Original Article

Comparison of Basophil Activation Test and Skin Testing Performances in NMBA Allergy

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What is already known about this topic? Neuromuscular blocking agents (NMBAs) are the main agents involved during perioperative allergy in France. The etiological diagnosis is linked to the clinical presentation, along with histamine and tryptase and skin test results. The role of BAT is still debated in this setting.

What does this article add to our knowledge? Combined CD63 and CD203c markers did not increase BAT sensitivity compared with CD203c only. BAT allowed identification of the culprit in 80% of NMBA-allergic patients and yielded concordant cross-reactivity results in 60% of the cases.

How does the study impact current management guidelines? BAT combining CD63 and CD203c markers does not replace skin testing in the assessment of NMBA allergy.

BACKGROUND: Neuromuscular blocking agents (NMBAs) are the main agents involved during perioperative immediate hypersensitivity. The etiological diagnosis (IgE-mediated allergy *vs* nonallergy) is linked to the clinical presentation together with tryptase and histamine levels and skin test results. The role of basophil activation test (BAT) needs to be better defined in this setting.

OBJECTIVES: To assess the role of BAT compared with the results of skin testing in 31 patients experiencing immediate NMBA hypersensitivity and compare skin test results and BAT performances in the identification of alternative NMBAs.

METHODS: Histamine and tryptase levels were quantified. Anesthetic drugs, including NMBAs, were skin-tested. Basophil CD63 and CD203c expressions were measured in response to serial dilutions of the different NMBAs.

RESULTS: Allergy and Nonallergy groups involved 19 and 12 patients, respectively. Circulating histamine and tryptase levels were significantly increased in allergic patients. In the Allergy group, while skin test results were positive in 100% (19 of 19) of the cases, BAT positivity to the culprit NMBA reached 78.9% (15 of 19) when combining CD63 and CD203c. NMBAs cross-reactivity was identified through skin testing and BAT in 36.8% (7 of 19) and 26.3% (5 of 19) of the cases, respectively. The concordance (culprit and cross-reactive NMBAs) between skin tests and BATs was between 73.6% (14 of 19) and 100% (19 of 19) for each NMBA. Negative skin-tested NMBAs were uneventfully used in 7 NMBA-allergic patients. In the Nonallergy group, skin test results were negative in 100% of the cases while BAT result was positive once (CD63 upregulation).

CONCLUSION: In our technical conditions, BAT does not replace skin testing in the assessment of NMBA allergy. © 2018 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2018;■:■-■)

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INTRODUCTION

Neuromuscular blocking agents (NMBAs) are the main triggers involved during perioperative immediate hypersensitivity in France.[1] The clinical presentation, together with biological parameters and skin tests, help identify the culprit drug and the pathomechanism involved (IgE-mediated allergy *vs* nonallergy). Skin testing remains the definitive standard for the detection of IgE-mediated NMBA allergy. To complete the assessment, skin cross-reactivity with other commercialized NMBAs is investigated to identify safe alternative regimens.[1-4]

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Abbreviations used
BAT- basophil activation test
IDT- intradermal test
MFI- median fluorescence intensity
NMBA- neuromuscular blocking agent
NPV- negative predictive value
PPV- positive predictive value
PT- prick test
QAI- quaternary ammonium ion

Flow cytometry allows quantifying the *ex vivo* capacity of sensitized blood basophil activation.[5,6] The basophil activation test (BAT) is based on the upregulation of 2 markers (CD63 and CD203c) expressed at the basophil membrane on *ex vivo* activation by the suspected drug. BAT might add to the etiological diagnosis of immediate NMBA hypersensitivity and help to identify both cross-reactive and safe alternative compounds.[6-11] However, its role needs to be better defined in this setting because skin testing is considered to be more sensitive than *in vitro* procedures.[1-3,12]

We conducted a retrospective study in 31 patients experiencing perioperative immediate hypersensitivity occurring within 5 minutes of NMBA injection. The objectives of the study were to (1) identify the pathomechanism of the clinical event on the basis of current guidelines[2,3,12,13]; (2) compare the results of skin testing with those obtained using BAT (identification of the culprit and cross-reactive NMBAs); (3) compare the positive and negative predictive values of skin testing and BAT in the diagnosis of immediate NMBA hypersensitivity; (4) assess the role of BAT in this clinical setting; and thus (5) evaluate the performance of both tests for predicting safe alternative regimens for further anesthesia.

METHODS

This observational retrospective study was performed in patients referred to an anesthesia-allergy center, between 2009 and 2011, following immediate hypersensitivity occurring within 5 minutes of NMBA injection.

Demographic and clinical features

The following data were collected: age, sex, administered NMBA. The severity of the reactions was based on clinical features using the *Ring and Messmer* scale.[1,2,14]

Skin testing

Skin tests were carried out at least 4 weeks after the clinical reaction.[1,2,13] The delay between the onset of clinical features and skin testing was compiled. All the drugs, including the culprit NMBA, administered within 5 minutes before the occurrence of the clinical reaction were skin tested through prick tests (PTs) followed by intradermal tests (IDTs) if no reaction was elicited by the PTs.[1,13] Diagnostic criteria for a positive skin test result (including PT and IDT) and maximum recommended concentrations were used according to current French guidelines.[1] A PT result is considered positive if, within 15 to 20 minutes of injecting the drug solution on the forearm, a wheal with a diameter equal to at least half that achieved with the positive control and larger (>3 mm) than that achieved with the negative control appears. IDT result is considered positive if, within 20 minutes of injecting the drug solution, a wheal (usually pruriginous) with a diameter equal to at least the double of the injection bleb appears and is surrounded by a flare.

NMBAs cross-reactivity was evaluated through PTs and IDTs using the commercially available NMBAs: suxamethonium (Aguettant, Lyon, France), rocuronium and vecuronium (MSD, Courbevoie, France), atracurium (Hospira, Meudon-La-Forêt, France), and cisatracurium (GSK, Marly-le-Roi, France).

Blood sampling timing

Circulating tryptase and histamine levels were both quantified at the time of the reaction as recommended.[1-4] Baseline tryptase levels, specific IgE levels, and BAT results were evaluated when the patient was referred for investigation.

Plasma tryptase and histamine

Acute plasma histamine (N < 10 nmol/L) (enzyme immunoassay (EIA), Immunotech, Beckman Coulter, Marseille, France) and total tryptase (ThermoFisher Scientific, Saint-Quentin-en-Yvelines, France) concentrations were measured. An increased acute over baseline tryptase level was defined when greater than [2 + 1.2 × baseline tryptase level], as recently suggested.[15,16] Information on the delay between the onset of the clinical signs and blood sampling was collected.

Serum specific IgE

The concentrations of specific IgE to quaternary ammonium ion (QAI) and suxamethonium were determined (ThermoFisher Scientific).[17] Drug-specific decision thresholds were set up at 0.35 kU/L and 0.11 kU/L for QAI and suxamethonium, respectively, as previously recommended.[9,17] Concentrations below 0.1 kU/L were assigned a value of 0.1.

Basophil activation test

BAT was done blind immediately after blood sampling.[8] We developed a whole blood technique adapted from a commercial test (FlowCAST Bühlmann Laboratories, Schönenbuch, Switzerland) measuring the increased expression of CD63 and CD203c. We used the stimulation buffer (containing IL-3) as a negative control, anti-FcεRI as a positive control, and serial dilutions of 5 NMBAs: suxamethonium (5000-5 μg/mL), rocuronium (500-5 μg/mL), vecuronium (500-5 μg/mL), atracurium (250-2.5 μg/mL), and cisatracurium (100-1 μg/mL). Cells were first stained with anti−CCR3-phycoerythrin (PE) and anti−CD63-fluorescein isothiocyanate (FITC) mAbs (from the FlowCAST kit) and then with anti−CD203c-allophycocyanin (APC) mAb (Miltenyi Biotec, Paris, France). Flow cytometric analysis was performed on a FACSCanto II flow cytometer (BD Immunocytometry Systems, San Jose, Calif) by acquiring at least 500 basophils per sample. Basophils identification relied on the combination of low side scatter and high CCR3 expression. The percentage of CD63-positive basophils was evaluated in response to the drugs, and a stimulation index was calculated (ratio between the percentage of CD63-positive basophils in response to each drug and the negative control). CD203c increase was expressed as the ratio of the median fluorescence intensity (MFI) obtained with the different NMBAs to the MFI obtained with the negative control. Results were considered positive when more than 5% of basophils were CD63-positive with a stimulation index greater than or equal to 2, or if the CD203 MFI ratio was greater than or equal to 2 with at least 1 dilution of the NMBA.[18]