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ORIGINAL INVESTIGATION

The basophil activation test in the diagnosis and management of adverse drug reactions in the elderly

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Background and aims. The use of multi-therapeutic regimes in the elderly predisposes to frequent adverse drug reactions. The objective of the present study was to evaluate the predictive value of the basophil activation test to prevent the risk of hypersensitivity reactions in the case of potentially dangerous drugs in the elderly.

Method. This study has been conducted in the Immuno-Allergy Unit of the Policlinico Hospital, in Bari. Patients over 65 years with hypersensitivity reactions were considered. The basophil activation Flow Cast test, performed following the manufacturer's instructions, measured the degranulation of basophils, using the anti-CD63 and anti-CD203c monoclonal antibodies.

Results. 61 patients, suffering from urticaria-angioedema or anaphylaxis due to Beta-Lactam (BL) antibiotics (Group A: 28 females and 9 men; mean age 71.3) and non-steroidal anti-inflammatory drugs, NSAIDs (Group B: 20 females and 4 men; mean age 73.2), were included, as well as 2 control groups. Group C consisted of 17 women and 4 men tolerating BL and NSAIDs. Group D comprised 51 female and 19 male younger (mean age 39.7) patients with proven BL and/or NSAIDs hypersensitivity. Sensitivity and specificity were respectively 64.9% and 90.5% in group A with positive and negative predictive values equal to 92.3% and 59.4%, respectively. In the group B the respective figures were 54%, 80.9%, 6.5% and 60.7%.

Conclusions. Even though more evidences are needed to assess the suitability of the basophil activation test technique for the diagnosis of allergic reactions, this test gives promising results in the field of hypersensitivity to drugs in the elderly.

Key words: Adverse drug reactions, Basophil activation test, Elderly

INTRODUCTION

The aging population around the world is often characterized by an increased number of multiple diseases (diabetes mellitus, cardiovascular diseases, hypertension) and the necessity of a polytherapeutic regime [1]. The comorbidities and correlated polytherapeutic regime cause an increased incidence of adverse drug reactions including hypersensitivity and allergic drug reactions (ADRs). It is important therefore that a careful management of therapeutics should be put in place, by means of educational campaigns for patients and guidelines for doctors [2]. Our previous observation confirms the possibility of ADRs in the elderly, even if the guidelines to manage these manifestations are missing in this population [3 4].

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The elderly patient often needs to take some drugs, responsible for a previous adverse reaction and for which there are no valid laboratory tests [5]. Another relevant factor is the alteration in the pharmacokinetics (absorption, distribution, metabolism, excretion) and pharmacodynamics as well as the changes in the body mass in terms of total body fat and water [6]. In addition, submitting geriatric patients to allergy tests may pose a higher risk for their health (especially in older patients with type 2 diabetes mellitus or if they take drugs such as ß-blockers and ACE inhibitors, responsible for particularly severe hypersensitivity reactions) [7].

The present study suggests that the basophil activation test (BAT) can help the clinician in his diagnostic and therapeutic decisions (check drug responsibility and mechanism of the reaction, choose an alternative drug) [8]. Our aim was to evaluate the BAT in terms of sensitivity and specificity and in the light of its possible clinical use in the diagnosis of allergic or pseudo allergic reactions to non-steroidal anti-inflammatory drugs (NSAIDs) and ß-lactam (BL) antibiotics [9].

MATERIALS AND METHODS

Patients

In the present study were retrospectively included patients who suffered from ADRs to either BL (group A) or NSAIDs (group B) and visiting in the Immunoallergology Unit of Policlinico Hospital, University of Bari. The BAT was conducted on geriatric subjects and compared with younger patients seen at the same unit.

For each patient has been drawn up the clinical history, the familial history, drugs taken, laboratory data and, when indicated, skin tests according to EAACI criteria [10].

Depending on the drug involved, in vitro tests were performed, including BAT (Buhlmann Lab., Basel, Switzerland), specific IgE (CAP system, Thermo Fisher Diagnostic Uppsala Sweden), determination of complement factors and circulating immune complexes, histaminemia and tryptasemia (CAP FEIA Thermo Fisher Diagnostic Uppsala Sweden).

A comparison was made between populations of young patients and geriatric patients in order to assess differences in ADR risk factors, chronic medications, pre-existing pathologies and responsible drugs. The observed patients were divided into two groups, patients under the age of 65, and patients over 65 years of age. Each group was further divided into: male and female; number of medications taken (Tab. I); verified and reported pathologies (Tab. II); drug that has probably or certainly caused ADR (Tab. III); clinical picture with which ADR is manifested (Tab. III).

Patients were selected following a careful anamnestic evaluation and with the following inclusion criteria: a reaction that occurred within 2 hours after taking the drug and occurred only when the active substance was intake; allergic reactions occurred from 1 month to 2 years before the test was performed.

All patients had suspended the use of any systemic antiallergic drugs, such as corticosteroids, cromoglycate and H1 antihistamines at least 24 to 48 hours before blood sampling.

Non allergic exposed subjects were added as controls.

Flow cytometry BAT

Flow Cast Kit, which uses CD63 as a basophil activation marker, has been employed. It uses a stimulating buffer containing IL-3 in the cell isolation and incubation phases of the allergen. The tests were performed according to the method described by Saint-Laudy et al. [11].

Tested allergens were: penicillin V, penicillin G, amoxicillin, ampicillin, penicillin G major (PPL) and minor (MDM) determinants, cefuroxime with regard to BL antibiotics; ibuprofen, metamizole, aspirin and acetaminophen for NSAIDs. For each patient a negative control, incubating the cells only with IL-3 stimulation buffer and a positive one, incubating cells with an anti IgE (from Sigma Aldrich, Poole, United Kingdom), were performed. BATs for a drug were considered positive when triggered an activation of more than 5% of basophils and at least double the negative control. Specific IgE for ampicillin, amoxicillin, penicillin G and penicillin V has been determined (CAP-FEIA Thermo Fisher Diagnostic, Uppsala, Sweden).

Table I. Chronic therapy: differences between patients aged 65 years or over and younger patients.

Numbers of drugs	Females below the age of 65 years	Males below the age of 65 years	Females aged 65 years or over	Males aged 65 years or over
0	26	19	13	4
(1-3)	14	4	43	5
(4-6)	1	1	28	13
(7-9)	0	0	12	2
≥ 10	0	0	5	1

Table II. Pathologies verified and reported: differences between patients aged 65 years or over and younger patients.

Pathologies verified and reported	Females below the age of 65 years	Males below the age of 65 years	Females aged 65 years or over	Males aged 65 years or over
Rheumatic diseases	3	1	0	1
Allergic diseases	9	7	4	1
Autoimmune diseases	2	1	1	0
Cardiovascular diseases	13	2	30	9
Hepatic diseases	0	1	2	1
Kidney diseases	0	0	2	0
Dyslipidemia	15	5	19	7
Thyroid diseases	6	0	3	0
Diabetes mellitus	4	2	3	3
Osteoporosis	1	0	11	0
Neuropsychiatric disorders	1	1	3	1
Respiratory diseases	1	2	0	2
Hyperuricemia	0	0	1	1

Table III. Drugs responsible for adverse reactions: differences between patients aged 65 years or over and younger patients.

Drugs responsible for adverse reactions	Females below the age of 65 years	Males below the age of 65 years	Females aged 65 years or over	Males aged 65 years or over
Antibiotics	30	15	42	10
Antimycotics	0	0	1	0
Allopurinol	0	0	2	1
Antihypertensive drugs	4	4	2	0
Acetylsalicylic acid	0	0	15	9
Nonsteroidal anti-inflammatory drugs	0	0	41	9
Statins	0	0	2	1
Heparin	0	1	3	1
Contrast agents	0	0	4	1
Psychoanaleptics	9	0	4	1
Osteoporosis drugs	0	0	2	0
Oral anticoagulants	1	0	1	0
Muscle relaxants	1	0	1	0
Analgesics	5	1	4	1
Anesthetics	0	1	4	0
Antiacid drugs	1	0	6	0
Corticosteroids	0	0	5	0
Intravenous solutions	0	0	1	0
Drugs for gastrointestinal diseases	1	0	2	0
Iron therapy	0	0	1	0

Oral tolerance test

Subsequently, after signing an informed consent, patients underwent a tolerance test with the culprit drug in the manner and time schedules according to EAACI criteria [10]. All the procedures followed are in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2000. Oral provocation tests were performed only for those drugs that triggered activations below 5%.This cut-off value was chosen on the basis of our clinical experience, considering that it never caused adverse systemic reactions, that can be particularly dangerous in elderly patients.

RESULTS

The BAT was tested in 61 geriatric patients, of whom 37 had suffered from a documented ADR that was definitely related to a BL antibiotic (group A) and 24 had suffered from ADR surely referred to a drug related to NSAIDs (group B). The clinical manifestations of ADRs were mostly urticaria-angioedema and more rarely in the form of anaphylactic shock or a cell-mediated reaction (Tab. IV). Clinical manifestations were urticaria-angioedema in 35 cases and anaphylaxis in 2 in group A, and 24 had suffered from urticaria-angioedema in group B. As a control population, a group of 21 geriatric patients was selected, of whom 13 with allergies to inhalants, who had never had any allergic reactions to drugs and had taken NSAIDs and BL antibiotics in the last year (Group C). Group A consisted of 28 women and 9 men, averaging 71.3 years (range 68-82); 4 among them were also affected by allergy to inhalants and foods. Group B consisted of 20 women

Table IV. ADR clinical manifestations: differences between patients aged 65 years or over and younger patients.

ADR clinical manifestations	Females below the age of 65 years	Males below the age of 65 years	Females aged 65 years or over	Males aged 65 years or over
MPE	2	1	0	0
Urticaria angioedema syndrome	40	21	93	25
Anaphylaxis	1	0	8	1

ADR: allergic drug reaction; MPE: maculo-papular eruption

and 4 men, averaging 73.2 years (range 66-85); 1 was also affected by food allergy. Group C consisted of 17 women and 4 men, averaging 70.9 years of age (range 66-75). In 14 patients of group A and 5 of group B, who had reintroduced the active substance, the reaction was reproduced. We also considered a control group made of younger patients (Group D, 51 female patients, 19 male patients, average age 39.7).

Medications mostly used for a chronic disease were antibiotics, NSAIDs, antihypertensive, diuretics, insulin, oral hypoglycemic drugs, statins, psychoanaleptics, heparin. As age advances, more diseases develop and more medications were needed. Most of the medicines taken were for the treatment of cardiovascular diseases, diabetes and dyslipidemia.

Finally, with regard to the presence of other allergies (inhalants, foods etc.), only 20.6% of geriatric patients (groups A and B) exhibited an allergy-confirmed disease compared to 58.4% of non-geriatric patients (Group D).

With particular reference to Flow Cast, in group A 24 out of the 37 patients were positive with at least one of the culprit tested drugs (sensitivity = 64.9%). 10 patients were positive to PPL, 5 to MDD, 11 to amoxicillin, 8 to ampicillin, 10 to penicillin G, 12 to penicillin V and 4 to cefuroxime. Only in 7 cases the result was positive for one drug only: 2 PPL, 3 MDM, 1 amoxicillin and 1 penicillin G. In group C, 2 patients had activations above the established cut-off with drugs that they tolerated: 1 to ampicillin and 1 to amoxicillin (specificity = 90.5%). Specific IgEs were positive to at least one of the drugs tested in 9 cases (sensitivity = 24.3%). In group B, 13 patients out of 24 had significant activation of basophils with the drug responsible for the reaction (sensitivity = 54%). 10 patients were positive to aspirin, 6 to ibuprofen, 5 to metamizole, 9 to acetaminophen. In 9 cases activation was induced by one other drug not involved in the index reaction: 4 by aspirin, 2 by ibuprofen, 3 by metamizole. In group C, 4 patients had higher activation than the cut-off for aspirin (2 cases), metamizole (1 case), ibuprofen (1 case) (specificity = 80.9%). Positive predictive value was 92.3% for BL antibiotics and 76.5% for NSAIDs, while negative predictive value was 59.4% for BL antibiotics and 60.7% for NSAIDs (Tab. V).

In both groups of patients, the response was not influenced by the time elapsed since the allergic reaction, as positive responses were also found in cases of reactions dating back to 2 years earlier. The in vivo provocation tests performed, following Flow Cast and according to the above-mentioned selection criteria were negative in 5 patients with allergy to BL antibiotics (and challenged with another BL) and 4 with hypersensitivity to NSAIDs and challenges with another NSAID), thus confirming the good negative predictive value.

DISCUSSION

The identification of the antigens responsible for allergic reactions is essential both for diagnostic purposes and for effective prevention measures in relation to these manifestations. Diagnostic protocols require, in most cases, an accurate collection of anamnestic data, in vivo tests and when available, laboratory tests. As regards the diagnosis of drug allergy reactions, in vivo tests are not without risk, in particular for elderly patients with chronic pathologies, especially respiratory and cardiovascular, for whom the induction of an anaphylactic shock after oral provocation tests could be dangerous and even lethal [13]. Considerably lacking is laboratory diagnostics. In fact, in the laboratory, allergen-specific IgE are detected, but only immunoassays for a few drugs are available and scientifically validated; moreover, this test is not very sensitive and tends to become negative in a short time. Some drugs,

Table V. BAT in immediate BL- antibiotics and NSAIDs hypersensitivity.

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Group A (BL antibiotics)	64.9	90.5	92.3	59.4
Group B (NSAIDs)	54	80.9	76.5	60.7

particularly NSAIDs, may also induce degranulation of effector cells by means of leukotrienes, complement or by direct action of the drug and not by means of IgE [14]. In recent years, a lot of scientific studies have highlighted the potential use of cytofluorometry for in vitro diagnostics of allergic diseases [15]. It has been seen that, under pacing with a specific allergen, basophils release active mediators in quantifiable doses and regulate the expression of markers that can be easily measured by flow cytometry using specific monoclonal antibodies [16]. Basophils are granulocytes that develop from CD34 + pluripotent stem cells, mature in the bone marrow and then pass into the bloodstream where they represent less than 1% of the leukocytes. Basophils show a segmented nucleus and possess rounded granules containing glucosamminoglycans, heparin and histamine in their cytoplasm. These granules are called "basophilic" because they have a particular affinity for the basic dyes mainly due to the presence of heparin and hyaluronic acid. Basophils express receptors for interleukins, chemokines, complement proteins, prostaglandins and for the Fc fragment of IgE [17 18].

The base of the basophil activation test is a demonstration of the change in the membrane phenotype of basophils that after allergenic stimulation can have an up or a down regulation [15]. The basophil activation test, which can be performed with any suspected drugs, measures the activation of basophils after stimulation and is suitable for both IgE-mediated and non-IgE-mediated hypersensitivity. Clinical studies utilizing flow cytometry for measurement of markers of basophils activation have primarily focused on 2 markers, CD63 and CD203c:

CD203c, expressed exclusively on basophils and mast cells and their progenitor cells, is, as CD63, overexpressed during activation of these cell types [17].

CD63 (gp 53), present in different cells (basophils, platelets, monocytes, mast cells). It is contained in intracytoplasmic granules and when in activation it is expressed in high density on the cell surface [18].

The basophilic activation study technique makes use of a flow cytofluorometry method based on the use of fluorochromes with monoclonal antibodies, which specifically identifies surface markers expressed on the membrane of the cells, in this case basophils CD63 and CD203c. Subjects with a degranulation percentage of 5% or more should be considered susceptible to allergy or have a positive stimulation. The results we have obtained in the case of BL antibiotics reveal a higher BAT sensitivity compared to the results reported in the literature and a slightly lower specificity [19]. In the case of NSAIDs, however, the sensitivity found is greater than the results reported in the literature, with a lower specificity [20]. However, although the sensitivity found is not high, the negative predictive value is of interest since we have not found any adverse events to drug administration in the negative test patients, according to the criteria previously reported. However, an extended prospective study would be needed to confirm these results. BAT can be used to reduce pretest probability of having unwanted reactions to the provocation test, which remains the gold standard for diagnosing of drug allergy. Moreover, BAT is also useful for the diagnosis of those hypersensitivity reactions that do not recognize an IgE-mediated mechanism, as in the case of NSAIDs. As it is a relatively recent method, it has not been fully standardized yet. Some questions concern the optimal drug concentration to be used for the test (too low concentrations can give false negatives, as well as too high concentrations can give false positives, due to the possible cytotoxic effect); in addition, the sample of blood must be analyzed no later than 24 hours after collecting and on a minimum number of basophils. The use of BAT is however an advantage from a clinical point of view, especially when referring to a category of patients, such as the geriatric one, where it is imperative to recognize accurately any hypersensitivity to drugs in the elderly, often affected by multiple diseases [21 22]. The same would hold true for infants and for severe reactions in which a drug provocation is not permitted.

ADRs represent a major impact on society, resulting in significant morbidity, mortality, and health care costs. They can mimic the clinical picture of other illnesses, causing unnecessary investigations, or preclude the use of certain drugs by the doctor, and so postponing the therapeutic treatment. The budget can be addressed to the right direction if it joins simple guidelines [23] such as: 1) collect a careful pharmacological history: the disease to be treated can be iatrogenic or the drugs taken may interact with prescription; 2) prescribe only for a specific diagnosis; 3) define the purpose of the therapy and start with small doses titling on the desired response; 4) maintain a high level of suspicion of drug reactions and interactions and know what other drugs the patient is taking; 5) simplify the treatment regime as much as possible and limit the number of drugs to be taken.

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