

LABORATORY INVESTIGATION

Assessing cross-reactivity to neuromuscular blocking agents by skin and basophil activation tests in patients with neuromuscular blocking agent anaphylaxis

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Abstract

Background: Following diagnosis of neuromuscular blocking agent (NMBA) anaphylaxis, identifying safe alternatives for subsequent anaesthesia is critical. A patient with anaphylaxis to one NMBA can also have an allergic reaction to other NMBAs (cross-reactivity). Whilst drug provocation testing is standard for identifying or excluding allergy, there is significant risk. *In vitro*, after an allergen activates basophils, basophils express surface activation markers that can be measured by basophil activation testing (BAT). We compared cross-reactivity between NMBAs assessed by BAT against that by skin testing.

Methods: All patients attending an anaesthetic allergy clinic in Sydney, Australia between May 2017 and July 2018 diagnosed with NMBA anaphylaxis qualified for this study comparing intradermal skin tests and BAT with a panel of NMBAs (rocuronium, vecuronium, pancuronium, suxamethonium, cisatracurium).

Results: Of the 61 patients participating, sensitisation on skin testing and on BAT completely matched in only nine patients (15%). Sensitisation was not in agreement for pancuronium, cisatracurium and rocuronium, but was in agreement for vecuronium and suxamethonium. Nine patients with negative skin tests subsequently tolerated cisatracurium, and one false positive on BAT to cisatracurium was detected.

Conclusions: The utility of BAT in identifying safe NMBAs for subsequent anaesthesia needs further evaluation. BAT detects a different cross-reactivity profile to skin tests. Negative skin testing and BAT might increase confidence in performing drug provocation testing, but this and follow-up of subsequent anaesthesia in our cohort is necessary to determine the clinical significance of BAT sensitisation.

Keywords: anaphylaxis; drug hypersensitivity; neuromuscular blocking agents; skin tests; basophil degranulation test

Editor's key points

- Identifying safe anaesthetic drugs for use after perioperative anaphylaxis is critical, particularly for neuromuscular blocking agents (NMBAs) that exhibit cross-reactivity.
- Cross-reactivity between NMBAs was assessed by basophil activation testing (BAT) and by skin testing in 61 patients presenting to an Australian anaesthetic allergy clinic.
- Sensitisation on skin testing and on BAT completely matched in only nine patients (15%).
- Further study using drug provocation testing and follow-up of subsequent anaesthesia is needed to determine the clinical significance of sensitisation detected on skin testing and BAT.

Anaphylaxis to neuromuscular blocking agents (NMBAs) remains the leading cause of perioperative anaphylaxis in Australia.¹ Following diagnosis of NMBA anaphylaxis, identification of safe agents for subsequent anaesthesia is necessary. A patient with anaphylaxis to one NMBA can also have an allergic reaction to one or more other NMBAs (cross-reactivity).^{2–6} Whilst drug provocation testing is the standard for identifying or excluding allergy, there are no standardised algorithms for its use in identifying safe NMBAs,⁷ and there is a significant risk profile that needs to be carefully considered before use in this context.¹ Therefore, current standard practice in Australia and New Zealand to assess for cross-reactivity initially involves *in vivo* skin tests to a panel of other available NMBAs.¹ However, the clinical significance of sensitisation to these other NMBAs is not clear. This position is supported internationally.⁸

Basophil activation testing (BAT) is an *in vitro* test that has been used in investigation of drug allergy, including NMBA allergy.⁹ Following activation by allergen, mast cells and basophils degranulate, triggering release of mediators that create the clinical manifestations of allergy and anaphylaxis. Basophils, unlike mast cells, are found in sufficient numbers in peripheral blood. Activated basophils express surface markers, including CD63 and CD203c, that can be measured by flow cytometry. We examined the use of BAT for assessing cross-reactivity, its agreement with skin testing, and its role in identifying safe NMBAs for subsequent anaesthesia.

Methods

We recruited patients sequentially from the anaesthetic allergy clinic, Royal North Shore Hospital, New South Wales

(NSW), Australia, between May 2017 and July 2018, who were diagnosed with NMBA anaphylaxis. No patient declined participation. Patients received standard assessment comprising a review of clinical history, skin tests to all drugs and substances administered before the onset of anaphylaxis, skin tests to a standard panel of NMBAs (rocuronium, vecuronium, pancuronium, suxamethonium, cisatracurium), serum tryptase concentrations (ImmunoCAP, Phadia, Uppsala, Sweden), and specific IgE assays (sIgE) to morphine, pholcodine, latex, and chlorhexidine (ImmunoCAP). sIgE to morphine and pholcodine measure IgE antibodies directed against morphine and pholcodine moieties, respectively, which contain substituted ammonium groups, considered to be the antigenic determinant of NMBAs.^{3,10} Additionally, BAT was performed in our research laboratory, using CD63 and CD203c as surface activation markers.¹¹

A patient was diagnosed with NMBA anaphylaxis in one of two scenarios: (1) There was a suggestive clinical history, that is anaphylaxis occurs within 15 min of administration of the NMBA,¹² supported by positive skin tests and (2) there was a suggestive clinical history but only positive sIgE to morphine or pholcodine, and all other potential culprits had been excluded by skin and serum tests, and occasionally challenges on at least two separate occasions.

Skin tests

All patients had intradermal tests (IDT) to the suspected NMBA culprit and a panel of other available NMBAs (rocuronium, vecuronium, pancuronium, suxamethonium and cisatracurium) that reflects local patterns of use. IDT was performed at both standard and strong concentrations (see Table 1 in a recent study by Li and colleagues¹¹), as recommended in the Australian and New Zealand Anaesthetic Allergy Group Perioperative Anaphylaxis Investigation Guidelines.¹ IDT for each NMBA was classified as positive or negative for both standard and strong concentrations; a test was positive if the wheal doubled in size or increased by 3 mm.¹

BAT

Our method has been described in detail elsewhere.¹¹ To summarise, basophils were incubated with a negative control, positive control, and the standard NMBA panel above. Basophils were incubated with each NMBA at three serial dilutions.¹³ We measured the upregulation of CD63 and CD203c on basophils and calculated individual results for all NMBA-stimulated samples using both % upregulation above the negative control and stimulation index (SI), the ratio of the mean fluorescence intensity (MFI) of antigen expression on basophils in NMBA-stimulated samples to MFI of the negative

Table 1 Standard and strong concentrations of NMBAs used in the evaluation of perioperative anaphylaxis. NMBA, neuromuscular blocking agent. Adapted from Scolaro and colleagues.¹

	Standard concentration for intradermal testing ($\mu\text{g ml}^{-1}$)	Strong concentration for intradermal testing ($\mu\text{g ml}^{-1}$)	Skin prick test concentration (mg ml^{-1})
Rocuronium	10	100	10
Vecuronium	4	40	4
Pancuronium	2	20	2
Suxamethonium	50	100	10
Cisatracurium	2	20	2

control. We selected the maximum value obtained across the three serial dilutions for each NMBA. BAT results were then classified as positive or negative based on previously calculated cut-offs for a positive test in our cohort using receiver operating characteristic curve analysis.¹¹ For CD63, we applied a cut-off of 4.45% and SI=1.44, and for CD203c, 8.80% and SI=1.49.¹¹

Comparing skin test and BAT results

For each patient, the results of IDT at standard and strong concentrations, and BAT using CD63 and CD203c for rocuronium, vecuronium, pancuronium, suxamethonium and cisatracurium were recorded. Each result was then placed into one of four categories: BAT+Skin+, BAT+Skin-, BAT-Skin+, and BAT-Skin-.

Statistical analysis

SPSS version 24 (IBM, Armonk, NY, USA) was used to conduct the statistical analysis of the categorical variables. We used McNemar's test to compare the performance of skin testing and BAT in assessing cross-reactivity; this statistical test is used to determine if there are differences in dichotomous dependent variable data. A result with $P < 0.05$ was considered significant.

Results

Of 61 patients diagnosed with NMBA anaphylaxis, anaphylaxis was to rocuronium ($n=48$), vecuronium ($n=4$), or suxamethonium ($n=9$). No patients had pancuronium or cisatracurium anaphylaxis. Of the 61 patients, 51 had positive skin testing to the culprit NMBA, and 53 had positive sIgE, 51 to both morphine and pholcodine, one to morphine only, and one to pholcodine only (Table 1).

Of the 51 patients with positive skin testing to the culprit NMBA, 20 showed no cross-reactivity on skin testing, that is skin testing was positive to the culprit NMBA only, and 31 showed cross-reactivity on skin testing, that is skin testing was positive to at least one other NMBA. Of these 31 patients, 12 had sensitisation to one or more aminosteroids (rocuronium, vecuronium, pancuronium), 15 had sensitisation to aminosteroids and suxamethonium, and four had sensitisation to aminosteroids, suxamethonium and cisatracurium. Of the 61 patients, 10 had isolated sensitisation on sIgE to morphine and pholcodine, and none of these had skin sensitisation to any NMBA (Table 2).

Skin testing compared with BAT

Skin test and BAT results were categorised as positive or negative (Table 3). Of 61 patients, sensitisation on skin testing and BAT only completely matched in nine patients. There was a significant lack of agreement between skin testing and BAT for pancuronium and cisatracurium ($P < 0.05$) using McNemar's test (Table 4), with more sensitisation to pancuronium and cisatracurium on BAT compared with skin testing (Table 3). There were only three positive skin tests to cisatracurium, but 20 positive on BAT when we combined surface markers. There were 12 positive skin tests to pancuronium at standard or strong concentrations, but 23 positive on BAT. Of the 18 patients with a positive BAT for pancuronium and a negative skin test for pancuronium, nine had a positive skin test to

Table 2 Patient characteristics of 61 patients diagnosed with NMBA anaphylaxis.

Patient characteristics	
Total subjects	61
Age in years, average (range)	52 (16–75)
Female, n(%)	41 (67%)
Interval between reaction and assessment (days)	102
Atopy, n(%)	26 (43%)
Significant rise and fall in tryptase, n(%)	47 (77%)
Number of patients skin test (IDT) positive to NMBA administered	51
Positive at standard concentrations	33
Positive at strong concentrations only	18
Number of patients skin test negative to NMBA administered	10
NMBA exposed to	
Rocuronium	48
Vecuronium	4
Cisatracurium	0
Succinylcholine	9
Pancuronium	0
Sensitisation to standard NMBA panel on skin testing	
To culprit NMBA only	20
Amongst aminosteroids only	12
Across aminosteroids and succinylcholine	15
Across aminosteroids, succinylcholine and cisatracurium	4
No sensitisation to any NMBA	10

IDT, intradermal tests; NMBA, neuromuscular blocking agent.

rocuronium and vecuronium. However, the remaining nine patients did not have any sensitisation to aminosteroids on skin tests, but eight had sensitisation to two or more aminosteroids on BAT.

There was a significant lack of agreement between skin testing and BAT for rocuronium ($P < 0.05$) using McNemar's test (Table 4), with more sensitisation to rocuronium on skin testing compared with BAT (Table 3). There were 45 positive skin tests compared with 30 by BAT. However, if we analysed the results of skin testing using standard concentrations only, there was agreement between skin testing and BAT ($P > 0.05$).

There was agreement in sensitisation to vecuronium and suxamethonium using skin testing and BAT ($P > 0.05$) using McNemar's test (Table 4). There were 24 positive skin tests to vecuronium against 23 by BAT, and 21 positive skin tests to suxamethonium against 24 by BAT (Table 3).

Outcomes

We have limited follow-up from nine patients in the study (Table 5). All patients had a negative skin test to cisatracurium. Five patients received cisatracurium as part of their subsequent anaesthetic based on our recommendations. We performed cisatracurium drug provocation testing for four patients to identify it as a safe agent for subsequent anaesthesia. Of these nine patients, seven had negative BAT and no reaction to cisatracurium, one had positive BAT but uneventful cisatracurium drug provocation testing, and one with negative skin testing but positive BAT had cisatracurium anaphylaxis at the next anaesthetic (Table 6).

Table 3 Cross-reactivity of patients with NMBA anaphylaxis on skin tests. *Nine patients (15%) with rocuronium anaphylaxis had a suggestive clinical history and isolated sIgE to morphine and pholcodine that was negative on skin testing to rocuronium. No sensitisation was demonstrated to all NMBAs. †One patient (25%) with vecuronium anaphylaxis had a suggestive clinical history and isolated sIgE to morphine and pholcodine that was negative on skin testing to vecuronium. No sensitisation was demonstrated to all NMBAs. n/a, not applicable; NMBA, neuromuscular blocking agent.

NMBA anaphylaxis	Cross-reactivity (No. of patients), n (%)					
	No cross-reactivity	Rocuronium (%)	Vecuronium (%)	Pancuronium (%)	Suxamethonium (%)	Cisatracurium (%)
Rocuronium (n=48)*	13 (27)	n/a	20 (42)	9 (19)	13 (27)	3 (6)
Vecuronium (n=4)†	1 (25)	3 (75)	n/a	2 (50)	1 (25)	1 (25)
Suxamethonium (n=9)	6 (67)	3 (33)	2 (22)	1 (11)	n/a	0 (0)

Table 4 Results of skin tests and BAT basophil activation testing to NMBAs. BAT+Skin+, sensitisation demonstrated on both BAT and skin testing; BAT+Skin-, sensitisation demonstrated on BAT but not on skin testing; BAT-Skin+, sensitisation demonstrated on skin testing but not on BAT; BAT-Skin-, sensitisation not demonstrated on BAT or skin testing. BAT, basophil activation testing; NMBA, neuromuscular blocking agent.

Results of skin testing (by standard or strong concentrations) and BAT (by CD63 or CD203c)				
	BAT+Skin+ (n)	BAT+Skin- (n)	BAT-Skin+ (n)	BAT-Skin- (n)
Rocuronium	23	7	17	10
Vecuronium	14	9	10	24
Cisatracurium	2	18	1	36
Suxamethonium	13	11	8	25
Pancuronium	5	18	7	27
Results of skin testing (by standard concentrations) and BAT (by CD63 only)				
NMBA				
Rocuronium	11	11	10	25
Vecuronium	8	10	5	34
Cisatracurium	0	14	2	41
Suxamethonium	9	7	10	31
Pancuronium	2	12	5	38
Results of skin testing (by strong concentrations) and BAT (by CD63 only)				
Rocuronium	17	5	23	12
Vecuronium	11	7	13	26
Cisatracurium	1	13	2	41
Suxamethonium	10	6	11	30
Pancuronium	4	10	8	35
Results of skin testing (by standard concentrations) and BAT (by CD203c only)				
Rocuronium	11	14	9	23
Vecuronium	7	12	6	32
Cisatracurium	1	13	1	42
Suxamethonium	10	12	10	25
Pancuronium	3	14	4	36
Results of skin testing (by strong concentrations) and BAT (by CD203c only)				
Rocuronium	20	5	20	12
Vecuronium	10	9	14	24
Cisatracurium	1	13	2	41
Suxamethonium	11	11	10	25
Pancuronium	5	12	7	33

Discussion

Modern-day NMBAs commonly used in NSW, Australia comprise aminosteroids (rocuronium, vecuronium, and pancuronium), benzylisoquinolines (cisatracurium), and suxamethonium. A patient with NMBA anaphylaxis can potentially have allergic reactions to other NMBAs (cross-

reactivity), as the major antigenic determinant, the substituted ammonium moiety,^{2,3} is present in all of these. In our cohort, 20 patients (31%) had no cross-reactivity on skin testing, and they were sensitised only to the culprit NMBA. However, 31 (51%) had cross-reactivity on skin testing with sensitisation to at least one other NMBA. The majority of cross-reactivity occurred across aminosteroids and

Table 5 P-values for McNemar's test for agreement of proportion of positive skin tests and BAT. BAT, basophil activation testing.

Skin test concentration	BAT	NMBA				
		Rocuronium	Vecuronium	Cisatracurium	Suxamethonium	Pancuronium
Standard	CD63	1.000	0.302	0.004	0.629	0.143
Strong	CD63	0.001	0.263	0.007	0.332	0.815
Standard	CD203c	0.405	0.238	0.002	0.832	0.301
Strong	CD203c	0.004	0.405	0.007	1.000	0.359
Standard or strong	CD63 or CD203c	0.064	1.000	<0.001	0.648	0.043

suxamethonium, consistent with the literature⁶: 12 (20%) had cross-reactivity with the aminosteroid group only, and 15 (25%) across aminosteroids and suxamethonium. Only four cases (7%) involved cross-reactivity across aminosteroids, suxamethonium and benzylisoquinolines.

A few hypotheses have been suggested to explain the variation in cross-reactivity. The flexibility of the chain and distance between the substituted ammonium can affect immunogenicity.^{5,14,15} Suxamethonium, with a more flexible chain, is considered more potent in initiating mast cell mediator release than NMBAs with a rigid chain,¹⁴ for example vecuronium, pancuronium, and rocuronium. It is also possible that the antigenic determinant of NMBAs extends to structures adjacent to the substituted ammonium, or IgE antibodies recognise structures other than the ammonium group.³ There may be variation of these structures across different NMBAs.

The most critical part in the evaluation of perioperative anaphylaxis is advice on subsequent anaesthesia. We formulate advice in our service based on standard assessment comprising a review of clinical history, skin testing, and sIgE tests. BAT is not routine. We advise avoidance of all three aminosteroids (rocuronium, vecuronium, pancuronium) in

patients who have sensitisation on skin tests to two or more aminosteroids. If sensitisation is to only one aminosteroid, we advise avoidance of that aminosteroid only. We advise avoidance of suxamethonium and cisatracurium only if sensitisation to these is present.

A model incorporating a review of clinical history and skin and sIgE tests has been reasonably effective in successfully advising on subsequent anaesthesia.^{16–18} However, even in these case series, there are patients who have anaphylaxis at subsequent anaesthesia.^{17–19} This is concordant with our experience. Therefore, ongoing research into new potentially useful investigations for perioperative anaphylaxis is necessary. A prominent Belgian group currently dissuades administration of an NMBA where BAT is positive, despite negative skin testing, on the basis that neither has a significant positive predictive value for allergy.⁸

We found good agreement between BAT and skin testing for vecuronium and suxamethonium. There is a lack of agreement in relation to rocuronium, pancuronium, and cisatracurium, so if we incorporated BAT results for these drugs into our advice, we might offer different advice on safe subsequent anaesthesia than when considering skin testing and

Table 6 Follow up of nine patients with NMBA anaphylaxis. Allergic, patient had anaphylaxis to cisatracurium; not allergic, patient tolerated cisatracurium with no adverse reaction. BAT, basophil activation testing; DPT, drug provocation testing; NMBA, neuromuscular blocking agent; sIgE, specific IgE.

Patient	Cross-reactivity (number of patients)									
	Culprit NMBA	Skin test results					sIgE to morphine/ pholcodine	BAT to cisatracurium		Cisatracurium at next anaesthetic or DPT
		Rocuronium	Vecuronium	Pancuronium	Suxamethonium	Cisatracurium		CD63	CD203c	
DT	Rocuronium	+	+	–	+	–	+	+	+	Allergic (at next anaesthetic)
MP	Rocuronium	–	–	–	–	–	+	–	–	Not allergic (at next anaesthetic)
JT	Rocuronium	+	–	–	–	–	+	–	–	Not allergic (at next anaesthetic)
NR	Vecuronium	–	+	–	–	–	+	–	–	Not allergic (at next anaesthetic)
JS	Rocuronium	+	+	–	+	–	+	–	–	Not allergic (at next anaesthetic)
LH	Rocuronium	–	–	–	–	–	+	–	–	Not allergic (DPT)
ME	Rocuronium	+	+	+	+	–	+	–	–	Not allergic (DPT)
KD	Rocuronium	+	+	+	–	–	+	–	–	Not allergic (DPT)
MK	Rocuronium	–	–	–	–	–	+	+	+	Not allergic (DPT)

sIgE results only. The number of positive tests is biased toward skin testing for rocuronium, and therefore would not change our clinical practice. However, the number of positive tests is biased toward BAT for cisatracurium and pancuronium.

We report 18 patients who were skin test-negative but BAT-positive for pancuronium. Nine of these 18 patients were positive on skin testing to rocuronium and vecuronium, so we already advised avoidance of pancuronium, and the BAT result does not change this advice. However, the remaining nine patients were skin test-negative to all aminosteroids, and eight of these nine patients were BAT-positive to pancuronium plus another aminosteroid. If we adopted the approach of Sabato and Ebo,⁸ we would need to change our advice on subsequent anaesthesia.

There were 20 patients sensitised to cisatracurium by BAT compared with three by skin testing. We have limited follow-up data from nine patients who received cisatracurium at subsequent anaesthesia or drug provocation testing, and only have information on the clinical significance of cisatracurium sensitisation for these patients (Table 5). The seven patients with negative skin testing and BAT tolerated cisatracurium, but the dataset is small, and the clinical significance of sensitisation to cisatracurium on skin testing and BAT requires further study.

In patients with NMBA anaphylaxis, we assess cross-reactivity initially by performing skin testing to other NMBAs.¹ Recent studies have confounded our understanding of skin sensitisation, suggesting that it may not only reflect IgE-mediated anaphylaxis, but perhaps activation of other mast cell receptors such as MAS-related G protein coupled receptor-X2, where clinical manifestations may be less severe and dose-dependent.^{20–22}

Drug provocation testing (DPT) is the standard for diagnosing or negating drug allergy, but this is a challenging area of anaesthetic practice as there are no standard recommendations,⁷ and higher concentrations of the drugs can have pharmacologic effects. We use DPT when we believe that the reaction is consistent with anaphylaxis to NMBAs and that no conclusion can be made from skin testing that provides a high likelihood of safe future anaesthesia with an NMBA. DPT is performed only after consideration of risks and benefits.⁷ Our local health service does not support using DPT of an NMBA where patients have a positive skin test to the NMBA because of a perceived risk of a significant reaction.

We perform DPT to identify safe anaesthesia^{1,23} for a NMBA group (benzylisoquinolines, suxamethonium, or aminosteroids) where there is no sensitisation on skin testing, and that is unlikely to cause anaphylaxis. We currently have most experience with cisatracurium DPT, although we have performed suxamethonium and rocuronium DPT on occasion. Perhaps a negative BAT together with negative skin testing gives more reassurance that there is no excessive risk. A Belgian study in 2014 reported 19 patients with NMBA allergy who tolerated subsequent anaesthesia with negative skin testing and BAT for an NMBA.²⁴ Further study is needed, and at this stage BAT does not appear to be indicated to replace DPT in skin test-negative patients with suspected NMBA anaphylaxis.

Limitations

We have insufficient knowledge of the clinical significance of sensitisation on BAT or skin testing. Our study included only a small cohort of 61 patients, with follow-up data available for

only nine at the time of publication. Limited resources restricted the number of DPTs we were able to perform.

Conclusions

In 61 sequential patients diagnosed with NMBA anaphylaxis, BAT detected a different cross-reactivity profile than skin testing, in particular, increased sensitisation to pancuronium and cisatracurium detected using BAT. We are currently unsure of the significance of this increased sensitisation. The ultimate aim is to develop an algorithm for identifying safe anaesthetic approaches following NMBA anaphylaxis, integrating the use of DPT, skin testing, BAT, or all three, but we need further study using DPT and follow-up of subsequent anaesthesia to determine the clinical significance of sensitisation detected on skin testing and BAT.

Authors' contributions

Performed the experiments: JL, OGB.

Analysed the results and constructed the tables: all authors.

Designed the research: JL, OGB, MAR, SLF.

Wrote the paper: JL.

Read and approved the final manuscript: all authors.

Declaration of interests

The authors declare that they have no conflicts of interest.

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