

Original article

Diagnosis of neuromuscular blocking agent hypersensitivity reactions using cytofluorimetric analysis of basophils

Background: Immunoglobulin E (IgE)-mediated hypersensitivity reactions to neuromuscular blocking agents (NMBA) are common and life threatening. Basophil activation based upon the expression of CD63 in the presence of specific allergens was found to be of importance for the diagnosis of IgE-mediated hypersensitivity.

Methods: The Basotest[®] was evaluated for the diagnosis of NMBA in 47 patients with proven NMBA anaphylaxis, 40 atopic subjects nonallergic to NMBA and five healthy volunteers. Diagnosis of NMBA was made according to international standards on clinical history, skin tests and provocation tests when needed.

Results: In the NMBA allergic patients, sensitivity of Basotest[®] was 36.1%, but it increased to 85.7% for reactions which occurred within the last 3 years. The specificity was 93.3%.

Conclusion: Basotest[®] may be useful for the diagnosis of NMBA allergy in patients with a suspicion of recent IgE-mediated hypersensitivity reaction to NMBA.

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Hypersensitivity reactions to drugs are common and may be severe. These include immediate and nonimmediate reactions (1). The diagnosis of drug allergy is notoriously difficult and must be performed by specialized allergists with knowledge of anaesthesiology (1). Adverse reactions are not uncommon during general anaesthesia. Neuromuscular blocking agents (NMBA), or myorelaxants, remain the most common cause of these reactions (61%) (2). Most of the adverse reactions to NMBA are immediate type reactions induced by an immunoglobulin E (IgE)-mediated allergic response (3–6) or a nonspecific histamine release (7, 8). In NMBA allergy, skin tests with defined concentrations of drugs were found to be relevant (9) but they need to be carried out by specialists.

Biological tests would be highly desirable to establish the nature of the culprit agent, especially when the patient is receiving several drugs simultaneously. Except for NMBA, few available biological tools have been found relevant (10). They include serum drug-specific antibodies, histamine release (11), cysteinyl leukotriene release (12, 13) and T-cell-based assays (proliferation,

activation, clones) (14). Most of these tests are not predictive of a drug hypersensitivity reaction induced by haptens. Tests based on basophil activation have also been proposed and studied in selected patients (15). These tests are of interest for NMBA (16–18) but their value in a nonselected population of patients suspected of drug allergy has never been fully established. Many studies have shown that sensitization to drugs decreases with time (19). However, the delay between the reaction and the test is usually not considered in studies assessing the sensitivity of biological tests.

The Basotest[®] (Becton Dickinson, Pont de Claix, France) is a basophil activation test based upon the occurrence of CD63 (20, 21) in the presence of allergens or nonspecific stimuli. In this test, CD63 is measured using cytofluorimetry. We have validated the Basotest[®] in the diagnosis of cypress and grass pollen allergy (22, 23). In the present study, we first studied the predictive value of the Basotest[®] to NMBA according to the time course between the reaction and blood sampling. The two populations were compared with an atopic population (mite and grass pollen) nonallergic to NMBA.

Patients and methods

Patients allergic to NMBA

As patients with NMBA allergy are not very common, we had to use the retrospective analysis of all the files of 180 patients who had a diagnosis of NMBA allergy within the past 15 years at the Department of Anaesthesiology of the University hospital of Montpellier. We were able to find the addresses of 147 patients. Among them, 47 (34 women and 13 men, 25–76 years; mean $\pm$ SD: 53.2 ± 3.7 years) agreed to come to the clinic for a new *in vitro* blood testing. Thirty patients (23 women, 7 men) presented an anaphylactic shock during general anaesthesia, 10 (7 women, 3 men) a bronchospasm and seven (four women, three men) a generalized urticaria. Time elapsed from clinical history of NMBA allergy to skin tests ranged from 3 months to 15 years (mean $\pm$ SD: 1.8 ± 4.0 years), and from clinical history to Basotest[®] from 6 months to 16 years (7.6 ± 4.0 years).

The diagnosis of allergy to NMBA was established from a characteristic clinical history (urticaria, bronchospasm and/or anaphylactic shock a few minutes after the start of anaesthesia) and the positivity of NMBA skin tests as previously described using intradermal skin tests (11). Skin tests were only carried out during the first examination of the patients for diagnosis purposes. They were not repeated when the patients were seen for the second time for the performance of *in vitro* tests to avoid a new NMBA contact. Before the basotest assay, patients have a washed-out period of 6 days of antihistaminic compound. NMBA were used, same compound used for skin test, at the following dilutions: suxamethonium 1/500 (stock Celocurine[®] at 50 mg/ml), pancuronium 1/10 (stock Pavulon[®] at 2 mg/ml), vecuronium 1/10 (stock Norcuron[®] at 4 mg/ml; Organon SA, Fresnes, France), rocuronium 1/10 (stock Esmeron[®] at 10 mg/ml; Organon), mivacurium 1/1000 (stock Mivacron[®] at 2 mg/ml; Laboratoire Glaxo Wellcom, Havly-le-Roi, France), atracurium 1/1000 (stock Tracrium[®] at 10 mg/ml; Laboratoire Glaxo Wellcom). These concentrations have been validated by the French Society of Anaesthesiology (2).

Control subjects without sensitization to NMBA

Forty atopic subjects (19 women and 21 men, 19–63 years; 30.3 ± 12.0 years) with a diagnosis of allergy to mite or grass pollen (positive prick test and serum-specific IgE to at least one allergen), and five healthy nonatopic volunteers (two women and three men, 22–63 years; 39.4 ± 18.0 years) were used as controls and Basotest[®] was carried out at the same time. Fifteen atopic subjects and one healthy volunteer had had a general anaesthesia with NMBA and none of the control subjects had any history of drug allergy. To avoid sensitization none of the control subjects were tested with NMBA.

Basotest[®]

We used the Basotest[®] to allow the quantitative determination of *in vitro* basophil activation according to a previously described method (22). Heparinized blood (100 μ l) was incubated first with a stimulation buffer for 10 min at 37°C, then with 100 μ l allergen solution of uncoupled drug and/or ubiquitous allergen (grass pollen, mite). The chemotactic peptide formyl-methionin-leucine-phenylalanine (FMLP) was used as a positive control and phosphate-buffered saline (PBS) solution served as a negative control. The activation process was stopped by incubating the samples for 10 min at 4°C. The samples were then incubated for 20 min at +4°C with 20 μ l of antibody phycoerythrin (PE)-anti-IgE and fluorescein isothiocyanate (FITC)-anti-CD63. Erythrocytes were subsequently removed by addition of 2 ml of lysing solution (Becton Dickinson).

Cells were washed twice with PBS solution and resuspended in 200 μ l of PBS solution and analysed within 1 h by cytofluorimetry (FASCalibur; Becton Dickinson). The basophil population was gated by the expression of PE-anti-IgE. The expression of CD63 was analysed on this gated cell population. Acquisition was performed on 1000 cells for each sample. Results are given in percentage of basophils expressing the CD63. NMBA may induce nonspecific histamine release and may therefore induce Basotest[®] activation. Because atracurium is the NMBA with the highest nonspecific histamine release activity, we tested 16 NMBA allergic patients (11 of them with a suspicion of atracurium involvement), five atopic controls and five healthy volunteers with the highest concentration of atracurium (10 mg/ml).

Statistical analysis of data

With the exception of demographic characteristics, where means and SD were used, results were expressed in medians and 25th–75th percentiles in percentage of CD63 expression on basophils. Sensitivity and specificity, which are respectively true positive test/true positive test + false negative test, and true negative/true negative + false negative of the Basotest[®], were calculated according to the formula of Galen and Gambino (24).

Results

Basotest[®], basal information

Basophils of all samples were not activated spontaneously, as basal percentage of CD63 expression was always under 15% when they were incubated with PBS. No statistical difference in the basal CD63 expression was observed between basophils of NMBA allergic patients and control subjects. Nonspecific FMLP stimulation induced a CD63 expression comparable between the groups ($37.3 \pm 20\%$, mean $\pm$ SD).

Basotest[®] to NMBA

In the NMBA allergic patients, Basotest[®] was positive in 17 subjects (Fig. 1) corresponding to a sensitivity of

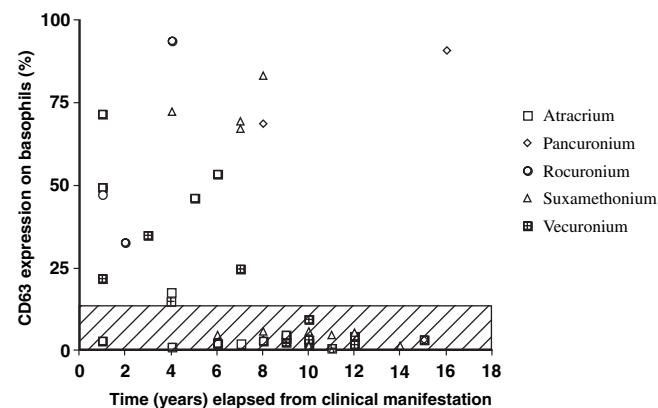


Figure 1. CD63 expression on basophils from patients treated with neuromuscular blocking agents according to time elapsed since clinical manifestation.

Table 1. Sensitivity of Basotest[®] according to time elapsed since clinical manifestation in relation to blood sampling

Time interval*	Sensitivity (%)	Suxamethonium‡	Pancuronium‡	Vecuronium‡	Rocuronium‡	Atracurium‡
0–3	85.7	0/0	0/0	2/2	2/2	2/3
4–8	47.6	4/7	1/4	1/2	1/1	3/7
9–16	5.2	0/7	1/2	0/9	0/0	0/1
0–16	36.1	4/14	2/6	3/13	0/1	5/11

*In years between allergic reaction and the Basotest[®] analysis.

‡Positive patients/total patients.

36.1%. The sensitivity of the Basotest[®] with regard to the clinical history was 43% for anaphylactic shocks, 40% for bronchospasms, and none of the patients with isolated generalized urticaria had a positive Basotest[®]. When the time elapsed between the allergic reaction to NMBA and the Basotest[®] was taken into account, the sensitivity increased to 47.6% for reactions which occurred from 4 to 8 years and up to 85.7% for reactions within the last 3 years (Table 1). At a dilution of NMBA of 1/10 000, no positive CD63 expression was detected on basophils. Maximal positive CD63 expression on basophils with NMBA stimulation was observed at dilutions ranging from 1/10 to 1/1000 (Fig. 2). In two patients, the maximal response was observed with undiluted NMBA compounds. Basotest[®] was positive in four of 14 patients for suxamethonium, two of six for pancuronium, three of 13 for vecuronium, three of three for rocuronium and five of 11 for atracurium (Table 1).

Basotest[®] on control subjects.

In the 40 atopic subjects nonallergic to NMBA, three patients had a positive Basotest[®] (at 1/1 dilution). In the five healthy nonatopic subjects, there was no positive Basotest[®]. The specificity of Basotest[®] in the 45 controls was 93.3%. CD63 expression after atracurium stimulation was negative in the five atopic control patients and

the five healthy volunteers. In the NMBA group, the five patients without suspicion of atracurium sensitization had a low CD63 expression (always under 10%).

Discussion

In the present study, we showed that Basotest[®] is an accurate method for the diagnosis of recent NMBA-induced immediate hypersensitivity. The low sensitivity of the test in the diagnosis of drug allergy is not related to the poor value of the Basotest[®] in the diagnosis of IgE-mediated allergic reactions as: (a) this method is validated for cypress or grass pollen-induced allergy (22, 23), (b) atopic patients involved in our study and sensitized to mite or grass pollens had positive Basotest[®] to these allergens (data not shown), and (c) patients with a recent reaction to NMBA tested positive for Basotest[®].

Previous studies have reported the detection of basophil activation by *in vitro* NMBA challenge using a flow cytometry method (16, 17). Abuaf et al. (16) found a sensitivity of 64% in their study, which is higher than ours. However, it is not possible to compare these two results, as they did not report the time elapsed between basophil activation and the clinical reaction. The direct effect of NMBA (suxamethonium, tubocurarine, vecuronium and atracurium) on the human leukocyte membrane has been previously reported (8, 25). The authors showed that atracurium caused a marked histamine release from mast cells in various concentrations than vecuronium and tubocurarine. However, none of them induced histamine release from human basophils. Therefore, we did not exclude atracurium from challenge. Indeed there was no false-positive result, not only in controls but also in patients allergic to NMBA with or without a positive skin test to atracurium, at any concentration.

The concentration of NMBA merits consideration. A 1/10 000 dilution was not positive for Basotest[®]. Undiluted compounds correspond to suxamethonium 10.5 mL, pancuronium 1.4 mL, atracurium 0.1 mL and vecuronium 3.2 mL. This strength may be considered to be too high as some subjects who were nonallergic to NMBA were positive for Basotest[®], and these concentrations are higher than those usually found in the peripheral blood of patients undergoing general anaesthesia (26).

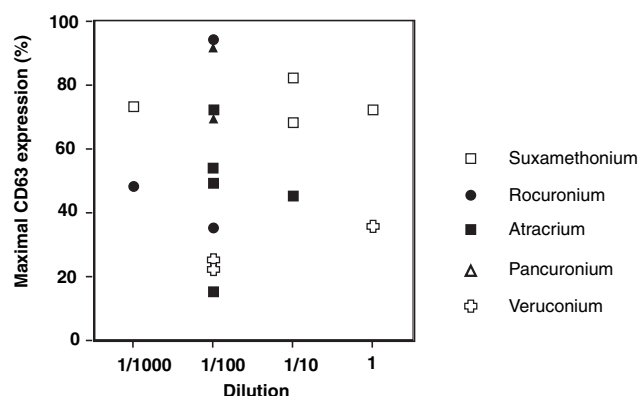


Figure 2. Dilution at which maximal CD63% was obtained for each of the 17 positives.

In the present study, the sensitivity of Basotest[®] to NMBA was found low when all allergic patients were analysed (36.1%), but it was dramatically improved when the time elapsed from the allergic reaction to NMBA was taken into account. The sensitivity calculated for the Basotest[®] performed in the last 3 years was 85.7%. These data raise several important hypotheses. On the one hand, some patients may have lost their NMBA sensitivity. Retrospective studies on penicillin allergy have shown that the longer the time interval between the initial reaction and the skin test, the less likely a positive response is obtained. However, it is impossible to confirm this hypothesis as a challenge with the culprit NMBA would be required and this cannot be performed to avoid systemic reactions and/or new sensitizations. On the other hand, it is possible that the sensitivity of the patients had decreased sufficiently to make basophil activation impos-

ible. We therefore advise diagnosing NMBA allergy rapidly after the reaction, and the Basotest[®] is of interest in this regard. The results of this study are in agreement with a previous study where it was reported the interest of basotest, with CD63 measurement, in the analysis of peri-anaesthetic anaphylaxis (27). This test could be useful in the detection of sensitivity to NMBA in patients presenting an hypersensitivity reaction during anaesthesia procedure as recommended by the ENDA group (28).

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