ORIGINAL ARTICLE

Assessment of the added value of basophil activation test in immediate drug allergy diagnosis

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Summary

Background. The diagnosis of immediate drug allergy (DA) relies on a combination of skin tests (ST), drug provocation tests (DPT), specific IgE levels (sIgE) and/or basophil activation tests (BAT). We aimed to compare BAT results with those of other allergy tests in patients with suspected immediate DA to a heterogeneous group of drugs, aiming to assess its diagnostic value. **Methods.** Patients who underwent BAT for suspected immediate DA at our hospital from January 2018 to December 2023 were included. Each case (suspected drug) was classified based on diagnostic tests performed – probable vs. improbable allergy (assessed by ST and/or sIgE only) or confirmed vs. excluded allergy (assessed by DPT). Inter-

method agreement was assessed with Cohen's kappa index (κ). **Results.** Eighty-five patients were included: 51 female (60.0%), median age 53.0 years [interquartile range (IQR) = 33.0, Q1-Q3=31.0-64.0]. Median time elapsed since index reaction was 1.0 year [IQR=3.0, Q1-Q3 = 1.0-3.0]. We identified 112 suspected drugs: out of 16 cases with positive BAT (14.3%), 6 were probable (37.5%) and 1 confirmed allergy (6.3%). From 89 drugs with negative BAT (79.5%), 41 were improbable (46.1%) and 5 excluded allergy (5.6%). Seven agents had an inconclusive BAT (6.3%). A slight agreement (κ = 0.201) between BAT and other studies was observed when combining probable/improbable and confirmed/excluded results (n = 76). When limiting these findings to confirmed/excluded results (n=6), we found a perfect agreement (κ = 1). **Conclusions.** We assessed BAT performance in a larger sample than those from previous studies. Slight agreement between methods increased to a perfect agreement when limiting to confirmed cases. Larger studies are needed to establish BAT's diagnostic value.

Key words

Allergy diagnosis; basophil; basophil activation test; drug allergy; flow cytometry.

IMPACT STATEMENT

This study evaluated BAT's diagnostic accuracy in a larger sample than previous studies, reinforcing its value in the diagnosis of immediate drug allergies, especially in high-risk patients.

Introduction

Drug hypersensitivity reactions (DHRs), accounting for about 15.0% of all adverse drug reactions, result from distinct immunologic and nonimmunologic mechanisms (1). Among immunologic DHRs, we encounter the term "drug allergy" (DA), based on the hapten concept, more frequently mediated by drug-specific IgE (sIgE) antibodies in immediate reactions (type I hypersensitivity), or drug-specific T lymphocytes in non-immediate reactions (type IV hypersensitivity) (1). Also framed into immunologic DHRs is isolated drug-induced immune system activation, which may occur through direct interaction with immune receptors (p-i concept) or receptors/enzymes of inflammatory cells (pseudo-allergy) (1). Recently, an European work group proposed a new nomenclature system for hypersensitivity reactions, particularly detailed regarding each DHR underlying mechanism (2).

Focusing on IgE-mediated reactions, these are initiated by a usually asymptomatic sensitization phase, when there is an interaction between a drug/drug hapten covalently bound to autologous proteins (hapten-carrier conjugate) and the immune system, resulting in sIgE production, which binds to tissue resident mast cells and circulating basophils (3). Subsequent exposure and cross-linking of sIgE leads to mast cell and basophil activation/degranulation, with release of inflammatory mediators and development of the typical clinical manifestations (3).

The proper diagnosis of these reactions poses a significant challenge and is generally guided by the anamnesis and the suspected underlying immune mechanisms, through a combination of *in vivo* and *in vitro* tests (4). Skin prick tests (SPT) and intradermal tests (IDT) are the most widely used methods to determine sensitization, as other *in vivo/in vitro* tests are less specific, less sensitive, or potentially harmful. However, their diagnostic value varies among the various drugs/drug classes, and maximum non-irritant drug concentrations are unknown or poorly validated for most drugs (4,5).

Drug provocation tests (DPT) are the gold standard for diagnosis confirmation. However, conducting a full-dose DPT can be challenging for certain agents due to their pharmacological effects. Moreover, DPT are time-consuming and not risk-free, particularly in patients who experienced a life-threatening reaction or in case of significant comorbidities, in whom they might even be contraindicated (4,6).

In challenging situations like these, opting for *in vitro* tests like serum sIgE levels may provide a safer alternative and contribute to physician decision-making. Unfortunately, only few sIgE assays for drugs are available and sufficiently validated to use in routine diagnostics and, depending on the drug involved, they generally have a low sensitivity and poor correlation with skin tests results (6, 7).

The basophil activation test (BAT) is another *in vitro* test which attempts to measure patients' functional response to allergen exposure. CD63 is a membrane protein located in the same lysosome-related secretory granules that contain histamine, and their translocation to the cell membrane during basophil activation and consequent degranulation can be measured through flow cytometry (8). As such, by incubating fresh patient's whole blood with the suspected drug and subsequently measuring the percentage of basophils that express activation marker CD63 at a given drug concentration, one is able to closely mirror the *in vivo* response (3, 8). Other activation markers have been proposed, such as CD203c, which has shown to be reliable; however, BAT with CD63 is currently the best clinically validated test (8). Despite this, BAT's sensitivity in drug allergy diagnosis is about 50.0% (specificity of up to 93.0%) and protocols are still not

fully standardized in terms of ideal timing, factors influencing activation, drug manipulation and concentrations and optimal cut-off values (3, 8). Given the difficulties in DA diagnosis, BAT has been proposed as a complementary test, although its place in the diagnostic algorithm of drug immediate reactions' investigation is not uniformly accepted and sometimes controversial (3, 8, 9).

The present study aimed to compare BAT results with those of other allergy tests in patients with suspected immediate DA to a heterogeneous group of drugs, aiming to assess BAT's diagnostic value.

Material and Methods

Study design and population

Patients who underwent BAT for suspected immediate DA to a heterogeneous group of drugs at our tertiary hospital from January 2018 to December 2023 were included in this cross-sectional study. Demographic and clinical data were collected from medical records. For each suspected drug (defined as a case), we analyzed results from allergy tests performed in addition to BAT. Cases were then classified as "probable allergy" if the patients had positive skin tests and/or sIgE levels ≥ 0.35 kU/L, or as "confirmed allergy" if the patient had a positive DPT. Conversely, cases were classified as "improbable allergy" if skin tests were negative and/or in case of below cut-off sIgE levels, or as "excluded allergy" following a negative DPT. The study protocol was reviewed and approved by our hospital's ethics committee [reference number: 2024.043(039-DEFI/039-CE)]. All procedures were conducted in accordance with the principles of the Declaration of Helsinki.

Data collection

Data collected included demographics, details regarding the index reaction - particularly the suspected drug, time of onset and clinical manifestations -, time elapsed until BAT, results of the allergy study for each drug and concurrent conditions or medications that could affect test outcomes. Allergic reactions were categorized based on organ system involvement (single vs. multi-organ). Anaphylaxis cases were identified using the World Allergy Organization (WAO) diagnostic criteria (10).

Additionally, information regarding BAT results, percentage of CD63+ basophils and stimulation index (SI, defined as proportion of activated basophils following allergenic stimulation compared to non-stimulated

basophils) was also gathered, along with absolute and relative count of serum basophils and acute/baseline tryptase levels.

Allergy study

The allergy evaluation in our department was conducted in accordance with the European Academy of Allergy and Clinical Immunology (EAACI) and European Network on Drug Allergy (ENDA) guidelines (5-7).

Depending on the nature of the index reaction, pharmacological class, drug-specific validated allergy study and patient's comorbidities, diagnostic work-up involved SPT and IDT, sIgE levels and/or DPTs. To further support the diagnosis of anaphylaxis cases, the established consensus equation for tryptase ratios was used (11).

Skin tests were performed following the recommendations of EAACI/ENDA regarding methodology and drug concentrations (5). Histamine at 10 mg/mL was used as a positive control, and 0.9% saline as a negative control. The tests were conducted using commercially available intravenous drug formulations, and for beta-lactams, we employed the commercial kit DAP® (Diater Allergy Products). A positive intradermal test result was defined as a ≥3 mm increase in the initial wheal diameter with concomitant flare reaction at 20 minutes. sIgE levels were quantified using the ImmunoCAP® system (Thermo Fisher Scientific), with a threshold of ≥0.35 kU/L defined as a positive result. DTPs protocols also followed EAACI/ENDA recommendations (7) and were deemed positive when signs and/or symptoms developed during the procedure.

All patients underwent BAT with the respective suspected drug(s) using the BÜHLMANN Flow CAST® kit (Bühlmann Laboratories). Tests were performed with commercially available intravenous drug formulations, assessed both as undiluted solutions and in serial dilutions (1:40–1:160), strictly following the manufacturer's protocols. Results were considered positive when the two cut-offs used in our center were met: percentage of absolute CD63+ basophils ≥ 5 and SI ≥ 2.

Statistical analysis

The data analysis was carried out using IBM® SPSS® Statistics, which encompassed descriptive analysis for qualitative and quantitative variables. We employed Cohen's kappa index (κ) to assess inter-method

agreement to compare performance of BAT with other diagnostic approaches combined. Cohen's kappa index was calculated as $\kappa = (pa-pe)/(1-pe)$, where pa = proportions of observation in agreement and pe = proportions of agreement due to chance, and was interpreted according to Landis & Koch scale (12). Statistical analyses were performed using one-way ANOVA to compare the means of numeric variables and chi-squared test was used to evaluate associations between categorical variables. Additionally, logistic regression was used to evaluate the influence of the time interval between the index reaction and BAT performance on both BAT positivity and concordance across studies. Statistical significance was set at a p-value threshold of 0.05.

Results

Eighty-five patients were included in the study: 34 male (40.0%) and 51 female (60.0%), with a median age of 53.0 years [interquartile range (IQR)=33.0, Q1-Q3=31.0-64.0] (as shown in Table I). Nine of these patients were under 18 years-old (10.6%).

Anamnesis revealed reactions occurring within one hour following drug exposure in 63 patients (74.1%) and between one and six hours in 22 (25.9%). Signs and symptoms compatible with anaphylaxis were presented by 44 patients (51.8%). Among non-anaphylaxis patients (n=41, 48.2%), the majority had developed isolated cutaneous symptoms (n=33, 80.5%); the remaining isolated respiratory (n=5, 12.2%) and gastrointestinal symptoms (n=3, 7.3%). The median time elapsed since the index reaction to BAT execution was 1.0 years [IQR=3.0, Q1-Q3=1.0-3.0] (as summarized in Table I).

A total of 112 suspected drugs were identified (a mean of 1.3 per patient), categorized as follows: 41 antibiotics (36.6%), including 32 beta-lactams and 9 from different pharmacological classes; 17 nonsteroidal anti-inflammatory drugs (NSAIDs) (15.2%); 4 iodinated contrast media (ICM) (3.5%); 3 proton pump inhibitors (2.7%); and 44 perioperative drugs (39.3%) (12 neuromuscular blockers, 3 neuromuscular blocker reversal agents, 14 general anesthetics, 2 local anesthetics, 2 antiemetics, 7 opioids, 3 antiseptics/latex, and 1 dye). Additionally, 3 supplements (2.7%) were also investigated (as presented in Supplementary Tables SI-SVI).

As previously stated, all 85 patients underwent BAT with the suspected drug(s). Among the 44 patients with anaphylaxis-compatible symptoms, only 11 (25.0%) had both acute and baseline tryptase measurements

available. Of these, 10 (90.9%) showed an elevation of acute tryptase levels. In the anaphylaxis group, BAT was positive in 7 cases (15.9%), compared to 9 positive BAT results (22.0%) among the 41 non-anaphylaxis patients. No statistical difference was found between these results (p=0.664).

The outcomes of the allergy investigation performed for each drug are detailed in the following sections, as well as in Supplementary Tables SI-SVI, and summarized in Figure 1.

Antibiotics

Out of 32 cases of suspected beta-lactam allergy, 13 (40.6%) were found to have a positive allergy study (12 classified as probable allergy and 1 as confirmed allergy). Among these, BAT results were as follows: 4 positive, 7 negative and 2 inconclusive. Conversely, 17 cases (53.1%) had a negative allergy evaluation (13 classified as improbable allergy and 4 as excluded allergy). Of these, 13 had a concordant negative BAT result, while 4 had a non-concordant positive result. Two cases (6.3%) were not submitted to additional study, despite a negative BAT result.

Allergy to other pharmacological classes was suspected in 9 cases. Of those, 2 (22.2%) had a positive allergy study (probable allergy), with associated negative and inconclusive BAT results. In 3 cases (33.3%) we documented a negative allergy evaluation (improbable allergy), all with concordant negative BAT results. The 4 remaining cases (44.4%) presented with a negative BAT result but were not submitted to further investigation.

The complete diagnostic workup performed in addition to BAT is presented in Supplementary Table SI.

Nonsteroidal anti-inflammatory drugs

NSAIDs allergy was suspected in 17 cases. Of those, 1 (5.9%) had a positive allergy study (confirmed allergy) with a positive BAT result, and 2 (11.8%) had a negative allergy study (improbable allergy) with negative BAT results. The remaining 14 cases (82.4%) were not further investigated (characterized in Supplementary Table SII).

Contrasts

Four patients reported reactions following the administration of an ICM: 1 (25.0%) with a positive allergy study (probable allergy) and negative BAT result, 2 (50.0%) with a negative allergy study (improbable

allergy), also with negative BAT results, and 1 (25.0%) without additional study (as shown in Supplementary Table SIII).

Perioperative agents

In the study population, 23 patients presented with suspected perioperative reactions with a total of 44 suspected drugs: a positive allergy evaluation was observed in 7 cases (15.9%) (probable allergy). Among these, BAT results were positive in only 1 case. Conversely, a negative allergy study was documented in 28 cases (63.6%) (27 improbable allergy, 1 excluded allergy), 26 with a concordant negative BAT result and 2 with a non-concordant positive result. Further allergy investigation was not conducted for the remaining 9 cases (20.5%), which included 4 negative, 2 positive, and 3 inconclusive BAT results.

The additional allergy workup performed alongside BAT is detailed in Supplementary Table SIV.

Proton pump inhibitors

Of 3 cases with suspected proton pump inhibitor allergy, 2 (66.6%) had a positive allergy evaluation (probable allergy) and negative BAT results. One case (33.3%) had a positive BAT result, with no additional evaluation (as shown in Supplementary Table SV).

Supplements

The suspected folic acid allergy case had a positive allergy evaluation (probable allergy) and concordant positive BAT result. In respect to cyanocobalamin, we observed a negative allergy study (improbable allergy), but we found BAT to be inconclusive. No additional study was performed in the case of suspected iron allergy (ferric carboxymaltose), but a negative BAT was documented (see Supplementary Table SVI).

Concordance assessment

* Categorization assessed by additional allergy study

Of a total of 16 cases with a positive BAT result (14.3%), a concordant positive allergy study was observed in 7 (43.8%) and a negative allergy study was observed in 6 (37.5%); 3 cases (18.7%) received no additional evaluation. Regarding the 89 cases with a negative BAT result (79.5%), 46 had a concordant negative allergy study (51.7%) and in 17 a positive allergy evaluation was recorded (19.1%); 26 agents (29.2%) received no additional evaluation. In respect to cases in which BAT results were inconclusive (n=7, 6.3%),

3 had a positive allergy study (42.9%), 1 a negative (14.3%) and 3 (42.8%) received no further evaluation (summarized in Table II).

A slight agreement (κ=0.201) between BAT and other allergy studies was observed when combining probable/improbable and confirmed/excluded allergy results without an inconclusive BAT outcome (n=76); in this group concordance was higher for negative results (73.0%) compared to positive ones (53.8%). When limiting these findings to confirmed results (n=6), we found a perfect agreement (κ=1) (as shown in Table III); this subgroup consisted of 5 excluded cases with a negative BAT (83.3%) and 1 confirmed case with a positive BAT (16.7%).

* Clinical presentation

When combining probable/improbable and confirmed/excluded results without an inconclusive BAT outcome (n=76), a slight agreement (κ=0.076) was found in anaphylaxis patients (n=45), and a fair agreement (κ=0.292) was observed in non-anaphylaxis cases (n=31) (Table III).

* Pharmacological class

In a sub-analysis by pharmacological class, the κ values indicated slight agreement for antibiotics (κ=0.143, n=32) and perioperative drugs (κ=0.091, n=35). In contrast, NSAIDs showed perfect agreement (κ=1, n=3) (summarized in Table III). It was not possible to calculate κ for cases involving ICM, proton pump inhibitors, or supplements due to sample size.

* Timing

A logistic regression analysis was conducted to assess the effect of the time elapsed since the index reaction on BAT positivity and its concordance with other allergy tests. Regarding BAT positivity, the time interval (in years) had no significant impact (Odds-ratio (OR)=1.03; 95% confidence interval (CI)=0.92–1.15; p=0.596). Similarly, for concordance, the time interval also did not show a significant effect (OR=1.06; 95% CI=0.92–1.22; p=0.431). In both analyses, the time elapsed since the adverse reaction did not significantly influence BAT results or its agreement with other tests.

Laboratory Findings

Of the 85 patients included in the study, information on the relative and absolute counts of serum basophils was available for 70 patients (82.4%), with a mean of 0.8% and 56.87/mm^3, respectively. Among these, a

sub-analysis of patients with positive BAT result (n=13) revealed a mean of 0.7% and 40.93/mm^3 serum basophils and, in those with a negative result (n=53), a mean of 0.8% and 61.67/mm^3 was observed. Patients in whom an inconclusive result was observed (n=4), a mean of 0.8% and 45.16/mm^3 serum basophils was detected. No statistical difference was found between the absolute counts of serum basophils (p=0.390).

In patients in whom acute phase and baseline tryptase levels were available (n=16), a median increase of 10.73µg/L was observed (max-min: -2.36-49.72).

The mean percentage of CD63+ basophils activation was 4.54 and the SI was 2.78, when considering the highest drug concentration for negative BAT results and the lowest drug concentration for positive BAT results. When restricting to positive BAT results, these values were 11.5% and 9.67, respectively, and in negative BAT results values of 1.9% and 1.65 were observed. Statistical difference was found between the different percentages of CD63+ basophils (p=0.005) and stimulation indexes (p=0.0003).

The inconclusive BAT results stemmed from a combination of factors, including a low basophil count, diminished CD63 expression, heightened basophil basal stimulation, and a lack of response to a strong positive control.

Discussion and Conclusions

In our study sample, a substantial number of cases was associated with the pharmacological categories most frequently implicated in suspected drug allergic reactions, namely beta-lactam antibiotics, and NSAIDs (13,14).

Concordance evaluation of all cases revealed a slight agreement beyond chance between BAT results and the results of the combinations of other allergy tests, when combining probable/improbable and confirmed/excluded results (κ=0.201). The fact that there was a high percentage of cases where allergy was not definitely confirmed or excluded may have significantly affected the level of concordance, since when limiting these findings to confirmed results (n=6), we found a perfect agreement (κ=1). While this value is noteworthy, it should be interpreted with caution given the very small sample size, remaining exploratory and requiring validation in larger cohorts with higher rates of DPT before firm conclusions can be drawn.

The results indicated a higher concordance rate for negative outcomes (73.0%) compared to positive ones (53.8%). In the positive results group, beta-lactam antibiotics were the predominant drug class (57.1%), while in the negative group, perioperative drugs (56.5%) and beta-lactam antibiotics (28.3%) were the most common. This suggests that BAT may be more useful in confirming beta-lactam allergy and in ruling out allergy to perioperative drugs. Further research is needed to validate these observations.

When sub-analyzing the findings by pharmacological category, we observed a similar level of agreement for antibiotics and perioperative drugs (κ=0.143 and κ=0.091, respectively). In contrast, NSAIDs demonstrated a perfect agreement (κ=1). Two recent studies which focused on penicillin found similar results (κ=0.2-0.4) (15,16), as well as an older study which included several pharmacological classes (κ=0.35) (17). The perfect agreement observed with NSAIDs may be explained by the fact that the ones included in this analysis, namely metamizole, are more commonly associated with IgE-mediated reactions within their class, rather than acting through the cyclooxygenase-1 inhibition mechanism (14); note that these drugs were not included in the sub-analysis because a complementary allergy evaluation was not conducted and thus analysis was not possible. Considering this limitation, along with the small sample size, the observed level of concordance may be overestimated, and additional studies with larger cohorts are needed to confirm this finding.

Contrary to the authors' expectations, concordance was higher in the group with non-anaphylaxis cases (κ=0.292 vs. κ=0.076), suggesting that BAT may have greater utility in evaluating milder reactions compared to severe ones. This observation has yet to be confirmed by further and more robust studies.

There are several limitations that can influence this study's results, which should be considered in its interpretation. BAT has limited sensitivity, in which several factors seem to play a role: firstly, the aforementioned test protocols, which are not standardized and vary among different institutions. These include not only the test's methodology *per se*, but also drug formulations/concentrations and designated cut-offs for positivity (3, 18). Its sensitivity is higher when evaluating classical IgE-mediated DHRs, decreasing in case of non-IgE mediated DHRs, such as in most cases of hypersensitivity to NSAIDs and quinolones (18). Also, a negative BAT does not fully exclude patient's sensitization to that drug's metabolite (18).

The time interval from index reaction to allergy study is also relevant, as a negative result following a reduced or prolonged time interval may reflect, respectively, basophils' anergy and sIgE progressive decline

over time. As such, BAT should be performed ideally 3-4 weeks after reaction occurrence (3, 18). In our sample, we observed a median time interval of 1 year [IQR=3.0, Q1-Q3=1.0-3.0], reflecting the real world of clinical practice, in which patients are often referred for allergy evaluation long after the initial event but report a history of severe immediate reactions. According to our analysis, the time interval did not significantly affect BAT positivity in our sample (p=0.596), contrary to expectations of decreased BAT positivity over time due to sIgE decline. Additionally, the time interval had no significant impact on the concordance between tests (p=0.431), which aligns with the expectation that a longer interval would reduce the sensitivity of all tests equally over time.

While this finding diverges from what has been described in prior studies (3,18), it may reflect characteristics specific to our patient population, such as the severity of index reactions or individual differences in immune response longevity. The small sample size and heterogenous time intervals may also influence the results. Therefore, these findings should be interpreted with caution and further studies with larger and more homogeneous cohorts, as well as prospective studies with standardized timing of BAT, are required to better understand the relationship between time interval and test sensitivity.

Lastly, patient-related factors can also decrease test sensitivity, such as chronic medication with systemic immunosuppressants (3). This was not a factor in our study sample, since only patients not chronically immunosuppressed were selected to perform BAT by their assistant physician.

Other limitations of our investigation concern a relatively small sample, though larger than the ones used on previous studies (14-16), the reliance on existing data, which may be incomplete or biased, and lack of control over study variables. Additionally, as previously stated, the low number of DPT may have affected our findings, since that is a crucial step for confirming or excluding hypersensitivity reactions. Its limited use hindered a more precise evaluation of BAT performance in comparison with other allergy tests, further constraining the robustness of our findings.

Despite these results and limitations, the authors consider that BAT may constitute an important diagnostic tool in cases where safe and clinically valid alternative diagnostic methods are unavailable, and in the investigation of patients who suffered life-threatening reactions, such as anaphylaxis, or with significant comorbidities, in whom performing *in vivo* tests constitutes a substantial risk (3). It may also play a significant part as a complementary test in case of suggestive anamnesis and discordant allergy evaluation.

Further studies with larger samples and thorough investigation protocols will help consolidate BAT's role in the diagnostic algorithm of immediate DA investigation.

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Contributions

ARP - Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Writing – review & editing; **IMC** - Conceptualization, Writing – review & editing; **SD** - Conceptualization, Writing – review & editing; **CIM** - Conceptualization, Investigation, Writing – review & editing; **EN** - Conceptualization, Writing – review & editing; **HF** - Conceptualization, Writing – review & editing; **ERG** - Conceptualization, Investigation, Writing – review & editing; **FC** - Conceptualization, Methodology, Investigation, Writing – review & editing.

Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

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Figure 1. Overview of basophil activation test results (BAT) and subsequent allergy study outcomes

BAT results in 85 patients across 112 cases, divided into positive BAT, inconclusive BAT, and negative BAT. Subsequent allergy studies further classified cases into positive, negative, or no additional study. The specific drugs involved in each category are detailed below the corresponding classifications.

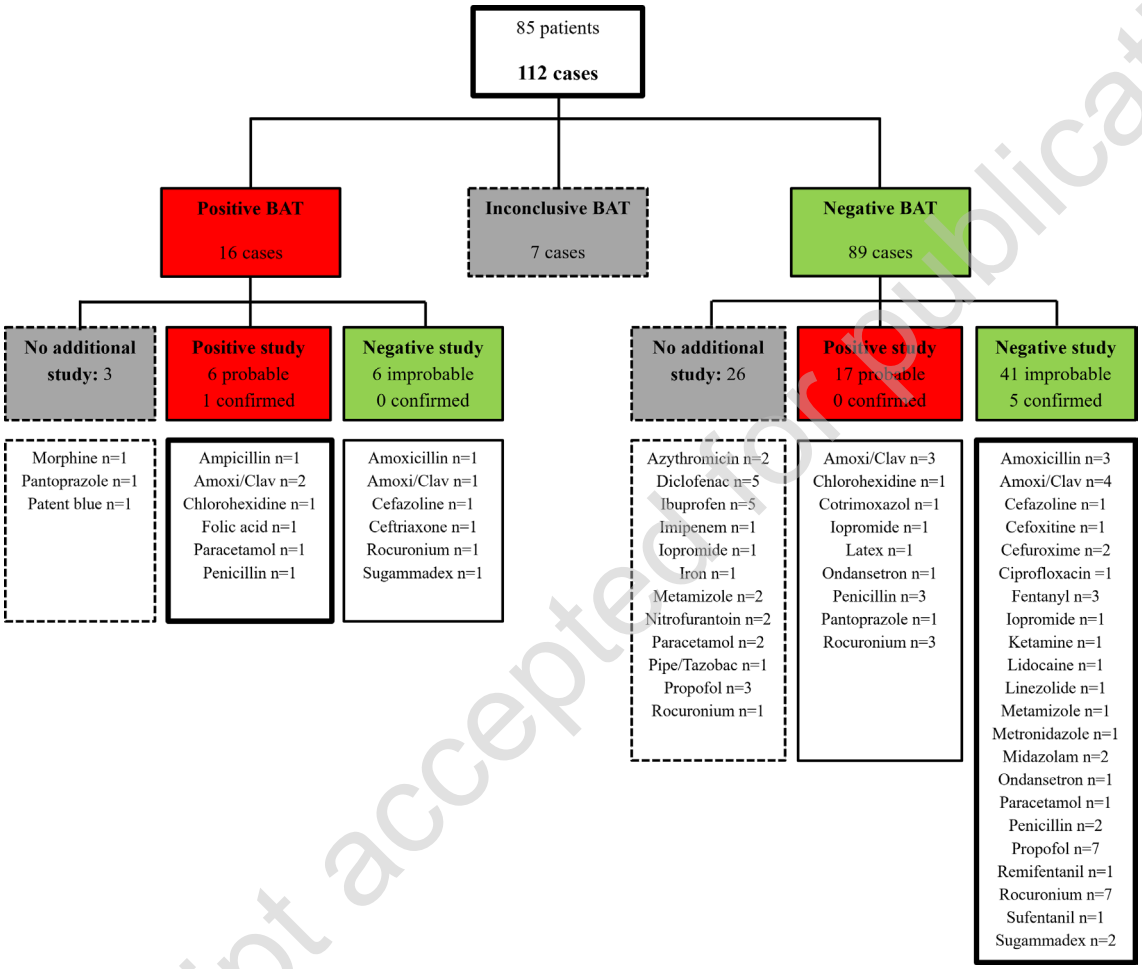

Table I. Demographic and clinical characteristics of the study population

Demographics	
Age (years)	Median: 53.0 IQR: 33.0, Q1-Q3: 31.0-64.0
Gender, n (%)	Male: 34 (40.0%) Female: 51 (60.0%)
Characteristics of suspected drug reactions	
Suspected drugs, n (mean per patient) ~~(n)~~	112 (1.3)
Time of onset, n (%)	≤1 hour: 63 (74.1%) 1–6 hours: 22 (25.9%)
Clinical presentation, n (%)	Anaphylaxis: 44 (51.8%) Non-anaphylaxis: 41 (48.2%)
	Non-anaphylaxis presentations: Cutaneous symptoms: 33 (80.5%) Respiratory symptoms: 5 (12.2%) Gastrointestinal symptoms: 3 (7.3%)
Time elapsed until BAT (years)	Median: 1.0 IQR: 3.0, Q1-Q3:1.0-3.0

BAT – basophil activation test, IQR – interquartile range.

Table II. Summary of basophil activation test results and concordance with additional allergy study findings

BAT result	Cases (n,%)	Positive study (n,%)		Negative study (n,%)		No additional study (n,%)
		Probable allergy [α]	Confirmed allergy [β]	Improbable allergy [δ]	Excluded allergy [μ]	
Positive	16 (14.3%)	6 (37.5%)	1 (6.3%)	6 (37.5%)	-	3 (18.7%)
Negative	89 (79.5%)	17 (19.1%)	-	41 (46.1%)	5 (5.6%)	26 (29.2%)
Inconclusive	7 (6.3%)	2 (28.6%)	1 (14.3%)	1 (14.3%)	-	3 (42.8%)

[α] Cases with positive skin tests and/or specific IgE levels ≥ 0.35 kU/L; [β] Cases with a positive drug provocation test; [δ] Cases with negative skin tests and/ or specific IgE levels < 0.35 kU/L; [μ] Cases with a negative drug provocation test.

Table III. Concordance analysis between basophil activation test results and additional allergy study findings

Concordance analysis			
	n	Cohen's κ	Interpretation [α]
Categorization			
Probable/Improbable + Confirmed/Excluded cases	76	0.201	Slight agreement
Confirmed/Excluded cases	6	1.000	Perfect agreement
Clinical presentation			
Anaphylaxis	45	0.076	Slight agreement
Non-anaphylaxis	31	0.292	Fair agreement
Pharmacological class			
Antibiotics	32	0.143	Slight agreement
Perioperative drugs	35	0.091	Slight agreement
Nonsteroidal anti-inflammatory drugs	3	1.000	Perfect agreement

[α] Cohen's kappa was interpreted according to Landis & Koch scale.

Supplementary Tables

Table SI. Results of the allergy study performed in cases of suspected immediate drug allergy to antibiotics.

Drug	No study	Probable allergy	Confirmed allergy	Improbable allergy	Excluded allergy	BAT	Total
Beta-lactams							32
Penicillins							24
Penicillin		Yes (sIgE)				Positive	1
Penicillin					Yes	Negative	1
Penicillin		Yes (sIgE)				Negative	2
Penicillin		Yes (ST)				Negative	1
Penicillin				Yes (sIgE, ST)		Negative	1
Ampicillin		Yes (ST)				Positive	1
Amoxicillin				Yes (ST)		Positive	1
Amoxicillin					Yes	Negative	1
Amoxicillin				Yes (sIgE)		Negative	2
Amoxi/Clav				Yes (sIgE)		Positive	1
Amoxi/Clav		Yes (sIgE)				Positive	2
Amoxi/Clav			Yes			Inconclusive	1
Amoxi/Clav		Yes (sIgE, ST)				Negative	1
Amoxi/Clav				Yes (sIgE)		Negative	4
Amoxi/Clav		Yes (ST)				Negative	1
Amoxi/Clav		Yes (sIgE)				Negative	1
Flucloxacillin		Yes (sIgE)				Inconclusive	1
Pipe/Tazo	Yes					Negative	1
Cephalosporins							7
Cefazoline				Yes (sIgE)		Positive	1
Cefazoline					Yes	Negative	1
Cefoxitine				Yes (sIgE, ST)		Negative	1
Ceftriaxone		Yes (ST)				Negative	1
Ceftriaxone				Yes (sIgE, ST)		Positive	1
Cefuroxime					Yes	Negative	1
Cefuroxime				Yes (sIgE)		Negative	1
Carbapenems							1
Imipenem	Yes					Negative	1
Other antibiotics							9
Azithromycin	Yes					Negative	2

Azithromycin		Yes (ST)			Inconclusive	1
Ciprofloxacin			Yes (ST)		Negative	1
Linezolide			Yes (ST)		Negative	1
Metronidazole			Yes (ST)		Negative	1
Nitrofurantoin	Yes				Negative	2
Cotrimoxazol		Yes (ST)			Negative	1

Amoxi/Clav – amoxicillin + clavulanate, BAT – basophil activation test, Pipe/Tazo - piperacillin + tazobactam, sIgE – specific drug IgE, ST – skin tests.

Table SII. Results of the allergy study performed in cases of suspected immediate drug allergy to nonsteroidal anti-inflammatory drugs.

Drug	No study	Probable allergy	Confirmed allergy	Improbable allergy	Excluded allergy	BAT	Total
Diclofenac	Yes					Negative	5
Ibuprofen	Yes					Negative	5
Metamizole	Yes					Negative	2
Metamizole				Yes (ST)		Negative	1
Paracetamol			Yes			Positive	1
Paracetamol	Yes					Negative	2
Paracetamol				Yes (ST)		Negative	1

BAT – basophil activation test, ST – skin tests.

Table SIII. Results of the allergy study performed in cases of suspected immediate drug allergy to iodinated contrasts.

Drug	No study	Probable allergy	Confirmed allergy	Improbable allergy	Excluded allergy	BAT	Total
Iopromide				Yes (ST)		Negative	2
Iopromide	Yes					Negative	1
Iopromide		Yes (ST)				Negative	1

BAT – basophil activation test, ST – skin tests.

Table SIV. Results of the allergy study performed in cases of suspected immediate drug allergy to perioperative agents.

Drug	No study	Probable allergy	Confirmed allergy	Improbable allergy	Excluded allergy	BAT	Total
Neuromuscular blockers							12
Rocuronium				Yes (sIgE)		Positive	1
Rocuronium		Yes (ST)				Negative	3
Rocuronium				Yes (ST)		Negative	7
Rocuronium	Yes					Negative	1
Neuromuscular blocker reversal agents							3
Sugammadex				Yes (ST)		Negative	2
Sugammadex				Yes (ST)		Positive	1
General anesthetics							14
Ketamine				Yes (ST)		Negative	1
Midazolam				Yes (ST)		Negative	2
Midazolam	Yes					Inconclusive	1
Propofol					Yes	Negative	1
Propofol				Yes (ST)		Negative	6
Propofol	Yes					Negative	3
Local anesthetics							2
Bupivacaine	Yes					Inconclusive	1
Lidocaine				Yes (ST)		Negative	1
Antiemetics							2
Ondansetron		Yes (ST)				Negative	1
Ondansetron				Yes (ST)		Negative	1
Opioids							7
Fentanyl	Yes					Inconclusive	1
Fentanyl				Yes (ST)		Negative	3
Morphine	Yes					Positive	1
Remifentanil				Yes (ST)		Negative	1
Sufentanil				Yes (ST)		Negative	1
Antiseptics/latex/dyes							4
Clorohexidine		Yes (sIgE, ST)				Positive	1
Clorohexidine		Yes (sIgE)				Negative	1
Latex		Yes (sIgE)				Negative	1
Patent blue dye	Yes					Positive	1

BAT – basophil activation test, ST – skin tests.

Table SV. Results of the allergy study performed in cases of suspected immediate drug allergy to proton pump inhibitors.

Drug	No study	Probable allergy	Confirmed allergy	Improbable allergy	Excuded allergy	BAT	Total
Pantoprazole		Yes (ST)				Negative	2
Pantoprazole	Yes					Positive	1

BAT – basophil activation test, ST – skin tests.

Table SVI. Results of the allergy study performed in cases of suspected immediate drug allergy to vitamins and minerals.

Drug	No study	Probable allergy	Confirmed allergy	Improbable allergy	Excluded allergy	BAT	Total
Cyanocobalamin				Yes (ST)		Inconclusive	1
Folic acid		Yes (ST)				Positive	1
Ferric carboxymaltose	Yes					Negative	1

BAT – basophil activation test, ST – skin tests.