

Comparison of Basophil Activation Test and Skin Testing Performances in NMBA Allergy



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What is already known about this topic? Neuromuscular blocking agents (NMBAs) are the main agents involved during perioperative allergy in France. The etiological diagnosis is linked to the clinical presentation, along with histamine and tryptase and skin test results. The role of BAT is still debated in this setting.

What does this article add to our knowledge? Combined CD63 and CD203c markers did not increase BAT sensitivity compared with CD203c only. BAT allowed identification of the culprit in 80% of NMBA-allergic patients and yielded concordant cross-reactivity results in 60% of the cases.

How does the study impact current management guidelines? BAT combining CD63 and CD203c markers does not replace skin testing in the assessment of NMBA allergy.

BACKGROUND: Neuromuscular blocking agents (NMBAs) are the main agents involved during perioperative immediate hypersensitivity. The etiological diagnosis (IgE-mediated allergy *vs* nonallergy) is linked to the clinical presentation together with tryptase and histamine levels and skin test results. The role of basophil activation test (BAT) needs to be better defined in this setting.

OBJECTIVES: To assess the role of BAT compared with the results of skin testing in 31 patients experiencing immediate NMBA hypersensitivity and compare skin test results and BAT performances in the identification of alternative NMBAs.

METHODS: Histamine and tryptase levels were quantified. Anesthetic drugs, including NMBAs, were skin-tested. Basophil

CD63 and CD203c expressions were measured in response to serial dilutions of the different NMBAs.

RESULTS: Allergy and Nonallergy groups involved 19 and 12 patients, respectively. Circulating histamine and tryptase levels were significantly increased in allergic patients. In the Allergy group, while skin test results were positive in 100% (19 of 19) of the cases, BAT positivity to the culprit NMBA reached 78.9% (15 of 19) when combining CD63 and CD203c. NMBAs cross-reactivity was identified through skin testing and BAT in 36.8% (7 of 19) and 26.3% (5 of 19) of the cases, respectively. The concordance (culprit and cross-reactive NMBAs) between skin tests and BATs was between 73.6% (14 of 19) and 100% (19 of 19) for each NMBA. Negative skin-tested NMBAs were uneventfully used in 7 NMBA-allergic patients. In the Nonallergy group, skin test results were negative in 100% of the cases while BAT result was positive once (CD63 upregulation).

CONCLUSION: In our technical conditions, BAT does not replace skin testing in the assessment of NMBA allergy. © 2018 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2018;6:1681-9)

Key words: Anaphylaxis; CD63 protein, human; CD203c protein, human; Flow cytometry; Histamine; Hypersensitivity; immediate; Neuromuscular blocking agents; Skin tests; Tryptase

INTRODUCTION

Neuromuscular blocking agents (NMBAs) are the main triggers involved during perioperative immediate hypersensitivity in France.¹ The clinical presentation, together with biological parameters and skin tests, help identify the culprit drug and the pathomechanism involved (IgE-mediated allergy *vs* nonallergy). Skin testing remains the definitive standard for the detection of IgE-mediated NMBA allergy. To complete the assessment, skin cross-reactivity with other commercialized NMBAs is investigated to identify safe alternative regimens.¹⁻⁴

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Abbreviations used

BAT- basophil activation test
 IDT- intradermal test
 MFI- median fluorescence intensity
 NMBA- neuromuscular blocking agent
 NPV- negative predictive value
 PPV- positive predictive value
 PT- prick test
 QAI- quaternary ammonium ion

Flow cytometry allows quantifying the *ex vivo* capacity of sensitized blood basophil activation.^{5,6} The basophil activation test (BAT) is based on the upregulation of 2 markers (CD63 and CD203c) expressed at the basophil membrane on *ex vivo* activation by the suspected drug. BAT might add to the etiological diagnosis of immediate NMBA hypersensitivity and help to identify both cross-reactive and safe alternative compounds.⁶⁻¹¹ However, its role needs to be better defined in this setting because skin testing is considered to be more sensitive than *in vitro* procedures.^{1-3,12}

We conducted a retrospective study in 31 patients experiencing perioperative immediate hypersensitivity occurring within 5 minutes of NMBA injection. The objectives of the study were to (1) identify the pathomechanism of the clinical event on the basis of current guidelines^{2,3,12,13}; (2) compare the results of skin testing with those obtained using BAT (identification of the culprit and cross-reactive NMBAs); (3) compare the positive and negative predictive values of skin testing and BAT in the diagnosis of immediate NMBA hypersensitivity; (4) assess the role of BAT in this clinical setting; and thus (5) evaluate the performance of both tests for predicting safe alternative regimens for further anesthesia.

METHODS

This observational retrospective study was performed in patients referred to an anesthesia-allergy center, between 2009 and 2011, following immediate hypersensitivity occurring within 5 minutes of NMBA injection.

Demographic and clinical features

The following data were collected: age, sex, administered NMBA. The severity of the reactions was based on clinical features using the *Ring and Messmer* scale.^{1,2,14}

Skin testing

Skin tests were carried out at least 4 weeks after the clinical reaction.^{1,2,13} The delay between the onset of clinical features and skin testing was compiled. All the drugs, including the culprit NMBA, administered within 5 minutes before the occurrence of the clinical reaction were skin tested through prick tests (PTs) followed by intradermal tests (IDTs) if no reaction was elicited by the PTs.^{1,13} Diagnostic criteria for a positive skin test result (including PT and IDT) and maximum recommended concentrations were used according to current French guidelines.¹ A PT result is considered positive if, within 15 to 20 minutes of injecting the drug solution on the forearm, a wheal with a diameter equal to at least half that achieved with the positive control and larger (>3 mm) than that achieved with the negative control appears. IDT result is considered positive if, within 20 minutes of injecting the drug solution, a wheal

(usually pruriginous) with a diameter equal to at least the double of the injection bleb appears and is surrounded by a flare.

NMBAs cross-reactivity was evaluated through PTs and IDTs using the commercially available NMBAs: suxamethonium (Aguettant, Lyon, France), rocuronium and vecuronium (MSD, Courbevoie, France), atracurium (Hospira, Meudon-La-Forêt, France), and cisatracurium (GSK, Marly-le-Roi, France).

Blood sampling timing

Circulating tryptase and histamine levels were both quantified at the time of the reaction as recommended.¹⁻⁴ Baseline tryptase levels, specific IgE levels, and BAT results were evaluated when the patient was referred for investigation.

Plasma tryptase and histamine

Acute plasma histamine (N < 10 nmol/L) (enzyme immunoassay (EIA), Immunotech, Beckman Coulter, Marseille, France) and total tryptase (ThermoFisher Scientific, Saint-Quentin-en-Yvelines, France) concentrations were measured. An increased acute over baseline tryptase level was defined when greater than $[2 + 1.2 \times \text{baseline tryptase level}]$, as recently suggested.^{15,16} Information on the delay between the onset of the clinical signs and blood sampling was collected.

Serum specific IgE

The concentrations of specific IgE to quaternary ammonium ion (QAI) and suxamethonium were determined (ThermoFisher Scientific).¹⁷ Drug-specific decision thresholds were set up at 0.35 kU/L and 0.11 kU/L for QAI and suxamethonium, respectively, as previously recommended.^{9,17} Concentrations below 0.1 kU/L were assigned a value of 0.1.

Basophil activation test

BAT was done blind immediately after blood sampling.⁸ We developed a whole blood technique adapted from a commercial test (FlowCAST Bühlmann Laboratories, Schönenbuch, Switzerland) measuring the increased expression of CD63 and CD203c. We used the stimulation buffer (containing IL-3) as a negative control, anti-FcεRI as a positive control, and serial dilutions of 5 NMBAs: suxamethonium (5000-5 µg/mL), rocuronium (500-5 µg/mL), vecuronium (500-5 µg/mL), atracurium (250-2.5 µg/mL), and cisatracurium (100-1 µg/mL). Cells were first stained with anti-CCR3-phycoerythrin (PE) and anti-CD63-fluorescein isothiocyanate (FITC) mAbs (from the FlowCAST kit) and then with anti-CD203c-allophycocyanin (APC) mAb (Miltenyi Biotec, Paris, France). Flow cytometric analysis was performed on a FACS-Canto II flow cytometer (BD Immunocytometry Systems, San Jose, Calif) by acquiring at least 500 basophils per sample. Basophils identification relied on the combination of low side scatter and high CCR3 expression. The percentage of CD63-positive basophils was evaluated in response to the drugs, and a stimulation index was calculated (ratio between the percentage of CD63-positive basophils in response to each drug and the negative control). CD203c increase was expressed as the ratio of the median fluorescence intensity (MFI) obtained with the different NMBAs to the MFI obtained with the negative control. Results were considered positive when more than 5% of basophils were CD63-positive with a stimulation index greater than or equal to 2, or if the CD203 MFI ratio was greater than or equal to 2 with at least 1 dilution of the NMBA.¹⁸

TABLE I. Patients' clinical history and tryptase results in NMBA-induced allergic hypersensitivity (n = 19)

Allergy					
Case	NMBA	Grade	Clinical features	Tryptase (N < 11.4 µg/L) (*)	Basal tryptase (N < 11.4 µg/L)
1	Suxamethonium	III	AP: 53/37 mm Hg, HR: 120/min Generalized erythema, bronchospasm	16.3 (130 min)	4.3
2	Cisatracurium	III	AP: 45/20 mm Hg, HR: 120/min Localized erythema	39.2 (?)	7.2
3	Suxamethonium	III	AP: 50/30 mm Hg, HR: 130/min No cutaneous signs	84.9 (75 min)	14.2
4	Suxamethonium	I	Generalized erythema	15.6 (60 min)	3.2
5	Suxamethonium	III	AP: 60/30 mm Hg, HR: 135/min Ventricular arrhythmia, localized erythema	1 (35 min)	2
6	Rocuronium	III	SAP: 40 mm Hg, HR: ? Generalized erythema when AP was restored	21.9 (?)	4.7
7	Suxamethonium	III	SAP: 50 mm Hg, HR: 150/min Localized erythema, palpebral angioedema	ND	5.4
8	Suxamethonium	III	SAP: 60 mm Hg, HR: 130/min Generalized erythema	ND	2.7
9	Suxamethonium	III	SAP: 40 mm Hg, HR: 130/min Localized erythema	27.2 (100 min)	4.7
10	Suxamethonium	III	SAP: 50 mm Hg, HR: 60/min Generalized erythema	149 (10 min)	5.6
11	Suxamethonium	III	AP: 50/30 mm Hg, HR: 60/min Bronchospasm, cutaneous signs ?	83.5 (15 min)	5.6
12	Atracurium	III	AP: 56/30 mm Hg, HR: 170/min No cutaneous signs	36.9 (70 min)	1.7
13	Suxamethonium	III	AP: 40/10 mm Hg, HR: 130/min Generalized erythema	60.8 (30 min)	2.2
14	Suxamethonium	III	AP: 40/20 mm Hg, HR: 150/min Generalized erythema	194 (30 min)	7.4
15	Rocuronium	III	SAP: 30 mm Hg, HR: 120/min Bronchospasm, localized erythema, vomiting	26.9 (>18 h)	6.5
16 [20]	Suxamethonium	III	Unmeasurable AP, HR: 40/min Ventricular arrhythmia, cutaneous signs when AP was restored	142 (?)	6.7
17	Suxamethonium	III	AP: 60/40 mm Hg, HR: 120/min Bronchospasm, generalized erythema	201 (45 min)	4.7
18	Suxamethonium	III	AP: 60/20 mm Hg, HR: 80/min Ventricular arrhythmia, generalized erythema when AP was restored	42.4 (90 min)	3.8
19	Suxamethonium	III	Unmeasurable AP, rapid thready pulse, bronchospasm, no cutaneous signs	52 (90 min)	4.8

AP, Arterial pressure; HR, heart rate; SAP, systolic arterial pressure.

*Onset delay between clinical signs and blood sampling.

Patients' classification and follow-up

Patients with positive skin test results to the culprit NMBA with or without increased histamine and/or tryptase levels were referred to the "Allergy" group (ie, IgE-mediated allergy). Patients with negative skin test results to the administered NMBA with or without elevated histamine and/or tryptase levels were referred to the "Nonallergy" group^{1-3,14}. In both groups, negative skin-tested NMBAs were proposed for further anesthesia. A phone call survey was then performed to compile the name(s) of the administered NMBA(s) in case of subsequent anesthesia together with the safety of the procedure.

Statistical analysis

Results are expressed as median (min-max) or (95th percentile; range). Between-group comparisons were performed using 2-tailed Mann-Whitney or chi-square tests (Instat, GraphPad Software, La

Jolla, Calif). Intragroup comparisons were performed using 2-tailed Wilcoxon or McNemar tests. Significance was assumed when *P* value was less than .05.

RESULTS

Overall cohort characteristics

Thirty-one patients (27 women, 4 men) who experienced immediate hypersensitivity occurring within 5 minutes of the NMBA injection were investigated between 2009 and 2011 (Tables I and II). Their median age was 59 years (range, 25-88 years). The clinical severity was as follows: grade I (n = 2 [6.4%]), grade II (n = 11 [35.5%]), grade III (n = 18 [58.1%]). No grade IV was reported. Administered NMBAs were suxamethonium (n = 21), atracurium (n = 8), rocuronium (n = 2),

TABLE II. Patients' clinical history and tryptase results in NMBA-induced nonallergic hypersensitivity (n = 12)

Nonallergy					
Case	NMBA	Grade	Clinical features	Tryptase (N < 11.4 µg/L) (*)	Basal tryptase (N < 11.4 µg/L)
20	Suxamethonium	II	HR: 95/min Mild bronchospasm, localized erythema	11.2 (15 min)	9.9
21	Atracurium	II	AP: 80/30 mm Hg, HR: 60/min Localized erythema	16.3 (15 min)	7.6
22	Atracurium	II	Hypotension, tachycardia, generalized erythema	5.5 (30 min)	4.1
23	Atracurium	II	AP: 80/50 mm Hg, HR: 80/min Localized erythema	3 (30 min)	4.6
24 [21]	Atracurium	II	AP: 77/50 mm Hg, HR: 120/min Generalized erythema	69 (90 min)	73
25	Suxamethonium	II	HR: 150/min Generalized erythema	4.1 (90 min)	4.5
26	Suxamethonium	II	Severe bronchospasm followed by moderate hypotension, tachycardia, palpebral angioedema, facial erythema	12.9 (120 min)	8.3
27 [19]	Suxamethonium	III	Severe bronchospasm then AP: 50/20 mm Hg, HR: 100/min Generalized erythema	5.4 (40 min)	5.8
28	Atracurium	II	AP: 79/45 mm Hg, HR: 55/min Facial erythema	3.3 (45 min)	1.8
29	Suxamethonium, atracurium	II	Mild bronchospasm, then AP: 60/30 mm Hg, no tachycardia, localized erythema	5.8 (210 min)	7.5
30	Suxamethonium	II	AP: 75/45 mm Hg, HR: 110 b/min Generalized erythema	3 (55 min)	1
31	Atracurium	I	Generalized erythema	17.3 (35 min)	12.9

AP, arterial pressure; HR, heart rate; SAP, systolic arterial pressure.

*Onset delay between clinical signs and blood sampling.

and cisatracurium (n = 1). In 1 case, suxamethonium and atracurium were injected one after the other (Table II, case 29).

Allergic and nonallergic patients' characteristics

A total of 19 of 31 patients (16 women, 3 men) with a median age of 61 years (range, 26-88 years) were included in the Allergy group (Table I). The Nonallergy group involved 12 patients (11 women, 1 man) with a median age of 58 years (range, 25-68 years) (Table II).

There was no between-groups difference in the distribution of age and sex ($P = .17$). Suxamethonium was the most frequent NMBA involved in the Allergic (79%) and Nonallergic (58%) groups ($P = .21$). Grade III reaction was reported in 90% (n = 17) of the allergic patients and 1 (8%) nonallergic case consistent with severe bronchospasm ($P < .0001$) (Table II, case 27). Epinephrine was required in 83% (15 of 18) of the allergic grade III reactions but in only 1 nonallergic case ($P < .0001$). No vasoconstrictor was required in 67% (8 of 12) of the nonallergic cases while ephedrine was used in 2 patients (17%) and neosynephrine (8%) or epinephrine (8%) in 1 case.

SKIN TESTS

The Allergy group involved 19 of 31 patients (61.3%) with positive skin test results to suxamethonium (n = 15 [79%]), rocuronium (n = 2 [11%]), atracurium (n = 1 [5%]), and cisatracurium (n = 1 [5%]). Skin test results were positive by PTs in 84% of the patients and by IDTs in the remaining cases

(16%). The assessment of NMBA skin cross-reactivity evidenced sensitization to 1 or 2 NMBA in 36.8% (n = 7) of the patients (Table III).

The Nonallergic group included 12 patients (38.7%) with negative PT and IDT results to atracurium (n = 7 [53.8%]) or suxamethonium (n = 6 [46.2%]) and to the other NMBA (data not shown). In both groups, skin test results remained negative to antibiotics, hypnotics, opioids, and latex. Three cases have been previously reported (cases 16, 24, and 27).¹⁹⁻²¹

Biological tests

Plasma histamine and tryptase. The individual values of plasma tryptase and histamine levels are shown in Figures 1 and 2. The median delay between clinical signs onset and blood sampling was similar: 30 and 15 minutes for histamine ($P = .068$) and 60 and 42 minutes for tryptase ($P = .49$) in the Allergic (Table I) and Nonallergic (Table II) groups, respectively. Acute tryptase and histamine levels were increased in allergic patients ($P < .001$). Results from blood sample taken 18 hours after the event in 1 allergic case (Table I, case 15) and from a patient with mastocytosis in the skin (Table II, case 24) were not included in the statistical analysis.

The Allergy group. The median histamine concentration measured in 13 of 19 patients was 100 nmol/L (100; 10-100). In 17 of 19 patients, the median concentration of tryptase showed a 9-fold increase (42.4 µg/L) (200; 1-201) when compared with baseline tryptase (4.7 µg/L) (7.0; 1.7-14.2) ($P < .001$). In 1 case,

TABLE III. Comparison of skin test and BAT (CD63 and CD203c) results in NMBA allergy (n = 19)

Case	Skin tests: Culprit NMBA and cross-reactivity			BAT: Culprit NMBA and cross-reactivity			Skin tests and BATs concordances		
	NMBA involved	Threshold positivity	Cross-reactivity	CD63* SI (%)	CD203c [†] MFI ratio	BAT conclusion (optimal positive concentration μ g/mL)	NMBA involved	Cross-reactivity	Safe subsequent injection: administered NMBA
1	sux	PT 10 ⁻¹ (1 mg/mL)	—	1.2 (6)	1.3	—	No	Yes	roc
2	cis	PT 10 ⁻¹ (0.2 mg/mL)	atra	cis: 8.9 (30) atra: 8.4 (29) roc: 5.1 (17) vec: 3.8 (13)	cis: 2.7 atra: 3.1 roc: 2.6 vec: 2.3	+ cis (100) atra (250) roc (500) vec (125)	Yes	No	roc
3	sux	IDT 10 ⁻² (0.1 mg/mL)	—	1 (2)	1	—	No	Yes	atra
4	sux	PT pure (10 mg/mL)	roc, vec	sux: 7.7 (59) vec: 2.2 (17)	sux: 4.5 vec: 2.1	+ sux (500) vec (125)	Yes	No	atra
5	sux	PT pure (10 mg/mL)	roc	1.3 (6)	2.7	+ sux (1000)	Yes	No	No surgery
6	roc	IDT 10 ⁻¹ (1 mg/mL)	vec	2 (10)	2.4	+ roc (500)	Yes	No	No surgery
7	sux	PT pure (10 mg/mL)	roc, vec	0.5 (2)	2.3	+ sux (50)	Yes	No	No surgery
8	sux	PT pure (10 mg/mL)	—	2.7 (9)	2.5	+ sux (50)	Yes	Yes	Lost to follow-up
9	sux	IDT 10 ⁻² (0.1 mg/mL)	—	0.9 (4)	0.9	-	No	Yes	No surgery
10	sux	PT 10 ⁻² (0.1 mg/mL)	—	8.2 (29)	2.6	+ sux (500)	Yes	Yes	atra
11	sux	PT 10 ⁻² (0.1 mg/mL)	—	2 (11)	2.1	+ sux (500)	Yes	Yes	Lost to follow-up
12	atra	PT 10 ⁻³ (0.01 mg/mL)	—	2 (12)	1.5	+ atra (10)	Yes	Yes	Lost to follow-up
13	sux	PT 10 ⁻² (0.1 mg/mL)	—	0.8 (2)	2	+ sux (50)	Yes	Yes	atra
14	sux	PT 10 ⁻² (0.1 mg/mL)	—	1 (4)	1.7	—	No	Yes	Lost to follow-up
15	roc	PT 10 ⁻³ (0.01 mg/mL)	vec	roc: 6.3 (60) vec: 6.1 (59)	roc: 2.2 vec: 2.2	+ roc (500) vec (500)	Yes	Yes	No surgery
16	sux	PT 10 ⁻¹ (1 mg/mL)	—	2 (9)	2	+ sux (50)	Yes	Yes	atra
17	sux	PT 10 ⁻² (0.1 mg/mL)	—	sux: 3.2 (17) roc: 2.7 (14) vec: 2.9 (15)	sux: 2 roc: 2.2 vec: 2.8	+ sux (500) roc (500) vec (125)	Yes	No	Lost to follow-up
18	sux	PT pure (10 mg/mL)	—	5.5 (22)	2.4	+ sux (500)	Yes	Yes	No surgery
19	sux	PT 10 ⁻³ (0.01 mg/mL)	roc, vec	sux: 4.9 (36)	sux: 4.5 roc: 2.6	+ sux (500) roc (500)	Yes	No	No surgery

atra, Atracurium; cis, cisatracurium; roc, rocuronium; SI, stimulation index; sux, suxamethonium; vec, vecuronium.

*CD63 increase is expressed as a SI. Result is positive when more than 5% of basophils are CD63 positive with an SI greater than or equal to 2.

†CD203c increase is expressed as a ratio of the MFI. Result is positive when CD203 MFI ratio is greater than or equal to 2 with at least 1 dilution of NMBA.

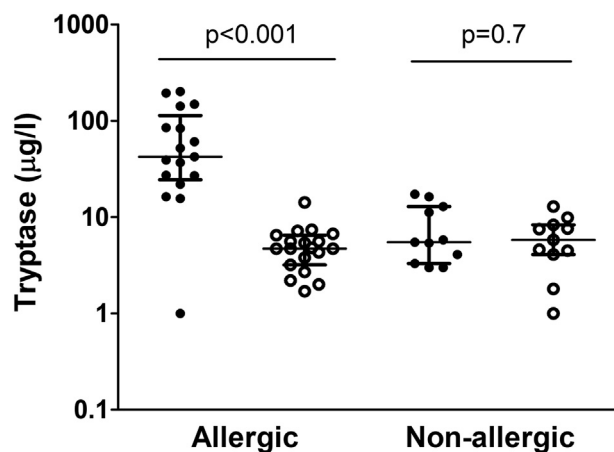


FIGURE 1. Plasma tryptase levels in allergic (17 of 19) and nonallergic (12 of 12) patients. Tryptase levels were determined at the time of the reaction (●) and at least 4 weeks after the reaction (baseline level) (○).

tryptase increase was lacking despite grade III reaction (Table I, case 5).

The Nonallergy group. The median histamine concentration measured in 11 of 12 patients was 4 nmol/L (95th percentile, 27; range, 3–27). In 12 of 12 patients, the median tryptase concentration (5.5 µg/L) (17.3; 3.0–17.3) was not different from that at baseline (5.8 µg/L) (12.9; 1.0–12.9) ($P = .37$).

Serum specific IgE. Specific IgE levels to QAI were increased (≥ 0.35 kU/L) in 16 of 19 allergic patients (sensitivity, 84.2%) and in 2 of 12 nonallergic cases (specificity, 83.3%) (data not shown). The median levels of IgE to QAI were different between allergic (0.8 kU/L [24.6; 0.1–24.6]) and nonallergic (0.1 kU/L [2.1; 0.1–3.5]) groups ($P < .01$). In NMBA allergy, the positive predictive value (PPV) and the negative predictive value (NPV) were 88.9% and 76.9%, respectively.

Specific IgE levels to suxamethonium were increased (≥ 0.11 kU/L) in 9 of 15 allergic patients to suxamethonium (0.2 kU/L) (26.4; 0.1–26.4) (sensitivity, 60%) and in 1 of 6 nonallergic patients exposed to suxamethonium (0.1 kU/L) (0.9; 0.1–0.9) (specificity, 83.3%) (data not shown) ($P = 0.08$). The PPV and NPV were 90% and 45.4%, respectively.

BAT results and comparison to skin tests. The flow cytometric representative results of allergic and nonallergic patients to rocuronium are displayed in Figure 3.

The Allergy group. Except in 2 patients studied 3 (case 6) and 6 (case 7) months after the reaction, the diagnosis was assessed within 7.6 weeks (4.3–13.7) after the clinical event (Table I). While skin test results were positive in 100% of the cases as defined in the study design, BAT result was positive to the culprit agent in 15 of 19 patients (78.9%) (Table III). An increase in CD63 expression in response to the culprit NMBA was observed in 12 of 19 patients (63.1%) and in 14 of 19 patients (73.6%) for CD203c ($P = .61$); this corresponds to isolated increases in CD203c in 3 cases (patients 5, 7, and 13) and CD63 in 1 case (patient 12). When combining both

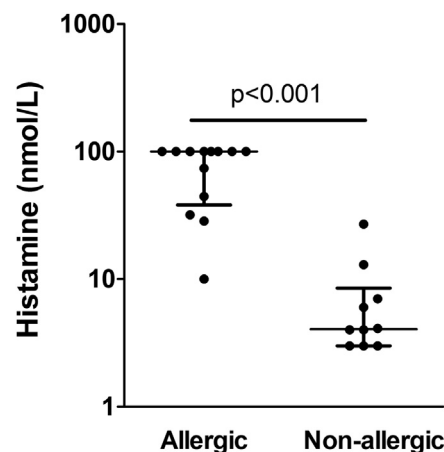


FIGURE 2. Plasma histamine levels measured at the time of the reaction in allergic (13 of 19) and nonallergic (11 of 12) patients.

markers, the sensitivity was 78.9%, close to that obtained with CD203c alone. The PPV and NPV of BAT, including CD63 and/or CD203c, to the culprit agent were, respectively, 93.7% and 73.3%. NMBA cross-reactivity assessed by skin testing was found in 7 of 19 patients (36.8%) and in 5 of 19 patients (26.3%) using BAT (Table III). The concordance for NMBA cross-reactivity between skin tests and BAT was 63.1% (12 of 19 patients). The differences mainly concerned steroidal NMBA (cases 2, 4, 5, 6, 7, 17, and 19). Altogether, the concordance between skin tests and BATs including the culprit and cross-reactive NMBA was 73.6% (14 of 19) for vecuronium, 78.9% (15 of 19) for rocuronium and suxamethonium, and 100% (19 of 19) for atracurium and cisatracurium.

The Nonallergy group. Except in 1 case (Table II, case 27) assessed 8 months after the reaction, the patients were investigated within 8.9 weeks (4.7–19.8). In these negative skin-tested patients, BAT result was negative for 11 of 12 of the administered NMBA, giving a specificity of 91.7% to the culprit agent. BAT result was negative for all other skin-tested NMBA, except for atracurium and cisatracurium, which were positive in 1 patient injected with atracurium (Table II, case 23); the concordance between skin tests and BATs was thus 91.7% with those NMBA.

Subsequent NMBA injection in allergic and nonallergic patients

The Allergy group. Negative skin-tested NMBA were safely injected during subsequent procedures in 7 of 19 NMBA-allergic patients (Table III). BAT yielded negative results to the NMBA subsequently administered in 6 of 7 of these allergic patients or positive results in 1 case (Table III, case 2). No further surgery was performed in 7 of 19 NMBA-allergic patients while 5 of 19 patients were lost to follow-up.

The Nonallergy group. Succinylcholine, that was negative by skin testing, was safely administered during subsequent anesthesia in this nonallergic patient who previously experienced severe bronchospasm following endotracheal intubation facilitated by succinylcholine (Table II, case 27). Eight nonallergic

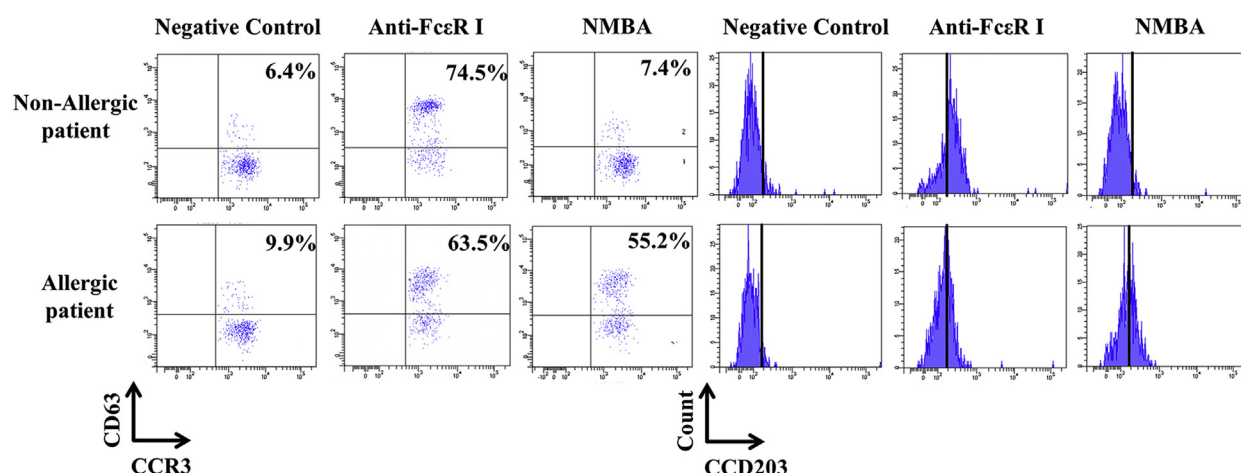


FIGURE 3. Whole blood basophil identification: combination of low side scatter and high CCR3 expression. Anti-FcεRI: positive control. IL-3-containing stimulation buffer: negative control. CD63 expression (upper panel) and CD203c expression (lower panel) are representative results obtained in a patient allergic to rocuronium (case 15) and in a patient nonallergic to rocuronium (case 21), out of the 31 tested patients.

patients were lost to follow-up while 3 were not reinjected with an NMBA during subsequent surgery.

DISCUSSION

The main findings of this retrospective study are the following: (1) despite a previously documented NMBA allergy, negative skin-tested NMBAs could be safely used during subsequent anesthesia; (2) a lower NMBA skin cross-reactivity rate (36%) than previously reported was observed; (3) BAT does not replace skin testing in the identification of the culprit agent in NMBA allergy, albeit it has a good sensitivity (80%) and specificity (91.7%); (4) combined CD63 and CD203c markers did not increase BAT sensitivity when compared with CD203c only; (5) the concordance between skin tests and BATs including the culprit and cross-reactive NMBAs was between 73.6% and 100% for each NMBA.

The combined analysis of the clinical history along with histamine/tryptase levels and skin tests results allowed identifying NMBA allergy in two-third of the cases and a nonallergic pathomechanism in the remaining cases. In our study, NMBA allergy occurred during anesthesia induction and, except in 1 case, the clinical presentation was life-threatening. Severe cardiovascular homeostasis disturbances, presumably the result of the additive cardiovascular effects of anesthesia and IgE-mediated drug allergy,²² were present in 95% of the cases. Epinephrine was required in almost 85% of these life-threatening reactions.

In contrast, nonallergic NMBA hypersensitivity was mild, except in 1 case of severe bronchospasm after endotracheal intubation in an obese patient with unknown uncontrolled asthma (case 27). No vasoconstrictor was required in almost 70% of these cases. Interestingly, it was recently shown in a mouse model that mivacurium and rocuronium, in contrast to suxamethonium and atracurium, can induce histamine release *in vitro* through mast cell degranulation via the activation of the secretagogue Mrgprb2 receptor.²³ However, this mechanism remains to be described in humans.

Tryptase and histamine levels correlate with the clinical presentation in allergic *versus* nonallergic patients. In 1 allergic grade III reaction, tryptase level was not increased. An increase in tryptase level is highly suggestive of immunological mast cell activation but its absence does not preclude the diagnosis of IgE-mediated allergy. In addition, although an increase in serum tryptase level (usually moderate) can be measured after onset of symptoms in cases of nonallergic immediate hypersensitivity, it may not be elevated.^{1-3,12} Our results are therefore in accordance with 2 studies supporting the place of both increased histamine and tryptase levels in the diagnosis of IgE-mediated allergy.^{24,25}

Interestingly, we showed a lower NMBAs skin cross-reactivity rate (36%) than reported by other groups with rates as high as 75%.²⁶ In addition, despite a previous NMBA allergy, negative skin-tested NMBAs could be safely injected during subsequent anesthesia in 7 patients from the Allergy group. The NPV of skin tests to NMBAs is actually debated. Less than 60 cases have been published in which, despite a previous NMBA allergy, NMBAs were safely injected during subsequent anesthesia on the sole basis of negative skin test results including either IDTs only^{27,28} or combined PTs and IDTs.²⁹⁻³² The NMBA concentrations used for skin testing were those currently recommended³⁰⁻³² or lower^{27,28,33} or remain not documented.^{29,32} In our patients, NMBAs yielding negative PT and IDT results up to the maximum recommended concentrations were used further^{1,13} despite increased specific IgE levels to QAI. The presence of circulating IgE to QAI is not predictive for subsequent NMBA allergy and thus does not preclude further use of NMBAs.^{3,34-36} Finally, second reactions to a negative skin-tested NMBA when using IDTs only, during investigation of a first reaction, have been reported.^{28,37-39} Failure to prevent further NMBA allergy might be explained by the use of lower dilutions of NMBAs than those recommended.^{1,13}

One of the main objectives of this study was to more closely examine the diagnostic performance of BAT in immediate NMBA hypersensitivity compared with skin testing. In contrast to previous studies, the originality of our study was to combine 2

activation markers (CD63 and CD203c). The expression of both proteins is regulated by different mechanisms. CD63 expression appears to be related to degranulation in contrast to that of CD203c, which can be weakly expressed by resting basophils.^{5,6} In the present study, the use of CD203c alone allowed a better sensitivity (79%) than that achieved with CD63 (70%), albeit not significant and without altering the specificity. Interestingly, combined markers did not improve BAT sensitivity. Most of the previous studies used CD63 upregulation for diagnosis purpose⁴⁰⁻⁴³ with a sensitivity ranging between 60% and 80% when compared with skin tests, depending on the cutoff and the delay between the clinical reaction and BAT sampling.^{42,43} Unexpectedly, as far as CD63 expression was used, BAT sensitivity was slightly weaker than in rocuronium-exposed patients,⁹ whereas suxamethonium was primarily involved in our patients. Interestingly, we found that CD203c alone had a better sensitivity than that previously reported in NMBA allergy⁴⁴; this could be related to the different cutoffs used and/or the expression of the results (percentage *vs* MFI in the present study). IL3 alone has been shown to induce CD203c expression.^{5,45} This might decrease the MFI ratio and explain why CD63 was the sole upregulated marker in one of our allergic patients with relatively high CD203c MFI in the presence of IL-3-containing buffer.

In a recent study, BAT was recommended in suspected atracurium allergy with negative or “equivocal” skin test results while further use of atracurium was not allowed in case of positive BAT result, irrespective of the skin test response⁴⁶; unfortunately, clinical history and tryptase results are lacking. One should keep in mind that perioperative immediate hypersensitivity is not always of allergic pathomechanism and skin test results need to be interpreted according to the clinical history. The clinical presentation together with or without tryptase increase and negative skin test results led us to diagnose nonallergic immediate reaction in 12 patients, in which further use of the culprit agent was allowed. Although a small rise in tryptase level was detected in 2 nonallergic patients, it was not considered sufficient to invalidate the diagnosis compared with the clinical history along with negative skin test and BAT results to the culprit NMBA. In addition, in 1 (case 23) of these 12 nonallergic cases injected with atracurium, BAT was positive to atracurium and cisatracurium. These latter agents were authorized for further use because skin testing yielded negative results. Unfortunately, this patient was lost to follow-up. However, 1 cisatracurium-induced allergic patient (case 2) with negative skin test results and positive BAT to rocuronium uneventfully received rocuronium during subsequent anesthesia. In contrast to Uyttebroek et al,⁴⁶ further use of NMBAs in our patients was based on skin test results irrespective of the BAT results. Finally, in a case consistent with mastocytosis in the skin, acute tryptase was not different from increased baseline tryptase.

This study also confirms that BAT sensitivity (80%) and specificity (91.7%) are better than those of specific IgE, especially to suxamethonium (60% and 83.3%, respectively). Using a cutoff of 0.35 kU/L for succinylcholine as suggested^{9,17} would decrease the sensitivity to 53% instead of 60% without any modification in the specificity. Nevertheless, we believe that the presence of specific IgE to QAI and/or suxamethonium must be interpreted together with the other clinical and biological features. The presence of drug-specific IgE is not in itself a proof that the drug is responsible for the reaction.³ In other words, the

presence of anti-suxamethonium IgE does not reflect a genuine suxamethonium allergy *per se* and might result in unnecessary avoidance measures.⁹ Thus, in a series published by Leysen et al,⁹ 5 patients with positive IgE to rocuronium but with negative skin test and BAT results safely received rocuronium later.

Another objective of this study was to evaluate the interest of combined skin tests and BATs in the identification of safe alternative NMBAs. We demonstrated that BAT has a good concordance to the negative skin-tested cross-reactive NMBAs because it was less sensitive in patients with positive skin-tested benzylisoquinolins. Few studies have evaluated BAT as a possible tool to identify a safe alternative. The use of the CD63 marker as the sole screening tool in selecting cisatracurium following a documented vecuronium allergy has been reported.⁴⁷ More recently, combined BAT and skin cross-reactivity negative results allowed the use of rocuronium uneventfully after cisatracurium allergy.⁴⁸ In both reports, the clinical history along with tryptase results suggested IgE-mediated NMBA allergy but the diagnosis was supported by positive skin test results to the culprit NMBA only in 1 case.⁴⁸ This has been confirmed in 15 NMBA-allergic patients uneventfully administered an NMBA negative in both skin test and BAT results; however, the clinical history and tryptase levels are also lacking in this study.⁹ In our series, 6 NMBA-allergic patients were uneventfully administered an NMBA negative in both skin test and BAT results during subsequent anesthesia while another allergic patient safely received a negative skin-tested NMBA that was positive in BAT. In addition, the sole nonallergic patient experiencing a life-threatening reaction consistent with severe bronchospasm following intubation facilitated by succinylcholine safely received this latter agent during further anesthesia.

In conclusion, skin testing allowed provision of a diagnosis and also suggested safe alternative regimens, especially in NMBA allergy. The performance of BATs was globally satisfactory with a better sensitivity and specificity than those of specific IgE while not reaching sensitivity and specificity of skin tests. However, BAT did not identify the culprit agent in 20% of NMBA-allergic patients and yielded divergent cross-reactivity results in almost 40% of the cases. We thus believe that BAT cannot replace skin testing in the assessment of NMBA immediate hypersensitivity but might be a useful “screening” tool when skin tests are not available, because they are not performed everywhere.

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