

Morphine and pholcodine-specific IgE have limited utility in the diagnosis of anaphylaxis to benzylisoquinolines

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Background: Investigation of immediate hypersensitivity reactions in the perioperative setting involves skin testing and measurement of specific IgE (sIgE) as standard practice. In the case of the neuromuscular blocking agents (NMBAs), the main allergenic epitopes have been shown to be substituted ammonium groups. Commercial assays are available for detection of sIgE to these epitopes using morphine and pholcodine substrates but questions have been raised about the effectiveness of these assays in the diagnosis of benzylisoquinoline anaphylaxis. This study was therefore undertaken to assess the effectiveness of these assays in the diagnosis of hypersensitivity reactions to this group of NMBAs.

Methods: Analysis was carried out on all available results for patients assessed at the Royal North Shore Hospital Anaesthetic Allergy Clinic during the period June 2009 to June 2016. Standardised intradermal skin tests were performed with a panel of NMBAs. Measurement of sIgE to morphine and pholcodine was performed via the Phadia ImmunoCAP[®] system.

Results: For all patients with positive skin test results to NMBAs which included a benzylisoquinoline NMBA ($n = 24$), 75% exhibited negative sIgE to both morphine and pholcodine. Where patients were reactive to benzylisoquinoline NMBAs alone ($n = 12$), 100% exhibited negative sIgE results, indicating 0% sensitivity of the assays relative to skin testing, in this subgroup.

Conclusion: Use of sIgE testing to morphine and pholcodine in the assessment of NMBA immediate hypersensitivity is a valuable tool particularly in the case of reactions to the aminosteroid NMBAs. However, these assays are unreliable in detecting sensitisation to benzylisoquinoline NMBAs.

Editorial comment

When sudden perioperative hypersensitivity reactions occur, follow-up skin testing should be done, and some cross-reactivity between certain drug classes is recognized. This study shows that morphine- and pholcodine-specific IgE assays may be helpful in distinguishing between different classes of neuromuscular blocker sensitivities.

Neuromuscular blocking agents (NMBAs) are the most commonly implicated agent in perioperative anaphylaxis in many countries.^{1–3} Structure-activity analysis by Baldo and Fisher⁴ in 1983 established that positively charged quaternary and tertiary ammonium ions were central to the allergenicity of the reacting NMBAs. Cross-sensitisation between NMBAs is common and seen in approximately 50% of patients with NMBA anaphylaxis.¹

The diagnostic algorithm for perioperative anaphylaxis typically involves pairing of intradermal and skin prick skin testing to substance potentially implicated in the aetiology of the anaphylactic reaction after consideration of timing of drugs given compared with the onset of clinical features. Confidence in the interpretation of the result is increased when positive skin tests correlate with clinical features of the reaction such as the timing of administration for intravenous antigens, and for positive skin tests found at concentrations that are at the dilute end of recommended valid concentrations. Skin testing itself does not carry absolute sensitivity and specificity^{5,6} and suffers from a need to delay testing for 4–6 weeks after the anaphylactic reaction to avoid the existence of a possible immune refractory period.⁷ Skin testing performed within this window period potentially confers a higher false negative rate with only positive results considered valid. Additionally, skin tests are contraindicated in the presence of certain medications and skin conditions.

Specific IgE (sIgE) assays may provide additional information in the investigation of perioperative anaphylaxis^{8,9} and can be used as an adjunct to positive skin test results to confirm NMBAs as the aetiology. Similarly, negative sIgE results increase confidence in the conclusion of an alternate agent as the cause for an IgE-mediated anaphylaxis or may suggest a non-IgE-mediated cause for the reaction. Unlike skin testing, sIgE assays are not affected by a 4–6 week refractory period and can be particularly valuable when urgent surgery is required within this period. Basophil activation tests have been used by some groups but these are not widely available and the diagnostic utility has not been fully assessed.¹⁰

Morphine and pholcodine are substances which at physiologic pH contain substituted

ammonium groups similar to the groups present on the NMBAs.^{11,12} Morphine contains one such group while pholcodine contains two amine groups of which one is more predominantly protonated at physiologic pH.¹² Testing of patient serum for the presence of sIgE to morphine and pholcodine has been subsequently shown to be of value in the detection of antibodies to NMBAs.^{11,13} It is not clear, however, whether morphine and pholcodine sIgE assays have equal diagnostic value in the investigation of allergic reactions to the different classes of NMBAs. Some earlier observations^{9,11,14} have suggested a lower sensitivity for the morphine sIgE assay in atracurium-allergic patients, with sensitivities ranging from 25% to 75%. In this study, we sought to investigate the diagnostic utility of both the morphine and pholcodine sIgE assays with respect to the different classes of NMBAs.

Methods

A retrospective analysis was performed to assess the correlation between the results of skin testing to NMBAs and measurement of sIgE to morphine and/or pholcodine. The patients had been referred to and attended the Royal North Shore Hospital Anaesthetic Allergy Clinic after a suspected episode of intraoperative anaphylaxis during the period of June 2009 to June 2016. Consent was obtained in all patients for retrospective use of their data for research and this study was approved by the Northern Sydney Local Health District Human Research Ethics Committee (LNR/14/HAWKE/315, approved 3 September 2014). The study excluded patients where sIgE testing was not performed (such as general anaesthesia without NMBA, spinal anaesthesia or dedicated local anaesthetic testing), sIgE testing was not done (such as patient refusal) or where sIgE testing was done by an external laboratory.

Skin testing followed published guidelines as outlined by the Australian and New Zealand Anaesthetic Allergy Group (ANZAAG)¹⁵ and involved intradermal with or without skin prick tests to all relevant antigens. Where a skin test result implicated an NMBA, skin testing to other commonly used NMBAs was undertaken to identify potential cross-reactivity to one or more other NMBAs. The NMBAs tested

routinely in cross-reactivity were rocuronium, vecuronium, pancuronium, suxamethonium and cisatracurium.

Atracurium was only tested if it was the NMBA given at the time of reaction. The rationale for this relates to atracurium's histamine-releasing properties, and the significant alterations in skin-testing concentrations required as a result. We considered cisatracurium to be clinically similar to atracurium, less complex to skin test and crucially, that a negative skin test to atracurium in the presence of a positive test to cisatracurium would not result in a recommendation to use atracurium in the future, given that cisatracurium is an isomer of atracurium.

Mivacurium is also highly histamine releasing and was only tested if it was the NMBA given at the time of anaphylaxis or if skin testing to the other five NMBAs were positive and a negative test to mivacurium could be clinically highly valuable.

Measurement of sIgE to morphine and pholcodine was performed using the Phadia ImmunoCAP system (Thermo Fisher Scientific, Uppsala, Sweden). The limit of quantitation is 0.10 kUA/l and the standard positive cut-off threshold is 0.35 kUA/l.

Patients were divided into four groups for further analysis, according to skin test results. Group A patients ($n = 106$) were those where the administered NMBA skin tested positive and the inciting or cross-reactive NMBAs did not include members of the benzylisoquinoline group. That is, inciting and cross-reactive NMBAs were only from the aminosteroidal group (rocuronium, vecuronium, pancuronium) and/or suxamethonium. Group B patients ($n = 12$) were those where the administered NMBA skin-tested positive and the inciting and any associated cross-reactive NMBAs included both benzylisoquinoline (cisatracurium, atracurium, mivacurium) as well aminosteroidal NMBAs and/or suxamethonium. Group C ($n = 12$) were patients who received benzylisoquinoline NMBAs at the time of reaction and were skin test positive to members of the benzylisoquinoline family only. Group D ($n = 292$) were skin test negative to all NMBAs tested and were judged not to be allergic to NMBAs.

Statistical analysis was performed using GraphPad Prism 7.01 (GraphPad, San Diego,

CA). The Mann–Whitney test was used for comparison of sIgE concentrations between groups. A P value of < 0.05 was considered significant. Analyse-it® (version 4.20.1, Analyse-it Software Ltd., Leeds, UK) for Microsoft Excel software was used for receiver operating characteristic (ROC) curve analysis and sensitivity/specificity determinations. ROC curves were generated, using sIgE values from all skin test positive patients (Groups A, B, C) and all skin test negative patients (Group D). Concentrations of sIgE are given as median (interquartile range).

Results

A total of 422 patients were included in the study. Of these, there were 130 patients who were skin test positive to NMBAs (31 male, 99 female). This represents 24% male, 76% female. A female preponderance is consistent with previous published studies.^{16–18}

Median concentrations of morphine and pholcodine sIgE for patients in Group A were 1.95 (0.46–6.38) kUA/l and 2.79 (0.45–8.18) kUA/l, respectively, compared to a median concentration in the skin test negative patients (group D) of 0.10 (0.1–0.1) kUA/l in both assays (Fig. 1).

Median concentrations for patients in Group C were also 0.10 (0.1–0.1) kUA/l in both assays and median concentrations for patients in Group B were 0.40 (0.11–1.13) kUA/l and 0.51 (0.14–1.58) kUA/l, respectively. Concentrations of sIgE were significantly higher for Group A patients as compared to each of Groups C and D ($P < 0.0001$) and Group B ($P < 0.05$) (Fig. 1).

A ROC curve was plotted for each assay with the AUC being determined as 0.868 for morphine sIgE and 0.880 for pholcodine. The calculated optimum threshold (by Youden's index) in each case was 0.23 kUA/l. Intra-assay and interassay coefficients of variation at this lower threshold were $< 10\%$ for both assays.

Using the recommended cut-off threshold of 0.35 kUA/l, sensitivity and specificity for morphine and pholcodine sIgE with reference to skin test for NMBAs are shown in Table 1. More importantly, a breakdown of sensitivity and specificity for each of the skin test groups A, B and C are shown in Tables 2 and 3 at both the recommended and calculated cut-off thresholds.

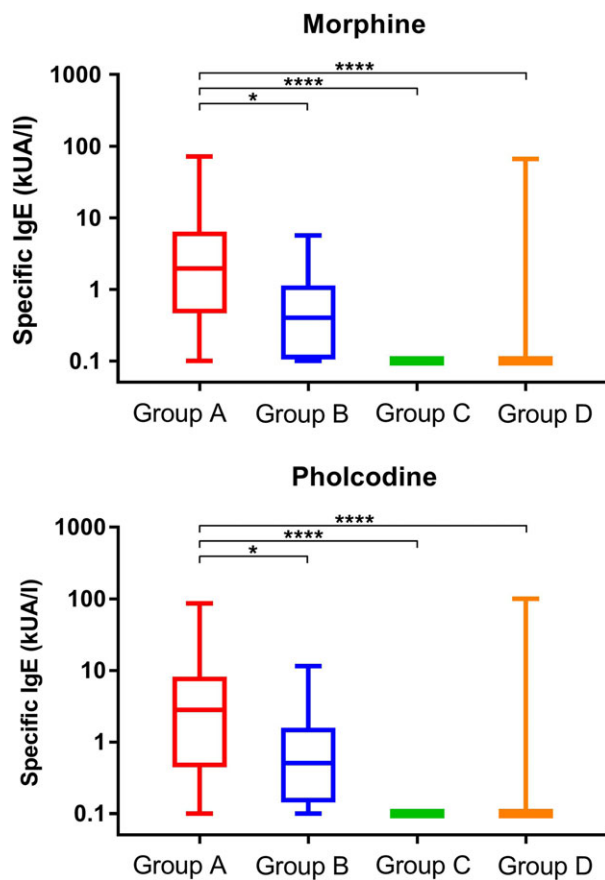


Fig. 1. Levels of morphine and pholcodine-specific IgE, showing aminosteroid and/or suxamethonium skin test positive patients (Group A) $n = 106$, patients skin test positive to an aminosteroid and/or suxamethonium as well as a benzylisoquinoline agent (Group B) $n = 12$, patients positive to benzylisoquinoline agents only (Group C) $n = 12$ and skin test negative patients (Group D) $n = 292$. Comparison between groups was performed using the Mann–Whitney test, **** $P < 0.0001$, * $P < 0.05$.

For all patients with positive skin test results to NMBAs which included a benzylisoquinoline NMBA (Groups B + C, $n = 24$), 75% exhibited negative sIgE to both morphine and pholcodine

at the recommended cut-off threshold. This figure increases to 100% ($n = 12$) for patients reactive to benzylisoquinoline NMBAs alone.

Discussion

Our study shows that there is an obvious contrast in the sensitivity of the morphine and pholcodine sIgE assays for detection of patients reactive to NMBAs of the benzylisoquinoline and the aminosteroid and suxamethonium groups, respectively. This study suggests that sIgE assays to both morphine and pholcodine are of benefit in the diagnosis of NMBA anaphylaxis where the inciting drug is aminosteroidal or suxamethonium, or where cross-reactivity exists with one of these NMBAs. In situations where skin tests suggest this to be the case, a positive morphine and/or pholcodine sIgE assay will help to confirm the diagnosis.

Equally, however, these results suggest that in cases where a benzylisoquinoline NMBA has been used and suspected anaphylaxis has occurred, a negative morphine and/or pholcodine sIgE assay should not dissuade the investigator from this diagnosis. The results additionally indicate that in cases where a patient has had an episode of anaphylaxis after the use of a benzylisoquinoline NMBA, positive morphine or pholcodine sIgE may suggest reactivity to one or more of the aminosteroid NMBAs and/or suxamethonium.

It has long-been established that the common feature of neuromuscular blockers which contributes to their antigenicity is the presence of the positively charged tertiary and/or quaternary amine groups found in these drugs. The ability to cause an IgE-mediated clinical anaphylactic reaction requires cross-linking of Fcε-bound IgE molecules on mast cells and basophils. As

Table 1 Performance characteristics of the morphine and pholcodine sIgE assays at the recommended and a ROC-adjusted threshold.

sIgE	Cut-off ≥ 0.35 kUAI/l		Cut-off ≥ 0.23 kUAI/l	
	Sensitivity % (95% CI)	Specificity % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)
Morphine	69.2 (60.8–85.9)	95.2 (92.1–97.1)	73.1 (64.9–80.0)	94.5 (91.3–96.6)
Pholcodine	67.7 (59.2–75.1)	94.5 (91.3–96.6)	73.1 (64.9–80.0)	93.5 (90.1–95.8)
Morphine OR Pholcodine	71.5 (63.3–78.6)	94.2 (90.9–96.3)	75.4 (67.3–82.0)	92.5 (88.9–95.0)

Table 2 Performance characteristics of the morphine and pholcodine sIgE assays between patient groups A, B and C. Recommended cut-off threshold is used (≥ 0.35 kUA/l).

sIgE	Sensitivity % (95% CI)			Specificity % (95% CI)
	Group A	Group B	Group C	
Morphine	79.2 (70.6–85.9)	50.0 (25.4–74.6)	0.0 (0.0–24.2)	95.2 (92.1–97.1)
Pholcodine	77.4 (68.5–84.3)	50.0 (25.4–74.6)	0.0 (0.0–24.2)	94.5 (91.3–96.6)
Morphine OR Pholcodine	82.1 (73.7–88.2)	50.0 (25.4–74.6)	0.0 (0.0–24.2)	94.2 (90.9–96.3)

Table 3 Performance characteristics of the morphine and pholcodine sIgE assays between patient groups A, B and C. Calculated cut-off threshold is used (≥ 0.23 kUA/l).

sIgE	Sensitivity % (95% CI)			Specificity % (95% CI)
	Group A	Group B	Group C	
Morphine	83.0 (74.7–89.0)	58.3 (32.0–80.7)	0.0 (0.0–24.2)	94.5 (91.3–96.6)
Pholcodine	81.1 (72.6–87.4)	75.0 (46.8–91.1)	0.0 (0.0–24.2)	93.5 (90.1–95.8)
Morphine OR Pholcodine	84.0 (75.8–89.7)	75.0 (46.8–91.1)	0.0 (0.0–24.2)	92.5 (88.9–95.0)

all NMBAs are at least divalent for these charged amine groups, they have the ability to initiate IgE cross-linking. This helps explain why drugs with single positively charged amine groups (such as morphine, codeine and pholcodine) will generally fail to trigger anaphylactic reactions in the presence of antibodies that bind to these groups.

In the case of the benzylisoquinoline NMBAs, however, clinical reactions and positive skin test results may occur without evidence of sensitisation to substituted ammonium epitopes, as we have shown here and has been indicated in previous smaller patient groups.^{14,19,20} This could of course indicate a non-IgE-mediated reaction, particularly given the recent description of direct mast cell activation by cationic substances, including some NMBAs, via the Mas-related G protein-coupled receptor X2 (MRGPRX2).²¹ However, It should be noted that mast cell activation in this reported study was indicated at drug concentrations significantly higher than those applied in the skin test protocols used here.

The absence of sIgE to substituted ammonium structures in these benzylisoquinoline reactive patients could also suggest the possibility of a separate allergenic epitope in this subgroup of

NMBAs.¹⁴ In some patients, cross-reactivity was also observed between the benzylisoquinoline and aminosteroid and/or suxamethonium NMBAs. This may indicate the presence of IgE antibodies towards substituted ammonium groups and another undetermined epitope particular to the benzylisoquinoline NMBAs or possibly in some cases, another cross-reactive epitope in both groups of drugs.

We found that using a combination of morphine and pholcodine sIgE assays allowed detection of an increased number of skin test positive patients within our cohort, compared to using either test alone. While confidence intervals did not indicate a combination of tests to be preferential, this did allow the identification of three extra NMBA allergic patients within the group investigated. Given the potentially serious nature of an anaphylactic reaction this may be helpful. Sensitivity of these combined assays, with respect to detection of all NMBA skin test positive patients, was 71.5% with a specificity of 94.2% at the 0.35 kUA/l cut-off threshold. Excluding patients with reactions to benzylisoquinoline NMBAs without cross-reactivity outside this group improved sensitivity to 82.1% without changing the specificity. This is in stark

contrast to when this subgroup of patients was examined, in which case the sensitivity of both the morphine and/or pholcodine sIgE assays was 0%. Additionally, where patients displayed skin test results indicating cross-reactivity to benzylisoquinoline and aminosteroid/suxamethonium NMBAs, 50% had sIgE levels to both morphine and pholcodine below the recommended positive cut-off threshold.

Use of a calculated-reduced optimum threshold was able to further increase sensitivity with only a slight corresponding decrease in specificity. This was particularly notable for group B patients using the pholcodine substrate and may indicate additional weak antibody binding sites on this molecule.

It should be noted that high total IgE levels have been shown to interfere with quantitation of morphine and pholcodine sIgE, particularly concentrations of 1500 kU/l and above.¹³ This may also have some impact on specificity and results should therefore be evaluated with caution in the presence of raised total IgE values.

The results of this study echo the overall sensitivity figures for morphine sIgE reported in slightly smaller series of NMBA reactive patients in the studies by Fisher and Baldo ($n = 100$) in 2000¹¹ and Laroche et al. ($n = 114$) in 2011.⁹ Fisher and Baldo demonstrated an overall sensitivity of 84.7% with a specificity of 98%. Interestingly in this study, sensitivity was excellent for suxamethonium, vecuronium and rocuronium but only 25% for atracurium. No information is provided regarding details of cross-reactivity for these 12 atracurium reactors, but we would suggest that the three patients with positive sIgE results may well have had cross-reactivity to aminosteroidal NMBAs, accounting for the positive in vitro results in these cases. Laroche et al. demonstrated a sensitivity of 84.2% for morphine sIgE in NMBA reactors but only 75% sensitivity for atracurium allergic patients.

Both these previous studies show lower sensitivities for morphine sIgE in benzylisoquinoline reactive patients but our study is the first to demonstrate a 0% sensitivity for patients reacting to a benzylisoquinoline without cross-reactivity to other NMBAs.

This study provides further evidence that the mechanisms involved in NMBA anaphylaxis

include more than just the tertiary or quaternary ammonium groups on these drugs. Conjecture as to the nature of alternative allergenic epitopes may include sites adjacent to and/or involving the substituted ammonium groups or could involve other structures. A recent study has suggested a possible cross-reactive epitope on rocuronium and atracurium which may not be associated with morphine reactive sIgE.²⁰ In the case of the benzylisoquinoline group of NMBAs particularly, the elucidation of other potential allergenic epitopes will be important to aid in the appropriate investigation of perioperative allergic reactions.

Conclusion

Morphine and pholcodine-specific IgE assays are useful in the diagnosis of NMBA anaphylaxis. They aid in the identification or exclusion of NMBAs as a cause of a perioperative reaction, as an adjunct to skin testing. Morphine and pholcodine sIgE, however, are not useful in cases where benzylisoquinolines NMBAs are the causative agents and cross-reactivity does not exist with the aminosteroidal NMBAs or suxamethonium.

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