Evaluation of a New Routine Diagnostic Test for Immunoglobulin E Sensitization to Neuromuscular Blocking Agents

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ABSTRACT

Background: Neuromuscular blocking agents (NMBA) are responsible for most immediate hypersensitivity reactions during anesthesia, as a result of the presence of a quaternary ammonium ion. The aim of this study was to evaluate the diagnostic performance of a commercial immunoglobulin E (IgE) test (quaternary ammonium morphine [QAM]) for diagnosing sensitivity to NMBA.

Methods: We tested 168 patients exposed to NMBAs during anesthesia. Of those patients, 54 had an uneventful procedure and 114 had immediate hypersensitivity reactions, and 57 patients had positive skin tests to the administered NMBA, whereas 57 had negative skin tests. Specific IgE concentrations determined with the QAM method based on a morphine solid phase were compared with those obtained with a recommended experimental method with a choline solid phase.

Results: For the QAM test, a 0.35 kU_A/l positivity cutoff was chosen from the receiver operating characteristics curve. QAM-specific IgE was found in 84.2% of skin test–positive reactors (80.7% with the recommended method; no significant difference), and binding was inhibited by the culprit NMBA in 80% of cases. The frequency of QAM-specific IgE positivity was significantly higher in skin test–negative reactors (24.6%) than in controls (9.3%), suggesting NMBA sensitivity.

Conclusion: Sensitivity of the QAM test (84.2%), together with its simplicity and suitability for routine laboratory use, makes it a valuable tool, in conjunction with skin tests, for diagnosing NMBA sensitivity in patients who react after NMBA injection. The QAM test is of particular interest when skin tests are not available or not reliable or give results poorly compatible with mediator release or clinical features.

What We Already Know about This Topic

- Neuromuscular blocking agents (NMBA) are responsible for most immediate hypersensitivity reactions during anesthesia, owing to the presence of a quaternary ammonium ion.

What This Article Tells Us That Is New

- The diagnostic performance of a commercial IgE test (quaternary ammonium morphine) for diagnosing sensitization to NMBA. The 84% sensitivity of this simple test makes it a valuable tool, in conjunction with skin tests, for diagnosing NMBA sensitization in patients who react following NMBA injection.

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Received from the Laboratory of Biophysics, University Hospital, Caen, France, and the Immunology Unit, Bichat-Claude Bernard University Hospital, Paris, France. Submitted for publication March 25, 2010. Accepted for publication September 22, 2010. Institutional support received from the different University Hospitals and Research Units. The sera of the control patients from Caen were obtained during another study sponsored by the University Hospital of Caen/Regional Council of Basse-Normandie, France (Grant no. 04-088). Laure Malzac received a research grant (no. 03-04-2008) from Phadia AB, Uppsala, Sweden for this work. The reagents used for the study were supplied free of charge by Phadia. Accepted as a poster at the XXVIII European Association of Allergy and Clinical Immunology (EAACI) Congress, June 5–9, 2010, London, England. Part of this work was presented as a poster at 4th Congrès Français d'Allergologie, April 14–17, 2009, Paris, France. A similar study using a different, less sensitive test was presented at the 2008 Annual Meeting of the American Society of Anesthesiologists, October 18–21, 2008, Orlando, Florida, USA; at 50th Congrès National d'Anesthésie et de Réanimation, September 24–28, 2008, Paris, France; and at the XXVII EAACI Congress, June 7–11, 2008, Barcelona, Spain.

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NEUROMUSCULAR blocking agents (NMBA) are the drugs most frequently responsible for immediate hypersensitivity reactions during anesthesia.[1] Most of these reactions are immunoglobulin E (IgE)–mediated (*i.e.*, anaphylactic).[2–4] The NMBA determinant mainly responsible for allergic reactions is the quaternary ammonium ion.[5] In France, the estimated incidence of reactions during general anesthesia is between 1/3,000 and 1/10,000.[6] Skin tests (STs) are currently the reference diagnostic tool, but their sensitivity is not perfect and their specificity controversial.[7–10] Detection of circulating IgE specific for NMBAs might therefore be helpful, either in addition to STs[11] or when STs are not available. Some tests have been developed with immobilized NMBAs or quaternary ammonium–containing molecules, the former being less sensitive than tests based on choline analogs coupled to Sepharose[12] or agarose.[13] The use of choline analogs is recommended in France,[14] but the relevant assays are not routinely available, thus there is a need for standardized commercial tests.

Morphine contains a single tertiary ammonium group that can bind IgE from NMBA-allergic patients.[15–16] A first immunofluorometric assay using immobilized morphine proved to be insufficiently sensitive,[17] and a new version of this assay was recently developed to enhance sensitivity (quaternary ammonium morphine [QAM] ImmunoCAP®; Phadia AB, Uppsala, Sweden). In this two-center retrospective study, we evaluated the QAM test in 168 selected patients who received NMBAs during an anesthetic procedure, 114 of whom had hypersensitivity reactions. We postulated that QAM-IgE could discriminate reactors with positive STs to NMBA from reactors with negative STs to NMBA and NMBA-exposed nonreacting controls. We therefore compared the results of the QAM test across the three patient groups and evaluated the diagnostic performance of the QAM test in reactors by comparison with a recommended test.

Materials and Methods

Patients

The study was conducted in the University Hospitals of Caen (France) and Bichat-Claude Bernard, Paris (France).

Patients with Hypersensitivity Reactions (Reactors)

We retrospectively selected from a cohort 114 patients (Caen: 94; Bichat: 20) who reacted during anesthesia in 2001–2007, had blood samples taken during the reaction for soluble mediator measurements, and, with their informed consent, had skin tests at least 4 weeks after the reaction. All had received NMBAs as part of the anesthetic protocol. The clinical severity of the reaction was assessed with the Ring and Messmer scale.[18] Fifty-seven reactors were selected on the basis of immediate reactions after NMBA injection, increased concentrations of histamine or tryptase, and a positive ST to the administered NMBA (group A). Fifty-seven reactors with negative skin tests to NMBAs during the same period composed group B.

Control Patients

Fifty-four consecutive patients (Caen: 29; Bichat: 25) exposed to NMBAs during an uneventful anesthetic procedure composed group C. They were recruited in 2006–2007 during studies approved by local ethics committees and gave their written informed consent for storage and analysis of sera sampled during anesthesia. For ethical reasons, the control patients were not asked to undergo STs.

Skin Tests

Prick tests and intradermal skin tests (IDSTs) were performed with the drugs administered immediately before the reaction, with all commercially available NMBAs, and with two latex extracts, in keeping with published guidelines.[19]

In Vitro Tests

Serum samples stored at −20°C were used for this study. Concentrations of QAM-specific IgE (QAM-IgE) and total IgE were measured in the 168 patients, using fluorometric immunoassays on an ImmunoCAP 100 or 250 apparatus (Phadia AB). The detection limit was 0.10 kU_A/l. The sera of the 114 reactors also were tested with the quaternary ammonium Sepharose-radioimmunoassay (QAS) test[12] that uses a choline analog and radiolabeled anti-IgE. Results were expressed as percentage binding to the solid phase, with a 2.3% positivity cutoff.[12] Inhibition curves were constructed with samples from five ST-positive reactors, each having reacted to a different NMBA, as follows: serum was mixed in equal volumes with the culprit NMBA solution at five different concentrations or with phosphate-buffered saline. After gentle shaking for 2 h at room temperature, QAM-IgE was assayed. The percentage response was calculated as follows: (response of serum with inhibitor)/(response of serum with buffer), after subtracting the system background response measured with buffer alone. Inhibition tests were then done with the optimal concentration of the culprit NMBA on samples from all patients with QAM-IgE concentrations exceeding 0.70 kU_A/l to take into account the serum dilution induced by the inhibition procedure. The percentage inhibition was similarly calculated as 100 minus percentage response. Percentage inhibition exceeding 20% was considered positive, as reported for the QAS test.[12] To document the allergic etiology, total plasma tryptase was measured during the reaction and 24 h afterward (basal concentration) with a fluoroenzyme immunoassay (ImmunoCAP tryptase, Phadia AB). Increased concentrations were defined as more than 12 μg/l (laboratory reference value) or as a value twice the basal concentration.[20] Plasma histamine concentrations were measured with a radioimmunoassay (RIA Histamine; Immunotech, Beckman Coulter, Marseille, France) and were considered increased if more than 6 nM (laboratory reference value).

Statistical Analysis

Statview 5.0 software (SAS Institute, Inc., Cary, NC) was used for statistical analysis. Results were expressed as medians

(95th percentile, range). Concentrations less than 0.1 kU_A/l were assigned a value of 0.1 for statistical analysis. The Kruskal–Wallis test or the Mann–Whitney test, as appropriate, was used to compare reactors and nonreactors. *P* values <0.05 were considered to denote significant differences. Positivity rates (%) were compared between the groups and methods by using the chi-square test. Correlations were identified by least-square regression analysis or by the Spearman test. Receiver operating characteristics (ROC) curve analysis was implemented with Prism 4.0.3 software (GraphPad Software, Inc., La Jolla, CA) to calculate the optimal QAM cutoff. The ROC curve was generated with data from group A and group C.

Results

Characteristics of the Patients

The three groups of patients are described in table 1. In group A, the reactions were life threatening in 50 of 57 patients and included four cardiac arrests. The median (95th percentile; range) histamine concentration was 138 nM (6,583; 1–12,820), and the median tryptase concentration was 88.5 μg/l (385; 2.3–629). The culprit NMBA was suxamethonium in 44 cases. In group B, the reactions were life threatening in 35 of 57 cases. Nineteen of these patients had positive skin tests with an administered drug other than NMBA (betalactam: n = 10; vancomycin: n = 1; propofol: n = 1) or with latex (n = 7). Among the other 38 patients who had negative skin tests to all administered agents and to latex, the reactions were moderate (grades 1–2) in 18 cases and severe (grade 3) in 20. The median histamine concentration was 4.5 nM (350; 1–823) and the median tryptase concentration 5.6 μg/l (24.2; 1–28.8). Fifteen patients had increased mediator concentrations (histamine: 14; tryptase: 8). Twenty patients received atracurium. In group C, most of the 54 patients received cisatracurium (recruited in Caen Hospital) or pancuronium (recruited in Bichat Hospital).

Measurement of Specific IgE with the QAM Method

The individual QAM-IgE concentrations in the three groups are shown in figure 1. The median concentration was 1.7 kU_A/l (42.3; 0.1–620) in group A, 0.1 kU_A/l (3.56; 0.1–14.4) in group B, and 0.1 kU_A/l (0.64; 0.1–15.3) in group C. The difference across the three groups was significant ($P < 0.0001$, Kruskal–Wallis test). The QAM-IgE concentrations in group A were significantly higher than those in group C ($P < 0.0001$, Mann–Whitney test) and group B ($P < 0.0001$).

The concentrations in groups A and C did not correlate with patients' age (Spearman test, $P = 0.09$)) or gender (Mann–Whitney test, $P = 0.35$). They were used to generate the ROC curve (fig. 2). The area under the curve was 0.923. A 0.35 kU_A/l cutoff was first investigated. QAM-IgE concentrations were less than 0.35 kU_A/l in 49 controls (90.7%) and exceeded 0.35 kU_A/l in the other five controls (0.44, 0.45, 0.64, 8.09, and 15.3 kU_A/l, respectively). QAM-IgE concentrations exceeded 0.35 kU_A/l in 48 patients in group A (84.2%) and in 14 patients in group B (24.6%). ROC curve analysis indicated that decreasing the cutoff to 0.15 kU_A/l would increase sensitivity to 87.7% without affecting specificity. However, when this cutoff was applied, three additional patients in group B became positive. As they were

Table 1. Characteristics of Patients Exposed to Neuromuscular Blocking Agents Who Had Hypersensitivity Reactions (Reactors) or Uneventful Anesthesia (Controls)

	Reactors (n = 114)			
Patient Characteristics	Positive IDST to NMBA Group A (n = 57)		Negative IDST to NMBA Group B (n = 57)	Controls (n = 54) Group C (n = 54)
Mean age (SD) (yr)	51 (15)		48 (17)	63 (16)
Range (yr)	19–82		10–82	24–88
Male/female	20/37		21/36	30/24
Severity of the reaction*	n		n	No reaction
Grade 4	4		0	
Grade 3	46		35	
Grade 2	7		8	
Grade 1	0		14	
NMBA†	Administered NMBA n	Positive Skin Test n	Administered NMBA n	Administered NMBA n
Suxamethonium	44	43	19	4
Atracurium	13	9	33	9
Pancuronium	3	3	1	18
Vecuronium	2	2	0	0
Rocuronium	3	1	1	0
Cisatracurium	0	0	5	26
Mivacurium	0	0	3	0

* Ring and Messmer scale (18). † Some patients received two different NMBAs.
IDST = intradermal skin test; NMBA = neuromuscular blocking agent.

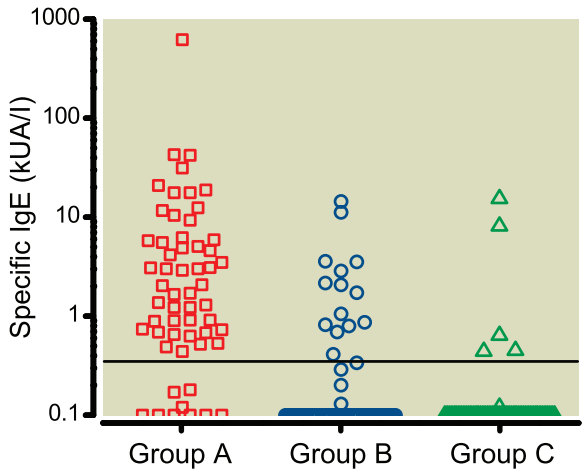

Fig. 1. Distribution of specific immunoglobulin E (IgE) concentrations (log scale) measured with the quaternary ammonium morphine test in the three groups of patients. Group A: Reactors with positive neuromuscular blocking agent (NMBA) intradermal skin tests (IDST); Group B: Reactors with negative NMBA IDST; Group C: Controls with uneventful anesthesia. The *line* corresponds to the positivity cutoff (0.35 kU_A/l).

shown to have reacted to antibiotics, we decided to retain the 0.35 kU_A/l cutoff to avoid such false-positive results.

Total IgE concentrations were measured in the 168 patients to detect possible interference by nonspecific IgE in the QAM-IgE assay. A weak linear correlation was found between total and specific IgE concentrations ($r^2 = 0.032$).

Inhibition curves were generated for each NMBA. Binding inhibition was concentration-dependent, as shown in figure 3. The decrease was at least 50% with suxamethonium 2.5 mg/ml, pancuronium 0.5 mg/ml, atracurium 2.5 mg/ml, cisatracurium 0.5 mg/ml, and

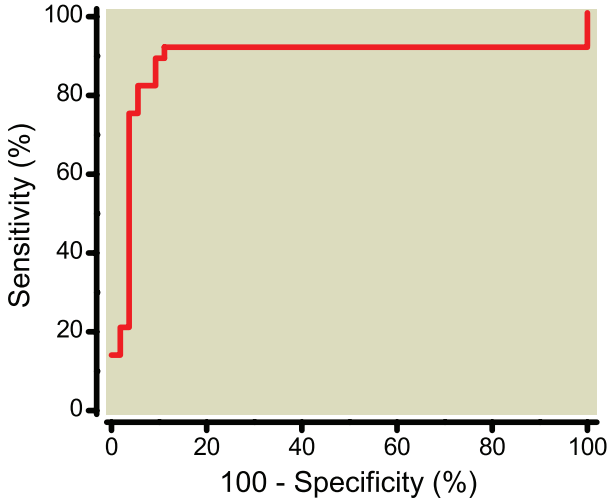

Fig. 2. Receiver operating characteristics (ROC) curve generated with quaternary ammonium morphine–IgE concentrations measured in neuromuscular blocking agent skin test–positive reactors and in controls with uneventful anesthesia. IgE = immunoglobulin E.

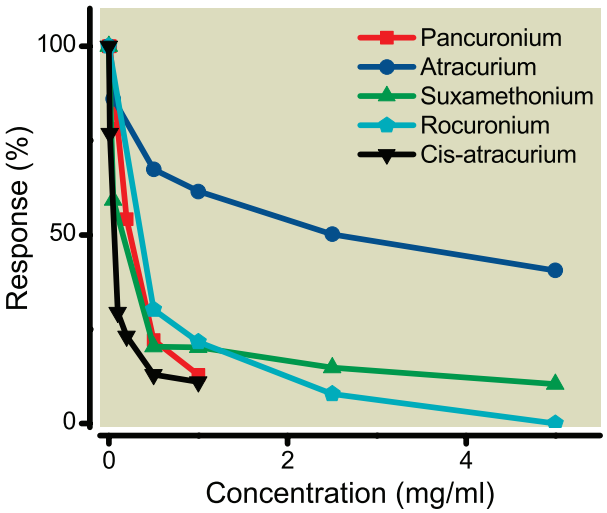

Fig. 3. Inhibition tests using the quaternary ammonium morphine–IgE test. Sera from five neuromuscular blocking agent (NMBA) skin test–positive reactors were incubated volume for volume with the administered NMBA(s) in serial dilution and pure solution or with buffer. Percent response: (response of serum with inhibitor)/(response of serum with buffer), after subtracting the system background response (buffer alone).

rocuronium 2.5 mg/ml, and these concentrations were used for subsequent inhibition tests. In group A (40 patients with QAM more than 0.7 kU_A/l), the percentage inhibition was 45.4% (92.4; 0–100) and inhibition was positive in 80% of cases. Two patients in group A were tested with four NMBAs different from the culprit NMBA. All of the NMBAs, including those who did not exhibit cross-reactivity in STs, gave positive inhibition, which was generally higher than the inhibition obtained with the culprit NMBA. In group B (11 patients tested), the median percentage inhibition was 34.7% (88.5; 7.5–89.6), and inhibition was positive in 82% of cases. Finally, specific positive inhibition by the NMBA administered during uneventful anesthesia was observed in the two control patients tested (45% and 50%, respectively).

A hook effect (by saturation of the solid phase) was observed in one patient who had cardiac arrest immediately after suxamethonium injection, with a tryptase concentration as high as 629 $\mu g/l$ and a positive IDST with suxamethonium diluted 1:100,000. The QAM-IgE concentration was 41 kU_A/l in undiluted serum but 620 kU_A/l in serially diluted samples (fig. 4).

Comparison of the QAM and QAS Tests

The QAM and QAS tests were compared in samples from patients in groups A and B. A linear correlation was observed (slope = 0.552; intercept = 3.642; $r^2 = 0.355$). In group A, the QAS binding value was 6.56% (22.9; 0.3–24.2), and 46 patients (80.7%) had positive results. Discrepancies were observed in six cases: two patients negative in QAM were positive in QAS, whereas four patients positive in QAM were negative in QAS. The QAS binding value was significantly

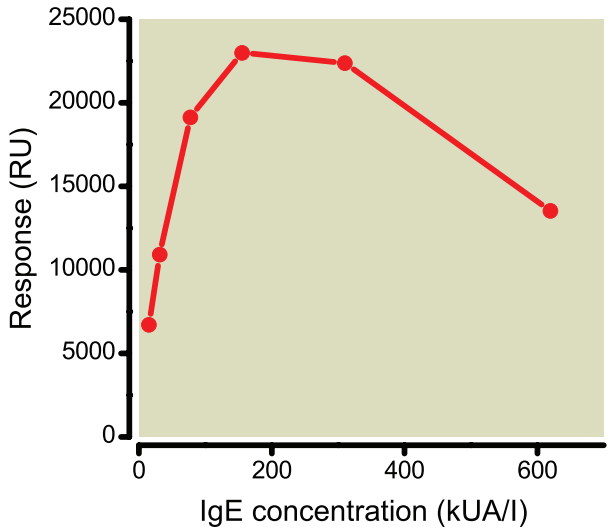

Fig. 4. Hook effect observed in a patient with a positive suxamethonium intradermal test. The observed response (fluorometric units) is plotted against the calculated quaternary ammonium morphine–immunoglobulin E (IgE) concentration in serially diluted serum. IgE concentration in undiluted serum, read from the calibration curve, was 41 kU_A/l compared with 620 kU_A/l, as calculated from diluted samples.

lower in group B (0.82%; 6.72; 0.39–18.5) than in group A ($P < 0.0001$). Forty-seven patients in group B (82.5%) had negative QAS tests, and 10 had positive QAS and QAM tests.

The diagnostic sensitivities of the two tests—84.2% for QAM and 80.7% for QAS—were not significantly different (Mac-Nemar test, Yates corrected chi-square, $P = 0.60$). Among patients who reacted to suxamethonium and atracurium, sensitivity was 88.9% and 75%, respectively, in the QAM test, and 86% and 66.7% in the QAS test.

In the overall group of 114 reactors, the positive predictive value of the QAM test (*i.e.*, the ability to predict positive skin tests) was 77.4% (*vs.* 82.1% for QAS), and the negative predictive value was 82.7% (*vs.* 81%).

Description of NMBA ST-negative Reactors with Positive QAM Results

Fourteen reactors in group B had increased concentrations of QAM-IgE. The frequency of positive tests (24.6%) was significantly higher in group B than in group C ($P < 0.05$, chi-square = 4.48). Four of these patients were probably false-positive: three had a positive ST to latex, vancomycin, or propofol (one case each), and one reacted 1 h after NMBA administration. The other 10 patients reacted less than 5 min after NMBA injection (table 2): six had increased histamine concentrations, and the tryptase concentration doubled in five cases.

Discussion

Diagnosis of immediate allergic hypersensitivity to NMBAs is crucial to prevent recurrences in patients who react during an anesthetic procedure. Although STs are the reference in this setting, the diagnosis is difficult because two pathways may be involved *via* IgE-dependent mechanism, namely anaphylaxis, or *via* nonspecific histamine release as a result of direct toxicity. Some NMBAs, such as atracurium, are known to induce nonspecific histamine release *in vivo* and to yield false-positive STs.[10] On the other hand, STs are unreliable during the first 4 weeks after a reaction and in patients on specific medications. Thus, a positive diagnosis should not rely only on STs, and simple serological tests would be helpful. The concentration of NMBA-specific IgE is stable during 6 weeks after a reaction,[21] which is interesting when surgery has been postponed but is still urgent.

Solid phases designed to mimic the quaternary ammonium epitope have been developed, using the ammonium

Table 2. Data for Patients Who Reacted Immediately after Neuromuscular Blocking Agent Injection and Had Negative Skin Tests but a Positive Quaternary Ammonium Morphine-IgE Test

Administered NMBA	Severity Grade*	QAM (kU_A/l)	QAM-inhibition (%)	QAS-binding (%)	QAS-inhibition (%)	Total IgE (kUI/l)	Histamine (nM) (Delay)	Tryptase ($\mu g/l$) (Reaction/Basal)
Atracurium	1	14.4	47.4	5.0	57	321	1.7 (25 min)	4.4/ND
Suxamethonium	1	3.56	34.7	4.9	67	100	9.3 (10 min)	10.4/5.9
Suxamethonium	3	3.54	78.7	5.3	75	506	30 (15 min)	22/2.2
Suxamethonium	3	2.85	7.5	6.7	68	207	12 (30 min)	5/<1
Cisatracurium	3	1.73	87	15.9	77	1,460	1.5 (22 min)	5/3
Suxamethonium and atracurium	3	1.05	87.4 ND	8.7	80 62	434	26 (5 min)	13/3.1
Atracurium	3	0.86	8.6	1.04	0	50	178 (25 min)	22/3
Rocuronium	3	0.8	66	3	63	172	13 (NS)	11/ND
Suxamethonium	1	0.69	ND	1.6	18	15	3 (10 min)	4.3/2.6
Suxamethonium	3	0.41	ND	0.78	0	675	H	11.8/3.3

Reference values: QAM test = <0.35 kU_A/l; QAM-inhibition = <20%; QAS-binding = <2.3%; QAS-inhibition = <20%; total IgE = <150 kUI/l; histamine = <6 nM; tryptase = <12 $\mu g/l$ (a doubling of the baseline concentration was considered positive).

* Ring and Messmer scale (18).

H = Hemolyzed sample; IgE = immunoglobulin E; ND = not done; NMBA = neuromuscular blocking agent; NS = not specified; QAM = quaternary ammonium morphine test; QAS = quaternary ammonium Sepharose test.

ion of choline[12,13] or morphine[22] to bind NMBA-specific IgE. These experimental radioimmunoassays have sensitivities close to 85% but are available in only a few laboratories. Recently, a fluorometric morphine-based assay (morphine-ImmunoCAP®; Phadia AB) showed 67.7% sensitivity in 65 patients with documented allergy to different NMBAs[17] and 88% in 25 rocuronium-allergic patients.[23] Here, we tested a modified version of this assay (QAM test) intended to have improved sensitivity.

A homogeneous cohort of reactors with a typical history, strong mediator release, and positive IDSTs to NMBAs was considered as the NMBA-allergic group. We compared the results for this group with exposed controls and with reactors with negative IDSTs to NMBA. QAM-IgE concentrations did not depend on age or gender, although a female predominance was observed among reactors, compared with controls, as previously described.[6] The three groups were markedly different with respect to the administered NMBAs, which were cisatracurium or pancuronium in controls (reflecting NMBA use in the two centers in 2006–2007); suxamethonium in most NMBA-allergic reactors (reflecting the higher frequency of anaphylaxis with this drug)[6]; and atracurium in most ST-negative reactors, possibly resulting from widespread use in 2001–2007 or to the histamine-releasing property of this drug.

We observed an overall sensitivity of 84.2% in the NMBA-allergic group, although sensitivity appeared higher in suxamethonium-allergic patients (88.9%) than in atracurium-allergic patients (75%). These results are consistent with the 84.7% sensitivity reported with an experimental radioimmunoassay using Sepharose-bound morphine in a group of 118 reactors with increased tryptase concentrations and NMBA ST positivity, which indicated 97% sensitivity in suxamethonium-allergic patients and only 25% in atracurium-allergic patients.[15] These differences may be explained by different presentations of the morphine epitope by the two solid phases and by different steric conformations of IgE against suxamethonium and IgE against atracurium. The atracurium epitope differs markedly from morphine, contrary to suxamethonium, which has a much simpler epitope. However, the 75% sensitivity observed here for atracurium-allergic reactors seems acceptable compared with the Sepharose assay.[15] Compared with the recommended QAS test, the QAM test appeared similarly sensitive, although a few discrepancies were observed.

Inhibition tests showed that IgE binding in this method could be reversed by the culprit NMBA but also by some NMBAs that did not elicit positive STs, confirming that inhibition tests cannot reliably be used to identify a safe alternative NMBA.[11,23]

We calculated a specificity of 90.7% in the control group, a value close to the 94.6% obtained in 1999 with an experimental choline-IgE radioimmunoassay in the French population.[24] Morphine-specific IgE has been detected in atopic patients with total IgE concentrations exceeding 3,000 kU_A/l,[23] but none of our patients had such high concentrations. A common pitfall of serological tests is that plasma IgE indicates sensitization to the allergen, whereas the reaction is a result of cell-bound IgE. The sensitization rate in France was evaluated as 7.5% in 2005–2007 with the previous morphine fluoroassay,[25] which is consistent with the 9.3% rate found here. NMBA-sensitive patients are at a higher risk of allergic reactions, but few of them react, as shown by the considerably higher frequency of sensitization than of reactions. This is why preoperative screening appears unreliable[26] and could inappropriately contraindicate NMBAs in numerous patients who could otherwise benefit from these drugs.

Finally, we compared reactors with and without positive IDSTs to NMBA. Reactors with negative STs are a particular concern in clinical practice because only 66% of reactions occurring during anesthesia are documented as allergic events,[6] with the mechanisms remaining elusive in other cases. In this study, strong mediator release was observed in allergic reactors, whereas reactors with negative STs had lower mediator concentrations and less severe reactions than reactors with positive STs. Most of the latter patients received atracurium, and nonspecific histamine release may have been responsible. However, the significantly higher frequency of positive QAM testing compared with the control group, confirmed by inhibition tests or positive QAS tests in most cases, points to an allergic mechanism in some QAM-positive patients. Ten patients reacted immediately after NMBA injection, seven of which had increased concentrations of tryptase or histamine. Increased tryptase concentrations are usually associated with allergic hypersensitivity,[27] and tryptase and IgE assays can be used for etiologic diagnosis in patients who die after a reaction.[28] This could apply when obvious discrepancies are observed among clinical history, mediator results, and STs. Our findings suggest that some allergic reactors can be identified by tryptase and IgE measurements, despite negative STs. In such cases, basophil activation tests[29] are the only means of confirming the diagnosis and identifying a safe alternative NMBA.

In conclusion, the sensitivity of the QAM test in NMBA-allergic reactors, together with its simplicity and suitability for routine laboratory use, make it a valuable tool, in conjunction with STs, for diagnosing sensitivity to NMBA. Associated with tryptase assay, the QAM test is of particular interest when STs are unreliable or not available, such as in fatal reactions and when STs give results that tend to conflict with clinical features.

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