

Immunoglobulin E Antibodies to Rocuronium

A New Diagnostic Tool

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Background: Diagnosis of allergy from neuromuscular blocking agents is not always straightforward. The objectives of the current study were to investigate the value of quantification of immunoglobulin E (IgE) by ImmunoCAP (Phadia AB, Uppsala, Sweden) in the diagnosis of rocuronium allergy and to study whether IgE inhibition tests can predict clinical cross-reactivity between neuromuscular blocking agents.

Methods: Twenty-five rocuronium-allergic patients and 30 control individuals exposed to rocuronium during uneventful anesthesia were included. Thirty-two sera (total IgE > 1,500 kU/l) were analyzed for potential interference of elevated total IgE titers. Results were compared with quantification of IgE for suxamethonium, morphine, and pholcodine. Cross-reactivity between drugs was assessed by IgE inhibition and skin tests.

Results: Sensitivity of IgE for rocuronium, suxamethonium, morphine, and pholcodine was 68, 60, 88, and 86%, respectively. Specificity was 100% for suxamethonium, morphine, and pholcodine IgE and 93% for rocuronium IgE. ROC analysis between patients and control individuals changed the threshold to 0.13 kUa/l for rocuronium, 0.11 kUa/l for suxamethonium, 0.36 kUa/l for morphine, and 0.43 kUa/l for pholcodine. Corresponding sensitivity was 92, 72, 88, and 86%, respectively. Specificity was unaltered. Interference of elevated total IgE with quantification of IgE was demonstrated by the analysis in sera with a total IgE greater than 1,500 kU/l. IgE inhibition did not predict clinical relevant cross-reactivity.

Conclusions: The rocuronium ImmunoCAP constitutes a reliable technique to diagnose rocuronium allergy, provided an assay-specific decision threshold is applied. IgE assays based on compounds bearing ammonium epitopes are confirmed to represent reliable tools to diagnose rocuronium allergy. High total IgE titers were observed to affect specificity of the assays.

DIAGNOSTIC management of anaphylaxis from neuromuscular blocking agents (NMBAs) generally rests on an evocative history corroborated by appropriate skin tests. However, disagreement exists on the specificity of skin tests with NMBAs,¹⁻⁴ these skin tests do not demonstrate absolute diagnostic accuracy, and results are not always predictive for the clinical outcome.^{5,6} False-positive test results may merely cause an inconvenience (due to unnecessary avoidance of safe drugs), whereas false-

negative or equivocal results may be extremely dangerous and severely undermine correct secondary prevention.

During the past decade, flow-assisted analysis and quantification of *in vitro* activated basophils^{7,8} has been shown to constitute a promising instrument to diagnose allergy from NMBAs.⁹⁻¹³ Moreover, the technique provides the physician with a quick and even safer tool that allows simultaneous assessment of cross-reactivity and tailoring of safe alternatives.^{9,13}

Apart from immunoglobulin E (IgE) for suxamethonium (Phadia AB, Uppsala, Sweden; c202), there is currently no drug-specific IgE assay readily available to document allergy from NMBAs. Unfortunately, quantification of IgE to suxamethonium has repeatedly been reported to be too insensitive to diagnose allergy from NMBAs in general (sensitivity varying between 30 and 60%).¹⁴⁻¹⁷ This implies that quaternary ammonium is not the only epitope, and a hydrophobic environment sometimes seems necessary in the binding of anti-NMBA IgE.¹⁴⁻¹⁸

To our knowledge, apart from the findings from Fisher *et al.*,¹⁴ who reported a positive rocuronium-based radioimmunoassay in only 3 of 13 rocuronium-allergic patients, no data on the diagnostic value of rocuronium IgE are available.

The primary purpose of this study was to assess the sensitivity and specificity of the ImmunoCAP technology for detection of IgE antibodies to rocuronium in anaphylaxis from rocuronium. Because allergy from rocuronium can be life-threatening, it is critical to establish the most sensitive cutoff value. Therefore, analysis was extended beyond the traditionally recommended cutoff value of 0.35 kUa/l. To ascertain whether the reliability of the assay could benefit from an individual threshold, receiver operating characteristic (ROC) analyses were performed.

In the absence of reliable NMBA-specific IgE assays, some authors have advocated application of a morphine-based immunoassay as an alternative instrument,^{14,16,19} in preference to different NMBA-specific IgE assays, to detect sensitization against the antigenic quaternary ammonium (NH₄⁺) epitope of NMBAs.²⁰ Morphine is an alkaloid with a single tertiary ammonium epitope that is easily protonized. The antitussive drug pholcodine is a morphine derivative containing two ammonium epitopes. Therefore, results were compared with IgE quantification for morphine and pholcodine.

Cross-reactivity between NMBAs is said to be common because of ubiquitous ammonium groups in these drugs.

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The estimated prevalence of cross-reactivity between NMBAs is approximately 75% by skin tests²¹⁻²³ and up to 100% by radioimmunoassay inhibition tests.²⁴ Although some pairings are common, the patterns of cross-reactivity vary considerably between patients.²³⁻²⁷ However, it is unusual for an individual to be allergic to all NMBAs.^{24,25,28} The second purpose of this study was to compare IgE inhibition experiments and skin tests in the prediction of clinically relevant cross-reactivity between NMBAs.

Materials and Methods

Study Population

Selection of patients and control individuals was performed as described previously.¹³ Twenty-five patients (PTS) who had experienced profound hypotension and severe bronchospasm within 5 min after injection of rocuronium and who demonstrated a positive skin test result for rocuronium were enrolled (see "Materials and methods: skin test procedures"). Potential alternative causes for anesthesia-related anaphylaxis (*e.g.*, latex, chlorhexidine, antibiotics, analgesics, hypnotics) were excluded as described elsewhere.^{29,30} Six patients had uneventful intravenous administration of cisatracurium during rescheduled surgery.

Thirty individuals exposed to rocuronium during uneventful anesthesia served as a control group (CTRL1). Sera of these control individuals were drawn 1 h after induction.

An additional analysis was performed in 22 individuals who were exposed to rocuronium and had experienced anesthesia-related anaphylaxis due to other agents (CTRL2). In those individuals, other causes (*e.g.*, latex, chlorhexidine, antibiotics, analgesics, hypnotics) were identified as described elsewhere,²⁹ whereas the skin test results to the five NMBAs (rocuronium, vecuronium, suxamethonium, atracurium, and cisatracurium) were negative. The study was approved by the Ethical Committee of the University Hospital Antwerp (Belgium), and participants gave their informed written consent.

Specificity analysis was expanded with an additional 95 sera of healthy controls with complete negative IgE and skin prick test results for classic inhalant allergens, 95 atopic control individuals with a documented inhalant allergy (history confirmed by IgE and skin prick test), and 9 individuals exposed to morphine. Finally, we selected 32 sera with a total IgE exceeding 1,500 kU/l to allow assessment of a potential effect of high total IgE on the quantification of IgE.

Specific IgE

Specific IgE to rocuronium, suxamethonium, morphine, and pholcodine was quantified by a low-level ImmunoCAP system (Phadia AB) with a detection limit

of 0.10 kUa/l. ImmunoCAP suxamethonium c202 was obtained from Phadia AB. ImmunoCAP rocuronium, morphine, and pholcodine were obtained as experimental prototypes made for research use.

Quantification of total serum IgE and specific IgE to inhalant allergens (house dust mite, timothy grass pollen, birch pollen, mugwort pollen, cat and/or dog epithelium, *Cladosporium herbarium*), *Hevea* latex, chlorhexidine was performed with the ImmunoCAP assay. All assays were performed according to the manufacturers' recommendations.

Inhibition assays were conducted by incubating 100 μ l of patients' serum with four different concentrations of rocuronium, vecuronium, atracurium, cisatracurium, suxamethonium, and morphine (0, 10, 100, and 1,000 μ g/ml) or buffer during 1 h at room temperature, followed by quantification of allergen-specific IgE using the ImmunoCAP technique.

Skin Tests

All individuals that presented anaphylaxis related to anesthesia (*i.e.*, the PTS and CTRL2 group) had skin tests as described.^{13,31} These implied rocuronium (Esmeron[®]; Organon, Brussels, Belgium), vecuronium (Norcuron[®]; Organon), atracurium (Tracrium[®]; GSK, Genval, Belgium), cisatracurium (Nimbex[®]; GSK), suxamethonium (Myoplegine[®]; Christiaens, Brussels, Belgium), latex (Stallergènes, Genval, Belgium), 2% chlorhexidine digluconate in 70% alcohol, the administered analgesics (generally Sufenta) and hypnotics (generally propofol), a negative (saline buffer), and a positive control (10 mg/ml histamine; HAL Allergy Benelux BV, Haarlem, The Netherlands). Skin prick test and intradermal test responses were considered positive when the wheal equaled or exceeded diameters of 3 and 8 mm (or doubling of injection bleb), respectively. For the skin prick test and intradermal test, commercially available drugs were diluted in a physiologic solution immediately before use. For the intradermal test, injection of 0.05 ml was performed through a hypodermic needle, and reactions were read after 20-30 min by measuring diameters of wheals and flares. A skin prick test for inhalant allergens implied house dust mite, timothy grass pollen, birch pollen, mugwort pollen, cat and/or dog epithelium, and *Cladosporium herbarum*.

Statistical Analysis

Statistical analysis was performed with SPSS 13 software (SPSS Inc., Chicago, IL). Results were expressed as median (range). After logarithmic transformation of the results, the Student *t* test and Pearson test were applied where appropriate. Differences were considered significant at a *P* value less than 0.05. ROC curve analysis was performed using the two-graph ROC method as described by Greiner³² to calculate the optimal cutoff value corresponding to the best sensitivity and specificity.

Table 1. Results of Skin Tests, Total IgE, and IgE in Patients

	Total IgE	Rocuronium					Vecuronium Skin Test
		Skin Test	IgE*	Suxa IgE*	Morphine IgE*	Pholcodine IgE*	
1†	100	+ SPT 1:100	3.30	4.00	3.13	1.72	Negative
2†	2,063	+ SPT 1:100	7.91	1.14	42.30	NA	+ SPT 1:1
3‡	370	+ SPT 1:100	0.65	4.47	5.96	0.64	+ SPT 1:1
4	64	+ SPT 1:10	0.08	0.01	0.05	0.06	Negative
5	63	+ SPT 1:10	4.22	0.12	1.80	Not available	+ SPT 1:1
6	342	+ SPT 1:10	0.23	0.92	1.06	2.69	Negative
7	120	+ SPT 1:10	5.28	0.03	1.46	2.49	Negative
8	137	+ SPT 1:1	0.60	0.03	0.05	0.01	+ SPT 1:10
9	171	+ SPT 1:1	0.19	0.18	0.59	Not available	Negative
10	176	+ SPT 1:1	0.42	0.16	1.68	1.17	+ SPT 1:1
11	40	+ SPT 1:1	0.10	0.03	0.07	0.07	Negative
12‡	121	+ SPT 1:1	0.58	5.27	7.17	2.69	Negative
13	Not available	+ SPT 1:1	0.21	0.37	22.0	8.31	+ SPT 1:1
14†‡	22,450	+ SPT 1:1	29.00	16.60	100.00	74.1	+ SPT 1:1
15	986	+ SPT 1:1	3.37	0.10	3.80	6.03	Negative
16‡	436	+ SPT 1:1	0.23	0.06	6.47	7.97	Negative
17†	2,643	+ SPT 1:1	1.71	1.46	35.50	15.7	Negative
18‡	37	+ SPT 1:1	0.39	6.19	0.86	1.04	+ SPT 1:1
19†‡	379	+ SPT 1:1	2.20	6.21	11.00	6.66	Negative
20	295	+ SPT 1:1	0.33	1.10	2.21	2.65	+ SPT 1:1
21	606	+ SPT 1:1	2.33	11.30	3.13	1.10	+ IDT 1:100
22	1,816	+ SPT 1:1	2.18	56.6	100.00	38.2	+ SPT 1:1
23	9	+ IDT 1:100	0.13	0.03	0.89	0.92	+ SPT 1:1
24	3,438	+ IDT 1:100	30.40	25.7	58.10	32.6	Negative
25	Not available	+ IDT 1:100	5.94	0.40	5.55	6.10	Negative

* Results of immunoglobulin E (IgE) are expressed in kUa/l. A result ≥ 0.35 kUa/l was considered positive. † Sera used for inhibition tests. ‡ Uneventful intravenous administration of cisatracurium. IDT = intradermal test; SPT = skin prick test; Suxa = suxamethonium.

Sample size calculation was performed according to a previous study.¹⁵ For an α of 0.05% and β (power) of 99%, we had to include 25 patients (δ 44%).

Results

Skin Tests

Individual results of skin tests for rocuronium and vecuronium in patients are summarized in table 1. In general, diagnosis of rocuronium allergy was documented by skin prick test. Only 3 of the 25 patients (12%) needed an additional intradermal test. Three patients demonstrated a positive skin test result for suxamethonium. All patients demonstrated negative skin test results for atracurium and cisatracurium. All individuals of the CTRL2 group demonstrated negative skin test results for the five NMBAs tested.

Total and Specific IgE

In the patients, the median (range) for total IgE was 295 (9–22,450) kUa/l and was significantly higher as compared with 27 (1–4,777) kUa/l in CTRL1, 62 (6–752) in CTRL2, and 37 (2–966) kUa/l in the 95 healthy controls ($P < 0.001$).

In the patients, the median (range) for IgE rocuronium, suxamethonium, morphine, and pholcodine was 0.65 (< 0.10 –30.40), 0.92 (< 0.10 –56.60), 3.10 (< 0.10 to >

100), and 2.70 (< 0.10 –74.10) kUa/l, respectively, significantly higher as compared with < 0.10 (< 0.1 –0.71), < 0.10 (< 0.10 –0.10), < 0.10 (< 0.10 –0.13), and < 0.10 (< 0.10 –0.24) kUa/l in control individuals (CTRL1) ($P < 0.001$; fig. 1). Intraassay coefficient of variation ranged from 9.7 to 10.3% ($n = 10$). Interassay coefficient of variation ranged from 21.7 to 27.2% ($n = 12$).

According to the 0.35-kUa/l threshold, comparison between PTS and CTRL1 revealed a sensitivity of IgE to rocuronium, suxamethonium, morphine, and pholcodine of 68, 60, 88, and 86%, respectively. Specificity was 100% for suxamethonium, morphine, and pholcodine IgE and 93% for rocuronium IgE. Comparable sensitivity and specificity data were observed for a comparison between PTS and CTRL2 (data not shown).

Figure 2 shows the ROC curves for rocuronium, suxamethonium, morphine, and pholcodine IgE, generated between PTS and CTRL1 (A) as well as the ROC curves generated between PTS and CTRL2 (B).

Receiver operating characteristic analysis between PTS and CTRL1 changed the threshold value to 0.13 kUa/l for rocuronium, 0.11 kUa/l for suxamethonium, 0.36 kUa/l for morphine, and 0.43 kUa/l for pholcodine IgE. Analysis between PTS and CTRL2 generated similar data (not shown). That is, ROC analyses had limited effect on the cutoff value for morphine and pholcodine based assay but lowered the decision threshold for rocuronium and

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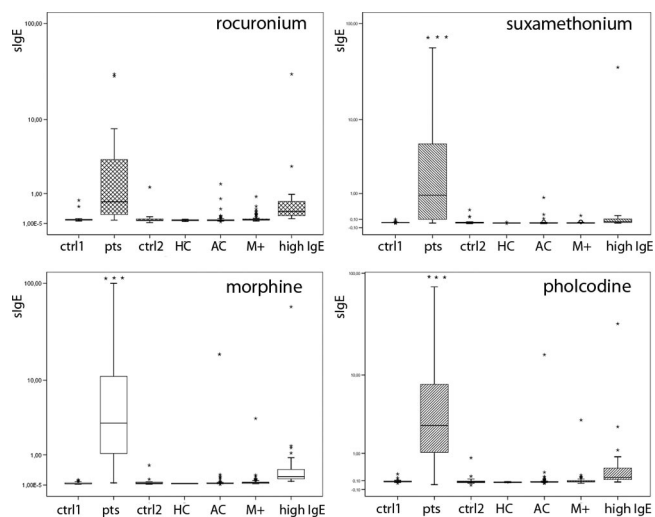


Fig. 1. Box plots of specific immunoglobulin E (sIgE) for rocuronium, suxamethonium, morphine, and pholcodine in different groups. AC = atopic controls with inhalant allergy; ctrl1 = control individuals exposed to rocuronium during uneventful anesthesia; ctrl2 = patients exposed to rocuronium having experienced anaphylaxis during anesthesia but not related to the myorelaxant; HC = healthy controls; high IgE = patients with total IgE titers exceeding 1,500 kUa/l; M+ = patients with prolonged intake of morphine; pts = patients allergic to rocuronium. * Outlier.

suxamethonium IgE. According to the ROC threshold values generated between PTS and CTRL1, sensitivity for rocuronium, suxamethonium, morphine, and pholcodine IgE was 92, 72, 88, and 86%, respectively. Corresponding specificity was 93, 100, 100, and 100%.

The expanded specificity analysis (95 healthy controls + 95 atopic controls + 9 patients exposed to morphine) revealed an IgE result exceeding 0.35 kUa/l for suxamethonium, morphine, and pholcodine in 0.5–1% of the samples, whereas 6 of the 199 (3%) showed reactivity for rocuronium. Lowering the cutoff to ROC-generated thresholds did not affect findings for suxamethonium. However, a positive IgE result for rocuronium was found in an additional 19 sera. Because the majority of these 25 positive results were found in sera with elevated titers of total IgE from the atopic control group, we sought to find the potential of interference of total IgE. Therefore, 32 additional randomly selected sera with total IgE concentrations exceeding 1,500 kU/l were added to the analysis. Fifteen (47%) and 31 (97%) of these 32 samples demonstrated a positive IgE for rocuronium, according to the 0.35 kUa/l and lowered ROC-generated threshold, respectively. Interference of high total IgE on quantification of IgE is shown in figure 3.

Approximately one third of the samples demonstrated morphine and pholcodine IgE antibodies, irrespective of the applied threshold.

Significant correlations between IgE rocuronium and IgE suxamethonium ($r = 0.52$, $P = 0.007$), IgE morphine ($r = 0.65$, $P < 0.001$), IgE pholcodine ($r = 0.61$, $P =$

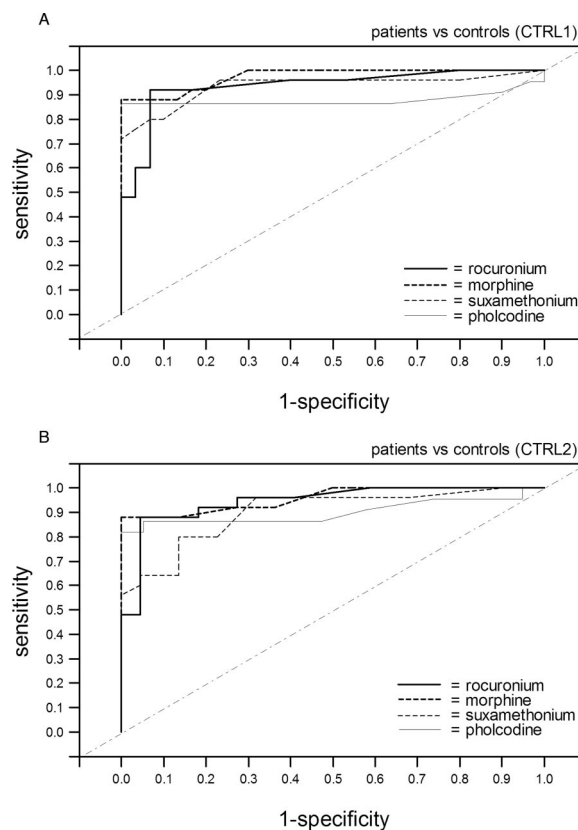


Fig. 2. (A) Receiver operating characteristic curves for immunoglobulin E (IgE) to rocuronium, suxamethonium, morphine, and pholcodine generated between rocuronium-allergic patients and rocuronium-tolerant individuals with uneventful anesthesia (CTRL1). (B) Receiver operating characteristic curves for IgE rocuronium, suxamethonium, morphine, and pholcodine generated between rocuronium-allergic patients and rocuronium-tolerant allergic control individuals who had anesthesia-related anaphylaxis not related to the neuromuscular blocking agents (CTRL2).

0.003), and total IgE ($r = 0.69$, $P < 0.001$) were observed.

In ImmunoCAP inhibition experiments, preincubation of sera (rocuronium, $n = 4$; morphine, $n = 3$) demonstrated a clear dose-related inhibition for all tested drugs (fig. 4). Cisatracurium and atracurium strongly inhibited IgE to rocuronium and morphine (up to 40–50%) in patients who demonstrated complete negative skin test results for both benzylisoquinoline-derived NMBA. Moreover, two of those patients had uneventful administration of cisatracurium during anesthesia for rescheduled surgery (table 1). No significant inhibition of IgE against latex was observed.

Discussion

The primary objective of this study was to evaluate the newly developed low-threshold ImmunoCAP IgE rocuronium assay in the diagnosis of anaphylaxis from rocuronium bromide. According to the traditionally recommended decision threshold (0.35 kUa/l), the assay

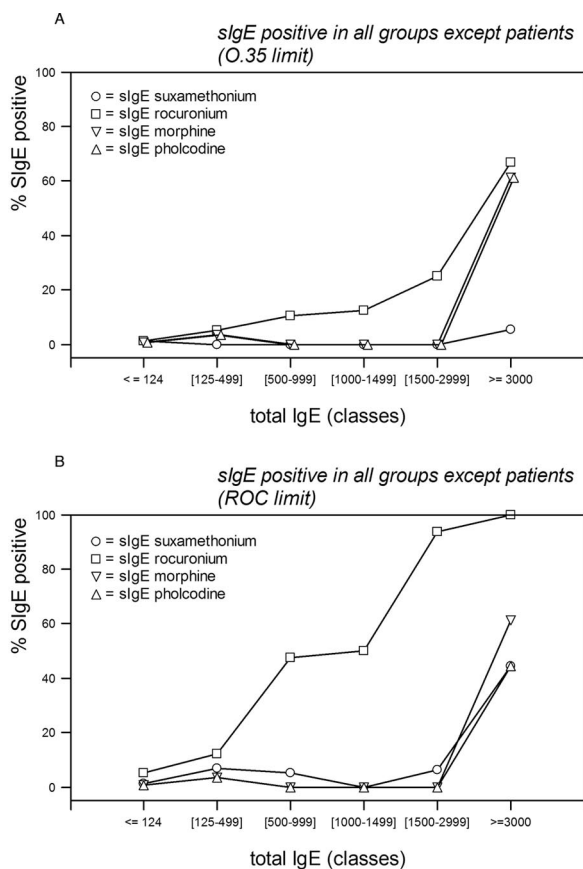


Fig. 3. Percentages of positive immunoglobulin E (IgE) found in all groups except patients according to total IgE (kU/l), divided in different classes of total IgE. IgE was scored positive when results were 0.35 kUa/l or greater (A) or according to receiver operating characteristic (ROC)-generated value (B). sIgE = specific IgE.

demonstrated a sensitivity of 68% and a specificity of 93%. Because anaphylaxis from rocuronium can be life-threatening, we wondered whether the diagnostic accuracy of the assay could be improved by adopting an allergen-specific cutoff. For this purpose, ROC analysis was applied. Comparison between patients and controls exposed to rocuronium during uneventful anesthesia revealed that lowering the cutoff for IgE positivity to a ROC-generated threshold of 0.13 kUa/l considerably increased sensitivity to 92%, whereas specificity remained unaltered.

In clinical practice, however, physicians will need to correctly identify the causative compound(s), rather than to dichotomize between patients and asymptomatic control individuals. Therefore, an additional evaluation was performed in control individuals exposed to rocuronium and having presented anaphylaxis during anesthesia unrelated to the NMBAs. This assessment revealed no significant differences with the analysis performed on control individuals exposed to rocuronium during uneventful anesthesia. Comparable sensitivity and specificity data of the different IgE assays were found, irrespective of the adopted decision threshold.

Specificity of rocuronium assay was further endorsed by the expanded analyses in healthy control individuals, atopic control individuals, and patients with prolonged morphine exposure without history of anesthesia-related anaphylaxis. However, probably as a result of nonspecific binding to the allergosorbent, elevated total serum IgE was shown to interfere with the specificity of the assay.

In the absence of reliable NMBA-specific IgE assays, different authors have advocated application of a quaternary ammonium assay (choline analog^{21,22,24,33} or p-aminophenyl-phosphoryl-choline [PAPPC]^{16,21,22,34}), or a morphine-based solid phase IgE^{14,16,19} assay in preference to different NMBA-specific IgE assays to diagnose allergy from quaternary ammonium determinants of NMBAs. However, the practice to adopt a morphine-based assay to diagnose allergy from NMBAs was recently called into question by Florvaag *et al.*,¹⁷ who demonstrated IgE antibodies against morphine in 5% and 10%, respectively, of Norwegian blood donors and probably allergic individuals. The most likely explanation for their findings was sought in the high consumption of pholcodine-containing syrups in Norway.

Our study provides additional support that a quaternary ammonium determinant-based immunoassay can constitute a performant diagnostic aid to document IgE-mediated allergy from rocuronium, because these assays reach a sensitivity of more than 85% and absolute specificity, irrespective of the applied decision threshold. There is a clear discrepancy between our findings and those of Florvaag *et al.*¹⁷ As in Norway, pholcodine-containing antitussives are readily available in Belgium, although consumption per capita is four times higher in Norway.³⁵ Almost no IgE antibodies against suxamethonium, morphine, and pholcodine were demonstrable in our control individuals. In our survey, apart from the patients, positive IgE for these drugs were almost exclusively observed in sera with elevated titers of total IgE. Therefore, in the absence of total IgE quantification in the series by Florvaag *et al.*, interference by total IgE cannot be excluded. An attractive alternative, but hypothetical, explanation could be ammonium-containing compounds such as pholcodine to boost synthesis of total and quaternary ammonium-specific IgE antibodies in sensitized patients.³⁶ This presumption seems to be endorsed by our unexpected observation that patients with definite rocuronium allergy demonstrated significantly increased titers of total IgE, as compared with all other control groups.

The diagnostic approach of allergy from rocuronium cannot be considered as complete when it does not address the possibility of cross-reactivity or identify safe alternative drugs. This assessment, however, is fraught with difficulties because results from diagnostic tests do not *per se* reflect the clinical outcome. This study confirms that one cannot rely on IgE inhibition tests to

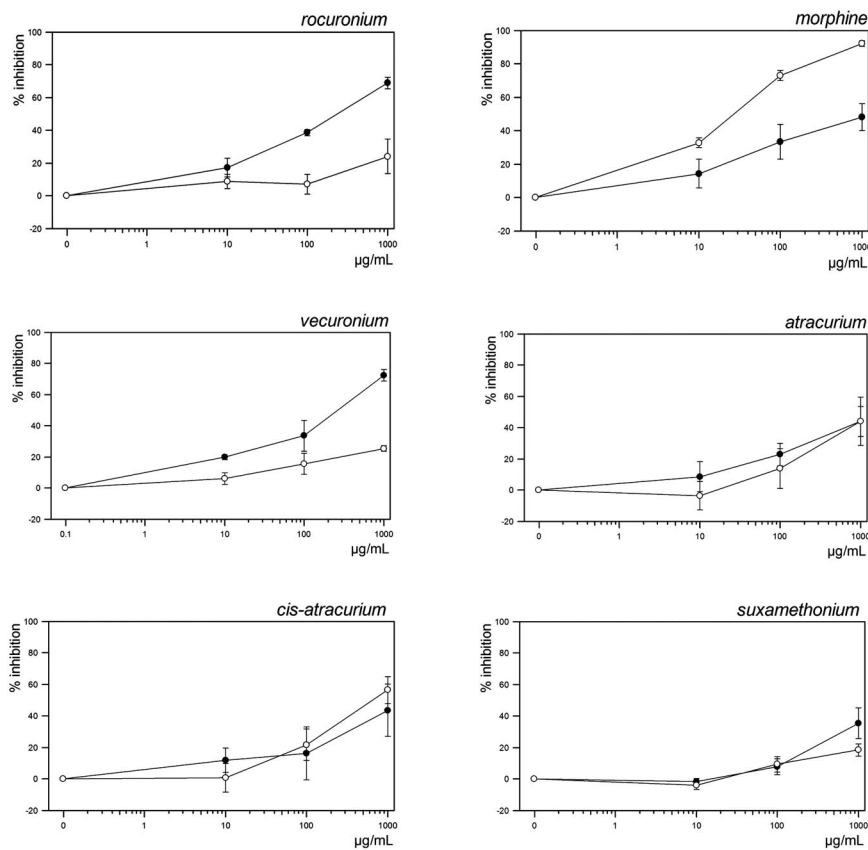


Fig. 4. In ImmunoCAP inhibition assays, preincubation of sera with five neuro-muscular blocking agents (rocuronium, vecuronium, atracurium, cisatracurium, suxamethonium) and morphine demonstrated a clear dose-dependent inhibition of immunoglobulin E (IgE) to rocuronium ($n = 4$, closed circles) and IgE to morphine ($n = 3$, open circles). There was no inhibition of IgE to latex.

assess cross-reactivity between different NMBAs, because they might simply reflect *in vitro* cross-reactivity without clinical relevance.²⁴ Cross-reactivity and identification of safe alternative NMBAs should be ascertained by skin tests and functional *in vitro* assays such as mediator release tests²⁴ or flow-assisted basophil analysis.⁹ Moreover, skin tests and functional *in vitro* tests might prove complementary in the evaluation of cross-reactivity between NMBAs and identification of a safe alternative curarizing agent.¹³

In conclusion, positive IgE antibodies to rocuronium, suxamethonium, morphine, and pholcodine mirror sensitization from quaternary ammonium ions. With respect to the diagnosis of allergy from rocuronium, quantification of IgE for rocuronium by ImmunoCAP constitutes a reliable adjunct technique, provided an assay-specific decision threshold is applied. However, attention should be paid to sera containing elevated titers of total IgE. Alternatively, our data confirm that quaternary ammonium determinant-based immunoassay IgE assays based on morphine can represent additional sensitive and specific tools to document diagnosis of allergy from rocuronium, irrespective of the decision threshold. For the assessment of clinically relevant cross-reactivity and determination of a safe alternative NMBA, IgE inhibition experiments are of limited help. Whether ImmunoCAP IgE assays based on compounds bearing ammonium

epitopes can also detect sensitization for other NMBAs remains to be confirmed.

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