitivity to opiate analgesics”³ and to constitute “simple and reliable methods to test hypersensitivity to opiates (morphine, heroin, codeine and pholcodine).”⁴ However, the authors appear to have overlooked various confounders that could have influenced the outcome of their studies and call their conclusions into question. For example, poppy seed extracts have been shown to harbor homologues of the major allergen Bet v 1 from birch pollen (*Betula verrucosa*), profilin, and various carbohydrate-bearing components (CCD)⁵ that are well known to undermine the specificity of the sIgE assay.⁶ In our region, where almost 20% of the

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Abbreviations used

BAT- Basophil activation test
 Bet v- Betula verrucosa (birch)
 CCD- Cross-reactive carbohydrate determinants
 DPT- Drug provocation test
 NMBA- Neuromuscular blocking agent
 ns-LTP- Nonspecific lipid transfer protein
 Phl p- Phleum pratense (Timothy grass)
 Pru p- Prunus persica (peach)
 sIgE- Specific immunoglobulin E

general population suffers from pollen allergy, sensitization to Bet v 1 is present in 96% of the patients with birch pollen allergy, and IgE reactivity to profilins and sensitization to complex α 1,3-fucose and β 1,2-xylose containing CCD is demonstrable in approximately 20% of patients with grass pollen allergy.⁶ We observed a positive sIgE to poppy seed in 33 out of 148 patients (22.30%) sensitized to tree and/or grass pollen (unpublished data). Sensitization to lipid transfer protein (LTP), in our region frequently seen in cannabis allergy,⁷ could be another possible explanation for false positive poppy seed sIgE results.⁸

Regarding morphine, it cannot be ignored that IgE reactivity to this opiate has been shown in patients sensitized to a variety of pharmacologically and structurally different drugs⁹ and that morphine sIgE antibodies are demonstrable in 1-10% of patients with classical inhalant allergies.¹⁰⁻¹² Moreover, nowadays the morphine solid-phase sIgE assay, by virtue of its overtly accessible tertiary ammonium epitope, is mostly being applied to demonstrate sensitization to neuromuscular blocking agents (NMBAs), especially suxamethonium and rocuronium.¹¹⁻¹³ As a matter of fact, sIgE reactivity to morphine is demonstrable in over 80% of the sera of patients with documented allergy to these NMBAs. Although, from clinical experience, it seems highly unlikely that patients sensitized to rocuronium and/or suxamethonium also suffer from opiate allergy, firm evidence is lacking, mostly as a result of the absence of reliable tests to document genuine opiate allergy. In our opinion, a basophil activation test (BAT) could be a valuable instrument to ascertain the clinical relevance of IgE serology that suggests opiate allergy as opiates do not elicit nonspecific histamine release from basophils.¹⁴⁻¹⁷ Moreover, we recently demonstrated in 3 pholcodine allergic patients without a positive sIgE to poppy seed that the BAT enables diagnosis of pholcodine allergy and is a better predictor than sIgE and skin tests to predict the negative challenge outcomes with the structurally almost similar opiate codeine.¹⁸

This study primarily aims at challenging the results of poppy seed and morphine sIgE antibody tests with the clinical outcome of controlled oral drug challenges with structurally almost identical opium alkaloids, ie, morphine and/or codeine.

MATERIALS AND METHODS

Study population

To verify the predictive value of a positive sIgE result to crude poppy seed or morphine, 22 individuals (men/women: 6/16; median age [range]: 42 [20-73] years) were included. Ten individuals with a positive sIgE to poppy seed were selected from a cohort of patients who attended our allergy outpatients' clinic for diagnostic evaluation

of a birch and/or grass pollen and/or cannabis allergy. Twelve patients with a positive sIgE to morphine were selected from a group of patients with rocuronium allergy. Pollen and cannabis allergy were established as detailed elsewhere.⁷ Rocuronium hypersensitivity was documented as described by Leysen et al.¹⁸ All patients gave informed consent approved by the local ethics committee [EC/12/2/24].

Total and specific IgE

Quantification of total and sIgE to whole poppy seed extract, morphine, the recombinant major birch (*Betula verrucosa*) pollen allergen rBet v 1, birch profilin rBet v 2, rPhl p 1 and rPhl p 5 from Timothy grass (*Phleum pratensis*), rPru p 3 (LTP), the nonspecific lipid transfer protein (ns-LTP) from peach (*Prunus persica*), and the MUXF-CCD biomarker bromelain was performed by an ImmunoCAP system according to the manufacturer's instructions (Thermo Fisher Scientific, Uppsala, Sweden). Values of sIgE $\geq$ 0.35 kUA/L were considered positive.

Drug provocation test

All patients had a standardized and controlled oral drug provocation test (DPT) with (an) opiate(s), namely, morphine (cumulative dose of 11 mg) and/or codeine (cumulative dose of 31 mg), to determine the clinical relevance of a positive sIgE result to poppy seed or morphine. In detail, during the DPT with morphine, 3 doses with an interval of 30 minutes were given, ie, 1 mg followed by a dose of 5 mg, and finally another dose of 5 mg. During the DPT with codeine, 4 doses with an interval of 30 minutes were given, ie, 1 mg, subsequently 5 mg, followed by a dose of 10 mg and a final dose of 15 mg. In all DPTs, the total observation time was at least 4 hours. A DPT was considered negative when neither objective nor subjective symptoms could be observed.

Basophil activation test

An additional BAT was performed in 18 of the 22 patients. *In vitro* basophil activation was quantified as detailed elsewhere.¹⁸ Briefly, 200 μ L endotoxin-free heparinized whole blood was challenged at 37°C for 20 minutes with 200 μ L buffer as control, 200 μ L anti-IgE (Pharmingen, BD Bioscience, Erembodegem, Belgium) as a positive control, and 200 μ L of serial dilutions of morphine (Stellorphone, Sterop, Brussels, Belgium) (10-100 μ g/mL) and codeine (Escapo C.V., Mechelen, Belgium) (1-1000 μ g/mL). Reactions were stopped by chilling on ice, adding 1 mL ice-cooled PBS with 10 mmol/L EDTA (PBS-EDTA), and spinning for 5 minutes (4°C, 200 g). To select and quantify basophil activation, cells were stained with 20 μ L of monoclonal antihuman IgE (clone GE-1, Sigma Aldrich GmbH, Steinheim, Germany) labeled with Alexa Fluor 405 (Molecular Probes, Thermo Fisher Scientific, Paisley, UK), 10 μ L of monoclonal antihuman CD63-fluorescein isothiocyanate (clone H5C6, BD Bioscience), and 10 μ L CD203c-allophycocyanin (clone NP4D6, BD Bioscience) for 20 minutes on ice. The cells were lysed/fixed with 2 mL BD FACS Lysing solution for 20 min (at room temperature). They were washed twice with PBS with 0.1% sodium azide and measured. Flow cytometric characterization of basophils relied on a combination of side scatter, anti-IgE, and CD203c. Results were expressed as %CD63 positive basophils and compared with spontaneous expression (ie, in response to buffer).

RESULTS

Total and specific IgE

The median [range] for total IgE was 326 [102-5500] kU/L in the sIgE to poppy seed positive individuals (Table 1) and 192

TABLE I. Patients with a positive specific IgE to poppy seed (demographics, laboratory, drug challenge, and basophil activation data)

PT	Age (y)	Sex	Total IgE (kU/L)	sIgE								DPT* (M/C)	BAT† (M/C)
				Poppy seed (kUA/L)	Morphine (kUA/L)	rBet v 1 (kUA/L)	rBet v 2 (kUA/L)	rPhl p 1 (kUA/L)	rPhl p 5 (kUA/L)	Bromelain (kUA/L)	rPru p 3 (kUA/L)		
1	37	M	NA	1.47	<0.10	<0.10	<0.10	0.22	0.65	1.40	<0.10	M	NA
2	25	F	166	4.37	<0.10	<0.10	0.15	<0.10	<0.10	0.81	34.40	C	M/C
3	20	M	490	1.72	<0.10	13.70	<0.10	<0.10	<0.10	<0.10	10.00	C	M/C
4	31	F	310	0.88	<0.10	14.90	<0.10	NA	NA	<0.10	2.36	C/M	M/C
5	38	F	1803	9.71	0.18	0.28	0.21	0.27	100	16.40	0.18	C	NR
6	29	F	5500	7.28	0.11	66.30	0.33	4.59	NA	2.63	67.90	C	M/C
7	24	F	885	3.35	<0.10	77.40	3.85	27.70	31.50	3.49	19.10	M	M/C
8	56	F	124	3.64	<0.10	<0.10	<0.10	0.55	NA	0.13	17.70	C	NR
9	31	F	326	1.74	<0.10	13.10	<0.10	15.20	9.69	1.15	4.19	M	NR
10‡	23	M	102	5.86	<0.10	0.11	<0.10	<0.10	<0.10	8.81	<0.10	C	M/C

PT, patient; M, male; F, female; NA, not available; sIgE, specific immunoglobulin E; r, recombinant; Bet v, *Betula verrucosa* (birch); Phl p, *Phleum pratense* (timothy grass); Pru p, *Prunus persica* (peach); NR, nonresponder.

*All patients had a negative drug provocation test (DPT) for morphine (M) and/or codeine (C).

†All basophil activation experiments with M and C yielded an upregulation of CD63 comparable to spontaneous expression.

‡Diagnosis of grass pollen allergy was documented by a skin prick test and a positive sIgE to negative Phl p 4 of 9.62 kUA/L.

TABLE II. Patients with a positive specific IgE to morphine (demographics, laboratory, drug challenge, and basophil activation data)

PT	Age (y)	Sex	Total IgE (kU/L)	sIgE		DPT* (M/C)	BAT† (M/C)
				Morphine (kUA/L)	Poppy seed (kUA/L)		
1	43	F	568	9.41	<0.10	M	M/C
2	42	F	192	1.95	<0.10	M	NA
3	57	M	32	0.82	<0.10	M	M/C
4	42	F	71	1.61	<0.10	M	NA
5	47	F	NA	1.68	<0.10	M	NA
6	60	M	108	4.18	<0.10	M	M/C
7	73	F	20.50	3.15	<0.10	C	M/C
8	62	F	121	0.54	<0.10	M	M/C
9	47	F	NA	10.70	<0.10	M	M/C
10	57	M	663	16.40	<0.10	M	NR
11	35	F	212	2.42	<0.10	C/M	M/C
12	59	F	NA	1.09	<0.10	C/M	M/C

PT, patient; M, male; F, female; NA, not available; sIgE, specific immunoglobulin E; NR, nonresponder.

*All patients had a negative drug provocation test (DPT) for morphine (M) and/or codeine (C).

†All basophil activation experiments with M and C yielded an upregulation of CD63 comparable to spontaneous expression.

[32-663] kU/L in the sIgE to morphine positive individuals (Table II). Table I also displays the individual sIgE results for poppy seed extract (median [range]: 3.5 [0.88-9.71] kUA/L), morphine, rBet v 1, rBet v 2, rPhl p 1, bromelain, and rPru p 3. According a cutoff value of ≥ 0.35 kUA/L, 5/10 patients (50%) were positive for rBet v 1, 1/10 patients (10%) were positive for rBet v 2, 4/9 patients (44%) were positive for rPhl p 1, 4/7 (57%) patients were positive for rPhl p 5, 7/10 patients (70%) were positive for the MUXF-CCD biomarker bromelain, and 7/10 patients (70%) were positive for sIgE to Pru p 3. None of these poppy seed positive patients demonstrated a positive sIgE to morphine.

Table II shows the individual morphine and poppy seed sIgE results in rocuronium-sensitized patients with a positive sIgE to morphine (median [range]: 2.19 [0.54-16.4] kUA/L). None of these patients demonstrated a positive sIgE to crude poppy seed extract.

Drug provocation test

As displayed in Tables I and II, 7 patients had a DPT with codeine, 12 with morphine, and 3 with both morphine and

codeine. The oral DPTs were invariably negative as neither objective nor subjective symptoms were reported.

Basophil activation test

The results of the 18 BATs are shown in Tables I and II. Note that 4 individuals were nonresponders, ie, nonresponsive to stimulation with the positive control and/or allergen. In the remaining 14 individuals, all BATs with codeine and morphine yielded an upregulation of CD63 comparable to spontaneous expression in response to buffer stimulation (<5%).

DISCUSSION

Correct diagnosis of IgE-mediated allergy to opiates poses a significant challenge, mostly due to uncertainties associated with skin testing with these potent cutaneous mast cell histamine releasers and the unavailability of reliable opiate drug-specific IgE assays.^{1,2} Although, in the absence of patients with established opiate allergy, we were unable to calculate sensitivity, specificity, and predictive values, our results indicate that neither sIgE to crude poppy seed extract nor sIgE to morphine constitutes

a reliable tool to correctly predict the outcome of graded oral opiate provocation tests. The explanation for clinical irrelevant poppy seed sIgE results probably has to be sought in the wide range of immunological cross-reactivities between plant allergens that harbor ubiquitous highly cross-reactive components such as Bet v 1 homologues, profilins, CCDs^{5,6} and ns-LTPs,⁸ and, for morphine sIgE, in the broad variety of drugs (and related compounds) that contain tertiary and quaternary substituted ammonium epitopes.⁹ As a matter of fact, our most important finding relates to the observation that patients who demonstrate positive sIgE results to poppy seed and/or morphine tolerate administration of pharmacologically effective doses of structurally related opiates, namely, morphine and/or codeine, that differ only at position 3, where morphine has a hydroxyl and codeine a methoxy group.² This observation is particularly relevant for physicians facing patients for whom correct identification of the causative drug is additionally impeded because of simultaneous intake or administration of different compounds, eg, during general anesthesia. Actually, erroneous opiate allergy diagnosis might not only entail unnecessary avoidance measures but also, most importantly, ultimately put patients at risk by overlooking alternative diagnoses. From our study, it emerges that morphine and codeine did not trigger basophil activation in individuals who demonstrated a positive sIgE result for morphine or poppy seed but having tolerated opiate challenges. Despite the absence of patients with genuine morphine and codeine allergy to validate the BAT with these opiates, we presume that the discrepancy between the positive sIgE results and the absence of basophil responses probably mirrors the underlying processes in these 2 tests. The sIgE solid phase assay simply measures the interaction between morphine and the paratope (epitope binding site) of the antibody, whereas BAT depicts drug-induced cross-linking of membrane-bound sIgE via the paratope and subsequent degranulation with the appearance of activation markers on the membrane and the release of mediators such as histamine. Moreover, together with our prior observation, ie, basophils from pholcodine allergic patients only to be responsive to this antitussive but not to the structurally related opiates codeine and morphine,¹⁸ and skin tests with opiates to be unreliable,¹ it seems that BAT might become an asset for discrimination between genuine opiate allergy and merely sensitization.

In conclusion, the detection of sIgE to poppy seed or morphine is not sufficient to establish the diagnosis of an opiate