

Predictive value of allergy tests for neuromuscular blocking agents: tackling an unmet need

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Summary

Background Neuromuscular blocking agents (NMBAs) are a predominant cause of perioperative anaphylaxis in Europe. Diagnosis of NMBA allergy relies upon the careful review of the anaesthetic report complemented with skin tests. Additional diagnostic tests are quantification of specific IgE antibodies (sIgE) and basophil activation test (BAT). However, data on the predictive value of the skin tests, the BAT and the sIgE assays (drug-specific and substituted ammonium structures) are limited or not available, mainly because such exploration requires dangerous NMBA provocation tests.

Methods In this study, the predictive value of skin test, BAT and measurement of sIgE to substituted ammonium structures is gathered from a review of anaesthetic records of subsequent surgical procedures with NMBA administration and/or occurrence of perioperative incidents.

Results We investigated a series of 272 patients with perioperative anaphylaxis, of whom 100 had undergone second general anaesthesia. Negative skin test and negative BAT assisted the selection of alternative NMBA, which were well tolerated in all cases. Five patients with a positive sIgE to rocuronium but with negative skin testing and BAT safely received rocuronium during second anaesthesia. Twelve patients with sIgE reactivity to morphine, but negative skin test and BAT to benzyloquinolines, tolerated administration of cisatracurium or atracurium. Alternatively, benzyloquinoline allergy went undetected in the morphine solid-phase assay.

Conclusions Skin test and BAT have an excellent negative predictive value in our series. The uneventful re-exposure of rocuronium in patients with an isolated positive sIgE result to rocuronium calls into question the predictive value of this assay and suggests sIgE serology to be less clinically predictive than the functional investigations relying upon activation of mast cells or basophils. The presence of a positive sIgE to substituted ammonium structures such as morphine does not preclude further use of benzyloquinolines.

Keywords BAT, NMBA, provocation test, sIgE, skin test

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Introduction

Allergy to neuromuscular blocking agents (NMBA) constitutes a major cause of potentially life-threatening perioperative anaphylaxis. In our series of 344 patients, NMBA accounted for 40% of all patients with an IgE-mediated perioperative allergy [1]. Hitherto, diagnosis of NMBA allergy rests generally upon review of anaesthetic notes and allergy skin testing with select NMBAs, the latter now supplemented in some

centres with quantification of drug-specific IgE (sIgE) antibodies or sIgE against tertiary or quaternary substituted ammoniums, as these represent a major antigenic epitope of NMBA [2]. Although these tests can provide valuable information, data on the predictive value of these tests are limited or not available. As a matter of fact, robust analysis of the predictive value of these tests requires drug provocation test (DPT) with NMBA as a gold standard. However, for obvious ethical reasons, DPT with NMBA should be

avoided. Therefore, to circumvent this hurdle, we took advantage of a 12-year follow-up of patients with a history of perioperative anaphylaxis who had a diagnostic work-up in our outpatient clinic of the Antwerp University Hospital. Information of both anaesthetic procedures was obtained by a thorough review of anaesthetic records.

There are only a few published results on subsequent anaesthesia after investigation at an anaesthetic clinic [3–7]. It appears from these studies that NMBA's yielding negative skin tests are generally well tolerated during new anaesthesia. However, data on the utilized NMBA are rather limited, and in most of these studies, there are no data on basophil activation test (BAT), drug sIgE and sIgE to substituted ammonium determinants.

Material and methods

Study population

Between January 2001 and June 2012, we systematically evaluated 272 patients with a history of perioperative allergy who had received a NMBA. During initial diagnostic work-up, all patients were investigated using a standardized protocol [8], including skin test and BAT for five different NMBA's [9, 10]. Note that quantification of sIgE was not part of initial diagnostic work-up, as sIgE to rocuronium and morphine only became available in 2006. Next, patients were called by telephone and asked whether they had undergone subsequent general anaesthesia, following our diagnostic work-up. If available, anaesthetic charts of all the subsequent surgical procedures were requested and reviewed in search of NMBA administration and/or perioperative incidents.

Diagnostic investigation for NMBA allergy

Skin tests. Skin prick tests (SPT) and intradermal tests (IDT) were performed according to the recommendations of the *Société Française d'Anesthésie et de Réanimation* [11]. Briefly, SPT were performed on the ventral part of the forearm and included histamine 10 mg/mL (HAL Allergy Benelux BV) as a positive control and a saline buffer solution as a negative control. The tested NMBA's included the following: atracurium [Tracrium (10 mg/mL), GSK, Genval, Belgium], cisatracurium [Nimbex (2 mg/mL), GSK, Genval, Belgium], rocuronium [Esmeron (10 mg/mL), Organon, the Netherlands], vecuronium [Norcuron (4 mg/mL), Merck Sharp and Dohme, Brussels, Belgium] and suxamethonium [Lys-thenon (50 mg/mL), Nycomed, Austria]. The maximal concentrations for SPT were as follows: atracurium 1/10, cisatracurium undiluted, rocuronium undiluted, ve-

curonium undiluted and suxamethonium 1/5. SPT were read after 15 min and considered positive when the wheal of the wheal and flare reaction exceeded 3 mm. Patients with negative SPT had additional IDT (maximal concentrations: atracurium 1/1000, cisatracurium 1/100, rocuronium 1/100, vecuronium 1/10 and suxamethonium 1/500) that were considered positive when the wheal equalled or exceeded 8 mm or had doubled as compared to the injection bleb.

Basophil activation tests. Flow cytometric analysis of *in vitro*-activated basophils was performed as described elsewhere [12]. The test included a negative buffer control without any drug, a positive control (anti-IgE) and stimulation with the aforementioned NMBA's (applied stimulation concentrations: rocuronium $0.5\text{--}5 \times 10^3 \mu\text{g/mL}$, vecuronium $0.5\text{--}2 \times 10^3 \mu\text{g/mL}$, atracurium $(0.5\text{--}5 \times 10^3 \mu\text{g/mL})$, cisatracurium $0.5\text{--}5 \times 10^3 \mu\text{g/mL}$ and suxamethonium $(0.5\text{--}5 \times 10^3 \mu\text{g/mL})$. Results are expressed as net percentage of CD63 positive basophils, that is, by subtracting spontaneous CD63 expression (negative control) from the value obtained with allergen (drug) stimulation. The threshold for positivity was set on 4% CD63 positive basophils [13].

Quantification of specific IgE. Specific IgE antibodies towards rocuronium, suxamethonium and morphine (used as a biomarker for sensitization to substituted ammonium epitopes) were quantified with the ImmunoCAP FEIA solid-phase technique (ThermoFisher Scientific, Uppsala, Sweden). In the majority of cases, thawed sera from the patients who were re-exposed to a NMBA after initial perioperative allergy were retrospectively analysed for the presence of sIgE antibodies to rocuronium and morphine [14], as these assays only became available from 2006. For sIgE to rocuronium, experimental prototypes of ImmunoCAP were obtained, kindly provided by ThermoFischer Scientific. Drug-specific decision thresholds were set at 0.35, 0.13 and 0.11 kUA/L for morphine, rocuronium and suxamethonium, respectively [14].

Results

Figure 1 displays our study population. One hundred and seventy-seven of 272 patients (65%) could be reached and queried whether they had undergone second or third general anaesthesia, following our diagnostic work-up. One hundred of them (56%) reported one or more new surgical interventions with general anaesthesia, and an anaesthetic report was obtained in 76 cases. From these anaesthetic notes, it appeared that all 76 had uneventful new anaesthesias, except one chlorhexidine allergic patient who was inadvertently

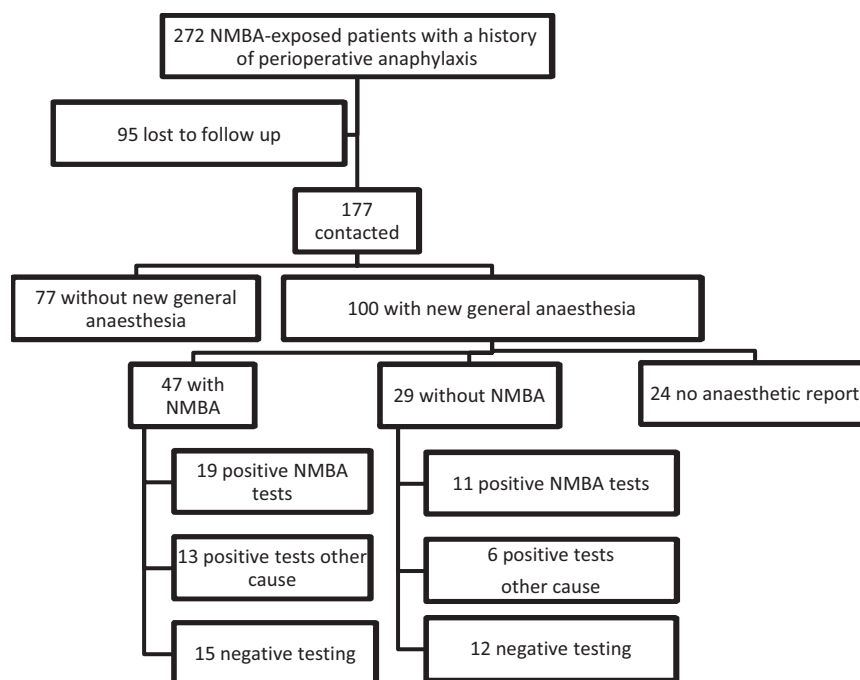


Fig. 1. Description of the patients included in the study. NMBA, neuromuscular blocking agent.

re-exposed to the antiseptic during insertion of a chlorhexidine-impregnated catheter [15].

From the 47 patients who were re-exposed to a NMBA, 19 were initially diagnosed with suspected NMBA allergy, 13 had another IgE-mediated allergy suspected, and in the remainder 15, no IgE-mediated allergy was identified. As shown in Table 1, 15 of the 19 NMBA allergic patients had reacted to the aminosteroid rocuronium and were subsequently uneventfully exposed to a benzylisoquinoline such as atracurium or cisatracurium. Table 1 also suggests that a suspected diagnosis in some patients could have been overlooked by skin testing. Seventeen patients had their suspected diagnosis confirmed by skin tests and/or BAT whereas two patients (# 16 & 17) required additional BAT to identify the NMBA as a potential cause for their perioperative reaction.

Figure 1 also shows that from the 29 patients who had a re-intervention without NMBA, 11 were initially diagnosed as NMBA allergic, six had another IgE-mediated allergy, and in 12, no IgE-mediated allergy was demonstrable. Of note is that all 27 patients without delineable IgE-mediated allergy had uneventful anaesthesias.

As already stated in the method section, drug sIgE to rocuronium and morphine in our patients was not part of initial diagnostic work-up. Table 1 reveals that 10 of 11 patients with positive skin test and/or BAT to rocuronium demonstrated a positive result for sIgE rocuronium. Moreover, nine of these 10 patients also

displayed IgE reactivity to morphine, indicating the presence of NMBA-reactive IgE antibodies likely to recognize the readily accessible tertiary N-methyl group of the opiate. Twelve rocuronium allergic patients had uneventful administration of a benzylisoquinoline that tested negative in both skin test and BAT.

Alternatively, one patient with positive skin testing to cisatracurium who showed an isolated positive sIgE rocuronium result (negative skin test and BAT to rocuronium, negative sIgE to morphine and suxamethonium) tolerated re-exposure to rocuronium during subsequent surgery (Table 1, patient 18).

Finally, Table 1 shows that the traditional suxamethonium-solid-phase assay was positive in only 10 of the 15 rocuronium allergic patients (66 and is therefore not a sensitive biomarker to detect rocuronium allergy).

Table 2 displays 28 patients with perioperative anaphylaxis with negative NMBA tests. From these 28 patients, 13 patients had suffered from another IgE-mediated allergy (e.g. natural rubber latex (NRL), antibiotics, chlorhexidine, dyes, corticosteroids), and in 15 patients, no IgE-mediated allergy was delineable. By far the most relevant finding is that four subjects without a reaction to rocuronium with re-exposure had a positive sIgE but negative prick and intradermal skin tests and negative BAT (Table 2, patients 6, 7, 15, 16 had a sIgE to rocuronium of respectively 0.19, 0.16, 0.43 and 3.38). Note that these patients demonstrate an increased titre of total serum IgE, which could result in non-specific binding to the solid-phase

Table 1. Patients with positive NMBA tests (N = 19)

		Delay		Specific IgE (kUa/L)				Skin test NMBA		BAT NMBA		NMBA	
		Diagnosis	Re-expo sure	IgE tot	S	R	Mor	+	–	+	–	Suspected offender	Alternative
1	F/69	13	60	9	–	0.14	0.89	R,S,V	A,C	Inc	Inc	R	A
2	F/48	1	24	176	0.16	0.42	1.68	R,V	A,C,S	R,V	A,C,S,V	R	A
3	M/41	3	3	174	2.07	NA	26.10	S,V	A,C,R	–	A,C,R,S,V	R	A
4	F/54	3	24	130	0.11	0.15	–	R,V	A,C,S	R,V	A,C,S,V	R	A
5	F/52	2	1	50	–	NA	0.87	C,R	A,S,V	Inc	Inc	R	A
6	F/56	1	1	121	5.27	0.58	7.17	R,S	A,C,V	R,S	A,C,V	R	A and C
7	F/64	3	48	169	0.82	0.46	4.16	R,S	A,C,V	R	A,C,S,V	R	C
8	F/18	1	24	436	–	0.23	6.47	R	A,C,S,V	R	A,C,S,V	R	C
9	F/52	6	72	37	6.19	0.39	0.86	A,R,V	C,S	R,V	A,C,S	R	C
10	M/60	1	12	75	1.57	NA	9.79	R,S,V	A,C	R,V	A,C,S	R	C
11	F/47	13	60	120	–	5.28	1.46	R	A,C,S,V	–	A,C,R,S,V	R	C
12	F/46	3	2	404	2.41	0.64	2.75	R	A,C,S,V	–	A,C,R,S,V	R	C
13	F/41	8	3	44	0.41	–	–	R,V	A,C,S	R,V	A,C,S	R	C
14	F/44	2	24	34	0.18	1.44	2.02	R,V	A,C,S	R,V	A,C,S	R	M
15	M/65	1	36	3217	–	NA	–	C,R,V	A,S	R,V	A,C,S	R	M
16	M/43	10	2	NA	–	–	–	S	A,C,R,V	V	A,C,R,S	V	C
17	F/34	13	48	57	–	–	–	–	A,C,R,S,V	A	C,R,S,V	A	R
18	F/50	4	36	145	–	2.42	–	C	A,R,S,V	–	A,C,R,S,V	C	R
19	F/50	2	24	325	NA	NA	0.66	S,V	A,C,R	–	A,C,R,S,V	S	A

Demographics, characteristics (age in years), laboratory findings (skin test, sIgE and BAT), offending and safe alternative NMBA.

Delay diagnosis, delay between reaction and diagnostic approach (in months); Delay re-exposure, delay between diagnostic approach and re-exposure (in months); +, positive result; –, negative result; NA, not available due to insufficient serum.

IgE tot, total IgE; Mor, morphine; A, atracurium; C, cisatracurium; M, mivacurium; R, rocuronium *, S, suxamethonium, V, vecuronium; NMBA, neuromuscular blocking agent. Inc, inconclusive that is cells non-responsive to stimulation with positive control and allergen (drug); BAT, basophil activation test.

*The rocuronium sIgE threshold of 0.13 kUa/L was calculated by ROC analysis between patients and controls and yielded a sensitivity of 92% and specificity of 93%. For a traditional threshold of 0.35 kUa/L, sensitivity and specificity were 68% and 93%, respectively [14].

assay. Note that 10 of these 28 patients had uneventful re-exposure to the same NMBA that was used during the first anaesthesia that was complicated by anaphylactic episode.

Discussion

To our knowledge, this is the first survey comparing the outcomes of second or third anaesthesia in subjects with a prior anaesthesia-related adverse event and the results of allergy tests, including prick and intradermal skin tests, *in vitro* basophil activation and sIgE to the suspected NMBA.

It includes 100 telephone enquiries from which 76 were completed by the attending anaesthesiologists. Our findings confirm prior publications suggesting that following an adverse event NMBAs may be re-administered if skin tests to the suspected causal agent are negative [16–18]. However, our data suggest that *in vitro* specific BAT may be more sensitive than skin tests in diagnosing NMBA allergy [13].

One of the major observations of this follow-up study relates to rocuronium and morphine-sIgE solid-phase

assays, the latter currently being recommended and applied as an easily accessible serologic biomarker for sensitization to substituted ammonium determinants [19, 20]. As already stated in the methods section, sIgE to rocuronium and morphine were not part of initial evaluation but were subsequently performed on thawed sera that were acquired during initial diagnostic work-up. It appears from our analyses that, albeit sIgE rocuronium may be helpful to diagnose rocuronium allergy [14], care should be taken when interpreting an isolated positive sIgE rocuronium result in patients with negative skin and BATs towards this NMBA. The uneventful re-exposure to rocuronium in different patients confirms that the serum sIgE antibody test for rocuronium can yield clinically irrelevant results that fail to correctly predict the safe outcome of future exposure to the drug [10]. In other words, a positive sIgE rocuronium result does not *per se* reflect a genuine rocuronium allergy and could lead to diagnostic errors resulting in unnecessary avoidance measures and, more importantly, failure to identify the real causative agent (s). Likewise, our data confirm that an isolated positive result for morphine does not serve as a reliable

Table 2. Patients with negative NMBA tests ($N = 28$)

N	Cause	Sex/age	Delay		IgE tot	Specific IgE (kUa/L)			NMBA exposed to	
			Diagnosis	Re-exposure		S	R	Mor	First	≥ Second
1	NRL	F/44	12	12	240	—	—	—	R	A
2	NRL	F/56	2	1	156	—	—	—	R	C
3	NRL	F/53	U	12	206	—	—	—	A	R
4	NRL	F/57	U	36	120	—	—	—	R	R
5	NRL	M/67			303	—	NA	—	C	C
6	NRL + AB	F/59	1	60	1175	—	0.19	—	R	R
7	AB + chlorhexidine	M/74	12	36	8550	—	0.16	—	A	R
8	AB	F/24	1	2	51	NA	—	NA	R	R
9	Patent blue	M/21	U	12	11	—	—	—	C	C
10	Patent blue	F/43	12	48	44	—	—	—	V	C
11	Chlorhexidine	M/46	2	24	41	—	NA	—	R	A
12	Chlorhexidine	M/62	1	1	NA	—	—	—	R	R
13	Solumedrol	M/57	72	12	21	—	—	—	C	A
14	Non-IgE	F/47	36	24	85	—	—	—	S	R
15	Non-IgE	M/57	U	3	741	1.52	0.43	3.82	A	R
16	Non-IgE	F/52	10	3	1529	3.05	3.38	19.20	R	R and A
17	Non-IgE	M/46	3	24	13	—	—	—	A	R
18	Non-IgE	F/42	U	3	11	—	—	—	A	R
19	Non-IgE	M/55	3	9	11	—	—	—	R	A
20	Non-IgE	F/45	9	12	18	—	—	—	R	M
21	Non-IgE	M/30	6	1	104	—	NA	—	R	A
22	Non-IgE	M/27	3	3	22	—	NA	—	R	R
23	Non-IgE	F/64	U	9	132	—	—	—	A	A
24	Non-IgE	F/57	2	36	86	—	—	—	A	R
25	Non-IgE	F/44	48	12	120	0.37	0.17	0.57	R	C
26	Non-IgE	F/41	60	2	56	—	—	—	R	R
27	Non-IgE	M/52	3	60	168	—	—	—	M	C
28	Non-IgE	M/63	3	4	368	—	NA	0.81	R	C

Demographics, characteristics and laboratory findings of 13 patients suspected with non-NMBA related IgE-mediated allergy and 15 patients with negative tests.

NRL, natural rubber latex; AB, antibiotics; First, first exposure; ≥ Second, second or third exposure; Delay diagnosis, delay between reaction and diagnostic approach (in months); Delay re-exposure, delay between diagnostic approach and re-exposure (in months). +, positive result; —, negative result; U, unavailable; NA, not available due to insufficient serum; IgE tot, total IgE; Mor, morphine; A, atracurium; C, cisatracurium; M, mivacurium; R, rocuronium; S, suxamethonium; V, vecuronium; NMBA, neuromuscular blocking agent.

predictor for genuine NMBA allergy. Clearly, these observations call into question the data of epidemiological surveys that have relied upon substituted ammonium sIgE assays to estimate sensitization and allergy to NMBA [21–23]. For example, in a recent survey by Dong et al. [21], 12 patients demonstrating negative NMBA skin tests were apparently diagnosed as allergic to curarizing myorelaxants because of an isolated positive sIgE against quaternary ammonium ions. These epidemiological studies may have ignored the pioneering contributions on the serological diagnosis of IgE-dependent allergy to NMBA. These studies clearly disclosed several uncertainties associated with NMBA sIgE binding and inhibition assays and discouraged the use of serological tests in isolation to diagnose NMBA allergy or to predict clinical outcome [24]. Furthermore, these epidemiological studies seem to have overlooked that in control populations, the prevalence of quaternary

ammonium ion-reactive serum IgE may be as high as 10% [19, 25]. The exact reason(s) for clinically irrelevant specific IgE remains elusive but probably relate(s) to the (mostly unknown) origin and extremely heterogeneous specificity of (highly cross-reactive) NMBA sIgE antibodies [2, 24, 26] and non-specific binding to the allergosorbent by high total IgE levels [10, 14, 24]. Keeping all this in mind, our current practice is not to eliminate the use of rocuronium in subjects with prior anaesthetic reactions and an isolated positive sIgE to rocuronium and/or morphine nor to recommend avoidance of morphine due to a positive sIgE to morphine.

As a matter of fact, our patients currently get BAT and undergo controlled opiate drug challenges to establish the clinical relevance of morphine-reactive sIgE antibodies and to assess whether they present a genuine allergy to this opiate and to closely related structures like codeine. Furthermore, our data demonstrate that

rocuronium allergic patients demonstrating a positive sIgE to substituted ammonium epitopes, as assessed by a morphine solid-phase assay, with negative skin tests and BAT for atracurium and cisatracurium tolerate administration of these benzyloquinolines. Together with the opposite observation of morphine-sIgE solid-phases assays generally not capturing patients with a benzyloquinoline allergy [20, 27], our findings argue against the model of a unique epitope accounting for NMBA allergy and that sensitization or allergy to NMBA can readily be predicted by a single solid-phase assay. Clearly, recognition of an NMBA by sIgE antibodies extends beyond the tertiary or quaternary ammonium structures and involves drug-specific neighbouring groups that remain to be identified [for comprehensive review: [2]]. Finally, our data indicate that although sIgE tests are easy to perform, they should not be used for preoperative allergy assessment during preoperative evaluation.

In conclusion, this is the first longitudinal follow-up study gathering information about the diagnostic reliability of skin test, BAT and sIgE to NMBA in patients with perioperative adverse events. It confirms that when NMBA yields a negative skin test and BAT, patients can safely be administered this NMBA during future anaesthesia. These data also reveal the potential of BAT in patients with difficulty of diagnosis. Furthermore, by demonstrating that isolated positive sIgE results to rocuronium and/or substituted ammonium compounds do not *per se* reflect genuine rocuronium allergy, our study re-emphasizes the difficulties involved in interpreting and applying the serological results to the clinical situation. Specific *in vitro* IgE tests to NMBAs should not be considered a substitute for skin testing or BAT. Spe-

cific IgE tests simply measure the interaction between the drug epitope and the antibody paratope, whereas skin testing and BAT probably more closely mirror the clinical outcome by depicting drug-induced cross-linking of adjacent membrane-bound sIgE with subsequent release of mediators. Erroneous interpretation of positive sIgE results might not only lead to unnecessary avoidance measures and overestimation of certain NMBA allergies but, more importantly, also entails a risk for diagnostic errors due to failures in identifying the genuine causative compound(s).

Author contributions

JLE, DGE, AU, LDC and CHB were involved in study design, data analysis and writing the manuscript. AU, VSO and DGE were involved in patient recruitment.

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Conflict of interest

The authors declare no conflict of interest.

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