

Original article

Investigation of drugs responsible for perioperative anaphylactic reactions using cellular allergen stimulation test

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Keywords: perioperative period; anaphylactic reaction; *in vitro*; flow cytometry; cellular allergen stimulation test

Background Anaphylactic reactions during anesthesia and operation are common and life threatening. Follow-up investigation is necessary for avoiding potential re-exposure of the patients to the offending drugs. The purpose of this study was to assess cellular allergen stimulation test (CAST) as a diagnostic instrument in immunoglobulin E (IgE)- and non-IgE-mediated anaphylactic reactions.

Methods This study included 25 patients who developed perioperative anaphylactic reactions and 10 subjects that tolerated anesthetics and other drugs during perioperative period from September 2009 to October 2013 in Peking Union Medical College Hospital. We performed skin tests and flow cytometric analysis of basophil activation-based CAST in all subjects.

Results Of the 25 patients, 17 had IgE-mediated anaphylactic reactions (causative agent identified by skin tests) and 8 had non-IgE-mediated anaphylactic reactions (negative skin tests). CAST showed a sensitivity of 42.9%, specificity of 90%, and negative predictive value of 80.6% for neuromuscular blocking agents.

Conclusions CAST may be useful for the diagnosis of anaphylactic reactions during perioperative period. Our findings call for further investigation to increase the sensitivity of the test.

Chin Med J 2014;127 (21): 3738-3743

Anaphylactic reactions in the perioperative period are common and life threatening. It has been calculated to range from 1 in 3 500 to 1 in 13 000 cases according to research in France.^{1,2} One report from Australia estimated the incidence to be between 1 in 10 000 and 1 in 20 000,³ whereas another report from Norway estimated the incidence to be 1 in 6 000.⁴ If causative drugs during anesthesia and operation are diagnosed promptly and optimally, severe adverse events may be avoidable during subsequent anesthetic procedures.

Several methods have been used in the diagnosis of anaphylactic reactions. Although skin tests remain the golden standard for the detection of drug allergies, they do not demonstrate absolute diagnosis because of a high false-positive rate.⁵ Radioallergosorbent test is the relatively common immunoglobulin E (IgE) antibody test which measures specific IgE antibodies in the blood. However, IgE measurement is only commercially available for very few drugs used during anesthesia. Therefore, additional reliable diagnostic tools are of tremendous interest. Currently, flow cytometry-assisted technique, cellular allergen stimulation test (CAST), may constitute a promising instrument for the diagnosis of perioperative anaphylactic reactions. The specificities were shown to be high (93%–100%), but the sensitivities were reported to vary (63%–89.7%) in several studies, which assessed CAST in the diagnosis of allergy from neuromuscular blocking agents (NMBAs).^{6,7}

We conducted this study to evaluate the efficacy of CAST in Chinese patients who were referred with possible perioperative anaphylactic reactions by determining its

sensitivity and specificity. The primary aim of this study was to assess the reliability of CAST as a safe diagnostic method for perioperative anaphylactic reactions.

METHODS

Subjects

This study included 25 patients who had presented clinical features in a few minutes after drug ingestion during the perioperative period between September 2009 and October 2013. The clinical features occurring may involve cardiovascular symptoms (tachycardia, bradycardia, cardiac arrhythmias, hypotension, cardiovascular collapse, or cardiac arrest), signs of difficulty with ventilation (bronchospasm, increased ventilator pressures, or respiratory distress in a non-intubated patient), and cutaneous-mucous signs (rash, flushing, erythema, urticaria, or angioedema). Ten control subjects had experienced uneventful general anesthesia before and none of them had any history of remarkable medical disease.

DOI: 10.3760/cma.j.issn.0366-6999.20141389

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This study was supported by the grant from Beijing Medicine Research and Development Fund (No. 2009-2002).

All subjects were recruited after informed written consent in our study, which was approved by the Research Ethics Committee of Peking Union Medical College Hospital. Skin tests and CAST were performed at least 6 weeks after the acute perioperative reactions.

Skin tests

Drugs tested in our study included NMBAs, induction drugs, opioids, local anesthetics, and antibiotics. The subjects received prick test (PT) first, by the same nurse who had significant experience. All tests were performed with 0.9% sodium chloride solution as a negative control and histamine (10 mg/ml, HAL Allergy Benelux BV, Haarlem, the Netherlands) as a positive control. A positive PT result was defined as positive if the diameter of the wheal was equal to or larger than one-third the diameter of the wheal induced by the positive control.

Suspected causative drugs that produce negative PT results were subsequently used in an intradermal test (IDT). The drugs were used as diluted (chlorure de sodium 0.9%; Lavoisier) solutions, beginning with the left arm and starting with the smallest concentration -1:1 000 solutions (NMBAs and morphine: 1:10 000 solution). The tests were performed by injecting about 0.05 ml solutions into the dermis of the forearm or back through a hypodermic needle. The circumferences of the wheal and flare were marked on the skin by using a blue ballpoint pen and covered with tape 20 minutes after reactions. IDT responses were considered positive if the diameter of the obtained wheal equaled or exceeded 5 mm and doubled with respect to the injection bleb. If the lowest dosage of the drugs had been performed without positive reactions, a 10-fold dosage was tested under the same conditions (maximal concentrations of available agents⁸ shown in Table 1). Any drug or substance

that induced a positive skin reaction (positive skin prick or intradermal reaction) is referred to as the "culprit drug."

Cellular allergen stimulation test

In all cases of study and control group, we used a panel of common anesthetics and antibiotics (CAST®-Allergens, Bühlmann Laboratories AG, Schönenbuch, Switzerland) to assess anaphylactic reactivity, including all presumed causative drugs.

Patients should avoid systemically administered antiallergenic drugs such as antihistamines, antileukotrienes, and steroids for at least 1 week prior to blood sampling. Sufficient blood was collected into K-EDTA tubes up to the dedicated volume. The blood samples were stimulated within 2 hours or refrigerated (2–8°C) for up to 24 hours. For each sample, we used both negative (phosphate-buffered saline) and positive (anti-FcεRI mAb and formyl-methionyl-leucyl-phenylalanine) controls. Experiments were performed using a commercially available Flow-CAST kit (Bühlmann Laboratories AG) following the manufacturer's instructions.⁹⁻¹²

The samples were analyzed using Accuri C6® flow cytometer. Basophils were selected on a CCR3 gate (A) and at least 300 basophils were counted (Figure 1A). The percentage of CD63-positive cells was calculated compared to the total amount of basophilic cells gated in A (Figure 1B). Results were considered positive if activated basophil percentage ≥5% and stimulation index (SI = percentage of basophils activated by the drug divided by percentage of activated basophils in the negative control) ≥2.

Statistical analysis

Data were expressed as mean ± standard deviation (SD) and Prism 6 software (La Jolla, CA, USA) was used to analyze the data. Mann-Whitney test was used to compare the differences in continuous variables and Fisher's exact test was used to analyze categorical variables. The correlation between skin tests and CAST was analyzed using Kappa coefficient. $P < 0.05$ were considered statistically significant.

The sensitivity was calculated as the ratio of the number of patients with a positive CAST result to the total number of drug-allergic patients with a positive skin test result. Specificity was calculated as the ratio of the number of control subjects with negative CAST results to the total number of control subjects tested. The negative predictive value was calculated as the ratio of the number of true-negative cases to the number of cases with a negative CAST result, where a "true negative" is the event that CAST makes a negative prediction, and the case has a negative result under the gold standard. (The cases had no clinical features after some drug ingestion and had negative skin test results for the drug.)

RESULTS

Demographic features and manifestations of perioperative reaction

The demographic characteristics of the study and control

Table 1. Maximal concentrations for intradermal test

Available agents	Concentration (mg/ml)	Dilution	Cmax (μg/ml)
NMBAs			
Atracurium	10	1/1 000	10
Cisatracurium	2	1/100	20
Rocuronium	10	1/100	100
Vecuronium	4	1/10	400
Induction drugs			
Etomidate	2	1/10	200
Midazolam	5	1/10	500
Propofol	10	1/10	1 000
Opioids			
Fentanyl	0.05	1/10	5
Morphine	10	1/1 000	10
Remifentanyl	0.05	1/10	5
Sufentanyl	0.005	1/10	0.5
Local anesthetics			
Bupivacaine	2.5	1/10	250
Lidocaine	10	1/10	1 000
Ropivacaine	2	1/10	200
Antibiotics			
PPL		Undiluted	35
MDM (penicillin)		Undiluted	1 000
Amoxicillin, ampicillin		Undiluted	(20–25) × 10 ³
Cephalosporins		Undiluted	(1–2) × 10 ³

C_{max}: maximal concentration; PPL: benzylpenicilloyl poly-L-lysine; MDM: minor determinant mixture; NMBAs: neuromuscular blocking agents.

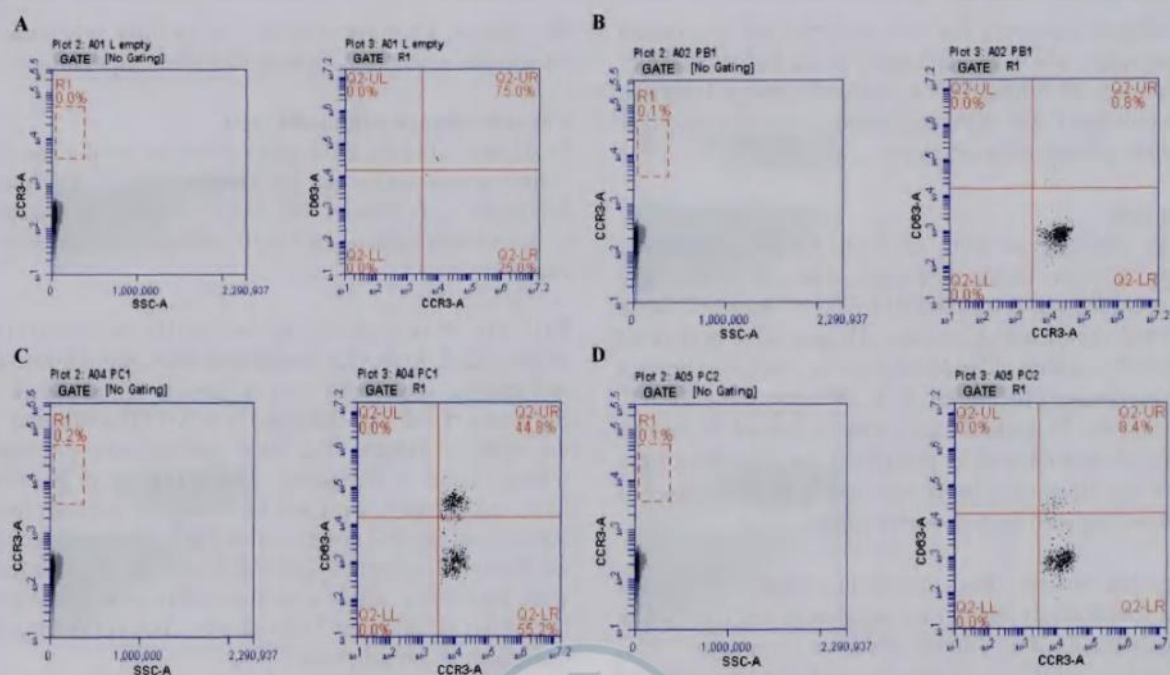


Figure 1. Flow cytometric analysis of basophil activation. **A:** Isotype control value; **B:** Negative control value; **C:** Positive control value; **D:** Presumed causative drug value. *Gate R1 denotes the total amount of basophilic cells. *Gate Q2-UR denotes the percentage of CD63-positive cells compared to the total amount of basophilic cells gated in R1.

groups, associated with history of drug allergy and reaction types, are displayed in Table 2. The prevalence of atopy was significantly lower in the study group with IgE-mediated anaphylactic reaction than in control group (35.3% vs. 60.0%; $P < 0.05$). In the IgE allergy group, four patients had a history of allergy to antibiotics and two had experienced hypersensitivity to other drugs, while in the non-IgE allergy group, five patients had a history of allergy to antibiotics and one had experienced hypersensitivity to other drugs. In the control group, six subjects (60.0%) had a history of hypersensitivity to antibiotics. Seven cases (41.2%) from IgE allergy group and four cases (50%) from non-IgE allergy group underwent subsequent procedures, the suspected causative agents were subsequently avoided, and all of them were uneventful.

Table 2. Demographic features and manifestations of perioperative reaction

Characteristics	IgE allergy group ($n=17$)	Non-IgE allergy group ($n=8$)	Control group ($n=10$)
Age (mean $\pm$ SD, years)	34 $\pm$ 13	56 $\pm$ 12	45 $\pm$ 21
Weight (mean $\pm$ SD, kg)	60.7 $\pm$ 20.5	61.8 $\pm$ 5.5	61.4 $\pm$ 6.4
M/F (n)	6/11	1/7	3/7
History of drug allergy (n (%))			
None	11 (64.7)	2 (25.0)	4 (40.0)
Anesthetics	0 (0.0)	0 (0.0)	0 (0.0)
Antibiotics	4 (23.5)	5 (62.5)	6 (60.0)
Other drugs	2 (11.8)	1 (12.5)	0
Characteristic reactions (n (%))			None
Cutaneous	9 (52.9)	4 (50.0)	
Respiratory	7 (41.2)	2 (25.0)	
Circulatory	16 (94.1)	7 (87.5)	
Subsequent anesthetic cases (n (%))	7 (41.2)	4 (50.0)	None

M: male; F: female; SD: standard deviation.

Skin test

In our study, all the patients needed additional IDT because of the negative PT results. According to the skin tests, 17 patients had an IgE-mediated anaphylactic reaction (the skin test was positive that identified a likely causative drug) and eight patients had a probable non-IgE-mediated anaphylactic reaction (the skin test results for all tested drugs were negative while the clinical syndromes suggested anaphylactic reactions) (Tables 3 and 4).

Of those who had an IgE-mediated anaphylactic reaction, a positive IDT for NMDAs (rocuronium, vecuronium, and cisatracurium) was found in 7/17 patients (41.2%), while four cases (23.5%) with local anesthetics (lidocaine and ropivacaine) hypersensitivity and 3 patients (17.6%) who over-reacted to antibiotics (penicillin and cefmetazole) tested positive to IDT. Only two patients who reacted to induction drug midazolam had a positive IDT and the one who reacted to opioid sufentanil showed IDT responsiveness. Of those who had a non-IgE-mediated anaphylactic reaction, all patients showed negative IDT response to their presumed causative drugs, respectively. All the control individuals demonstrated negative IDT results to the drugs tested (data not shown). For all patients who had an IgE-mediated or non-IgE-mediated anaphylactic reaction, the presumed causative drug was avoided in the subsequent operation.

Cellular allergen stimulation test

The details of CAST are displayed in Tables 3 and 4. In the IgE allergy group, three of the seven patients with positive skin test results to neuromuscular blocking drugs showed positive CAST results. Only one patient with midazolam

Table 3. CAST results in patients with IgE-mediated anaphylactic reactions

Patient No.	Gender	Culprit drug	Clinical events	Activated basophils in CAST		
				Control value (%)		Culprit drug value (% (SI))
				(-)	(+)	
1	M	Penicillin	Bronchospasm, hypotension	4.75	16.80	7.72 (1.63)
2	F	Ropivacaine	Hypotension, angioedema	23.57	86.53	80.26 (3.41)*
3	M	Midazolam	Profound hypotension, bronchospasm	4.55	21.94	3.78 (0.83)
4	F	Rocuronium	Bronchospasm	2.30	13.20	5.60 (2.43)*
5	M	Sufentanil	Profound hypotension, rash	2.67	10.63	2.21 (0.83)
6	M	Vecuronium	Profound hypotension	0.61	70.31	23.51 (38.54)*
7	F	Lidocaine	Tachycardia	1.14	36.18	0.67 (0.59)
8	F	Lidocaine	Hypotension	1.49	59.44	2.86 (1.92)
9	F	Cisatracurium	Bronchospasm, hypotension, rash	2.03	89.02	4.21 (2.07)
10	F	Rocuronium	Hypotension	1.98	13.76	11.05 (5.58)*
11	M	Rocuronium	Bronchospasm, hypotension, erythema,	1.37	12.00	1.45 (1.06)
12	F	Cefmetazole	Profound hypotension, rash, angioedema	0.15	38.39	0.24 (1.60)
13	F	Cefmetazole	Bronchospasm, hypotension, rash	2.36	24.24	2.91 (1.23)
14	F	Midazolam	Profound hypotension, rash	1.97	10.02	24.38 (12.38)*
15	M	Rocuronium	Profound hypotension, angioedema	0.36	64.49	1.60 (4.44)
16	F	Ropivacaine	Profound hypotension bronchospasm, rash	1.45	37.99	3.80 (2.62)
17	F	Rocuronium	Profound hypotension, cardiac arrest	3.04	22.60	2.89 (0.95)

CAST: cellular allergen stimulation test; SI: stimulation index; *Positive results: activated basophils $\geq 5\%$ and SI ≥ 2 ; M: male; F: female.

Table 4. CAST results in patients with non-IgE-mediated anaphylactic reactions

Patient No.	Gender	Presumed causative drug	Clinical events	Activated basophils in CAST		
				Control value (%)		Presumed causative drug value (% (SI))
				(-)	(+)	
1	F	Rocuronium	Profound hypotension, rash	3.11	13.64	2.60 (0.84)
2	F	Lidocaine	Hypotension, tachycardia	0.94	22.62	0.42 (0.45)
3	F	Ceftriaxone	Profound hypotension, high peak airway pressure	0.87	35.66	0.74 (0.85)
4	F	Articaine	Tachycardia, respiratory distress	1.36	65.34	1.22 (0.90)
5	F	Lidocaine	Profound hypotension, tachycardia	2.83	39.12	1.30 (0.46)
6	F	Lidocaine	Skin rash, syncope	2.26	11.67	1.62 (0.72)
7	M	Lidocaine	Tachycardia, rash	2.38	22.47	1.24 (0.52)
8	F	Rocuronium	Profound hypotension, angioedema	1.46	10.63	2.21 (1.51)

CAST: cellular allergen stimulation test; SI: stimulation index; *Positive results: activated basophils $\geq 5\%$ and SI ≥ 2 ; M: male; F: female

hypersensitivity was also positive for CAST, while the patients of local anesthetics, opioids, and antibiotics hyperreaction tested negative to CAST. As one patient (No. 2 in Table 3) responded to negative control stimulation, it is impossible to interpret positive drug stimulation in this patient. In the non-IgE allergy group, all the patients had negative CAST results, while in the control group, the subjects showed negative response to allergens except rocuronium (Table 5). Our study showed a fair correlation between CAST and skin tests ($\kappa = 0.243$). The CAST results showed a negative predictive value of 80.6% for neuromuscular blocking agents. The specificity of CAST for neuromuscular blocking agents was found to be 90% but the sensitivity was only 42.9%. Statistical analysis was not performed for other groups of drugs, as the numbers of subjects were too small. Within the study group, the CAST results were not affected by the history of allergy and did not differ between those with cutaneous-mucous signs and those with respiratory or cardiovascular symptoms (Table 6).

DISCUSSION

Anaphylactic reactions during perioperative period have immediate threat to patients. In most cases, correct identification of the causative factor is not always

Table 5. CAST results of NMBAs in control subjects

Activated basophils in CAST					
Patient No.	Gender	Control value (%)		Test drug value (% (SI))	
		(-)	(+)	Rocuronium	Cisatracurium
1	F	1.20	14.00	2.30 (1.92)	1.20 (1.00)
2	M	2.67	49.67	3.79 (1.42)	3.16 (1.18)
3	F	0.80	81.20	1.00 (1.25)	1.10 (1.38)
4	F	4.70	10.00	11.00 (2.34)*	9.10 (1.94)
5	M	1.27	53.80	0.01 (0.01)	0.47 (0.37)
6	F	0.76	70.54	1.23 (1.62)	0.72 (0.95)
7	F	1.71	13.40	0.31 (0.18)	0.29 (0.17)
8	F	1.92	44.77	1.20 (0.63)	0.68 (0.35)
9	M	0.23	24.43	0.17 (0.74)	0.18 (0.78)
10	F	2.87	10.23	4.42 (1.54)	3.38 (1.18)

CAST: cellular allergen stimulation test; SI: stimulation index; *Positive results: activated basophils $\geq 5\%$ and SI ≥ 2 ; F: female; M: male.

Table 6. Comparison of CAST results according to the type of characteristic reactions

Type of characteristic reactions	Positive results in CAST	
	IgE allergy group	Non-IgE allergy group
	(n=17, n/n (%))	(n=8, n/n)
Cutaneous	2/9 (22.2)	0/4
Respiratory	1/7 (14.3)	0/2
Circulatory	4/16 (25.0)	0/7

CAST: cellular allergen stimulation test.

straightforward because multiple drugs are administered during operation, making it necessary to test all drugs

taken.¹³⁻¹⁵ CAST is an analytic technique suitable to quantify the expression of cellular membrane markers on basophils. This technique was first described for perioperative allergy diagnosis about 20 years ago.^{16,17} There have been many reports regarding flow cytometric analysis of basophil activation using CD63 to test allergies to NMDAs and induction agents.¹⁸⁻²⁰ However, there are not enough studies to recommend the CAST assay for routine diagnosis of perioperative anaphylactic reactions. Furthermore, there are no studies known to us evaluating CAST in Chinese patients with anaphylactic reactions during anesthesia and operation. Therefore, our purpose was to assess the efficacy of CAST for the diagnosis of perioperative anaphylactic reactions in Chinese and to evaluate whether the test could contribute to identifying causative drugs.

In our study, only 25 patients were included and it illustrates the difficulty of studying perioperative anaphylactic reactions. In analogy to other studies, patients and control subjects had skin tests with different suspected causative drugs. We demonstrated that the proportion of IgE-mediated anaphylactic reactions was 68.0%.

Of the IgE-mediated anaphylactic reactions, NMBAs were the most frequent causative agents, which were consistent with previous reports.²¹⁻²⁴ In the present study using an SI cutoff of 2, the specificity of CAST for NMBAs was found to be 97.2% but sensitivity was only 42.9%. The specificities were reported to be nearly 100% in all of the previous studies and the sensitivity varied between 63% and 89.7%.^{6,7,18-20} The reasons for the low sensitivity in our study may be related to several factors, including the false-positive skin tests, concentrations of NMBAs for skin tests, and geographical or race differences. The sensitivity of skin tests remains unclear because of the eliciting effect of higher drug concentrations. We used the same concentrations for skin tests recommended by the French Society of Anesthesiology and may induce more positivity of skin tests in Chinese.

Only one patient with midazolam sensitivity was positive for CD63. Positive changes in basophil CD63 expression were not observed in any of the patients who were thought to have anaphylactic reactions to opioids, local anesthetics, or antibiotics. This study supported previous observations that midazolam rarely causes anaphylactic reactions²⁵ and only a small fraction of cases are really over-reacted to opioids or local anesthetics.²⁶ However, in some cases, antibiotics are the primary cause of perioperative anaphylactic reactions.²⁷ One possible explanation is most cases with antibiotics hypersensitivity were not included in this study and a larger population needs to be studied further.

The study has several limitations. First, serum tryptase levels were not routinely obtained. The Association of Anaesthetists of Great Britain and Ireland guidelines suggest collecting samples for testing serum tryptase levels at the time of the acute event.²⁸ Elevation of serum tryptase

levels indicates mast cell degranulation and typically suggests IgE-mediated anaphylactic reaction, although normal levels do not exclude it.²⁹ Nevertheless, there are lots of limitations for our departments to take and handle blood samples for tryptase assay. Second, the sample size in this study was small. It was hard to perform a retrospective study because some patients may refuse to receive skin tests and CAST after several weeks.

In conclusion, CAST may be useful for the diagnosis of anaphylactic reactions during perioperative period since it is a safe *in vitro* test method with a high specificity. However, the sensitivity of CAST remains unclear. This may explain why CAST has not become a widely used technique in daily clinical practice. These findings illustrates further evaluation of causative drugs is still quite difficult and would need more studies to further evaluate and improve the role of CAST in the diagnosis of perioperative anaphylactic reactions.

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(Received June 14, 2014)

Edited by Gao Jian

中華醫學會