ORIGINAL ARTICLE

Nonsteroidal Anti-inflammatory Drug Hypersensitivity Syndrome. A Multicenter Study
I. Clinical Findings and In Vitro Diagnosis

AL De Weck,[1] ML Sanz,[1] PM Gamboa,[2] W Aberer,[3] M Blanca,[4] S Correia,[7] S Erdman,[6] JM Jermann,[8] G Kanny,[9] M Kowalski,[10] L Mayorga,[4] W Medrala,[5] A Merk,[6] GJ Sturm,[3] J Sainte-Laudy,[11] MS Schneider,[8] A Sczczeklik,[12] JM Weber,[8] A Wedi,[13] and members of the ENDA group

[1] Department of Allergology and Clinical Immunology, Clínica Universidad de Navarra, Collaborative center of GA_2LEN, Pamplona, Spain
[2] Hospital Basurto, Division of Allergy, Bilbao, Spain
[3] Department of Dermatology, Medical University of Graz, Austria
[4] Hopital Haya, Malaga, Spain
[5] University Hospital, Allergology, Wroclaw, Poland
[6] Dermatology Clinic, University Hospital Aachen, Germany
[7] Allergy Division, Hospital, Setubal, Portugal
[8] Bühlmann Laboratories, Allschwil, Switzerland
[9] Centre Hospitalier Universitaire, Médecine, Nancy, France
[10] University Hospital, Lodz, Poland
[11] Centre Hospitalier Universitaire, Limoges, France
[12] University Hospital, Cracow, Poland
[13] Dermatology Clinic, University Hospital, Hanover, Germany

Abstract

Background: We present the results obtained from the largest series of in vitro diagnostic tests ever reported in patients with clinically validated hypersensitivity to acetylsalicylic acid (ASA)/nonsteroidal anti-inflammatory drugs (NSAID) compared with various categories of controls tolerating ASA/NSAIDs. This multicenter study, which was performed within the framework of the European Network for Drug Allergy (ENDA) group, showed that the basophil activation test (BAT), particularly when used with the 3 NSAIDs aspirin (ASA), diclofenac (DIC), and naproxen (NAP), allows us to confirm the diagnosis of NSAID hypersensitivity syndrome. The results of the cellular allergen stimulation test (CAST) frequently correlate with those of the BAT, although not always. An unexpected finding was that basophil activation by NSAIDs is not an all-or-nothing phenomenon restricted to clinically hypersensitive patients, but that it also occurs in a dose-related manner in some NSAID-tolerant control individuals. Therefore, NSAID hypersensitivity appears as a shift in the normal pharmacological response to NSAIDs. These findings allow us to formulate a new rational hypothesis about the mechanism of NSAID hypersensitivity syndrome, a mechanism that most authors continue to describe as "unknown."
Methods: We enrolled 152 patients with a history of hypersensitivity to NSAIDs and 136 control participants in 11 different centers between spring 2003 and spring 2006. Flowcytometric BAT was performed.
Results: The most noteworthy results of our study were that 57% of 140 patients presented very clear-cut positive BAT results to multiple NSAIDs, and 16% were entirely negative. In about 27% of cases, positive results were obtained with 1 or 2 concentrations of a single NSAID. There is clearly a correlation between the results of BAT and CAST.
Conclusions: BAT seems particularly indicated in patients with a clinical history of NSAID intolerance, and in whom a provocation test is not advisable for ethical, clinical, or other reasons. Clear-cut positive results can be considered as confirming a history of NSAID hypersensitivity, although negative results may not exclude it.

Key words: NSAID hypersensitivity syndrome. Clinical findings. In vitro diagnosis. Basophil activation test. Flowcytometry. Sulphidoleukotrienes.

Resumen

Introducción: En este estudio presentamos los resultados obtenidos en la mayor serie sobre diagnóstico in vitro en pacientes con hipersensibilidad a aspirina/AINEs comparados con diferentes categorías de controles tolerantes. Este estudio multicéntrico, realizado en el marco del grupo ENDA (European Network Drug Allergy) confirma que el TAB (Test de activación de basófilos), cuando se utiliza frente a aspirina, diclofenaco y naproxeno, permite confirmar el diagnóstico de este síndrome. El CAST se correlaciona en algunos casos con el TAB. Un hallazgo inesperado es que el TAB frente a AINES no es un fenómeno restringido a los pacientes hipersensibles clínicamente sino que también ocurre en algunos individuos tolerantes de forma dosis-dependiente. Así, la hipersensibilidad a AINES aparece como una modificación de la respuesta farmacológica normal en respuesta a AINES. Estos hallazgos nos permiten formular una nueva hipótesis racional sobre el mecanismo de hipersensibilidad a AINES sobre el que la mayoría de los autores consideran que es desconocido.
Métodos: En este estudio se incluyeron 152 pacientes con historia de hipersensibilidad a AINES y 136 sujetos control recogidos en 11 diferentes centros entre la primavera de 2003 y la de 2006. Se realizó el TAB.
Resultados: Los resultados más destacables de este estudio son los siguientes: el 57% de 140 pacientes presentó claros resultados positivos a múltiples AINES y en el 16% de los casos los resultados fueron completamente negativos. En aproximadamente un 27% de los casos, se obtuvieron resultados positivos con 1 o 2 concentraciones de un solo AINE. Existe una clara correlación entre los resultados del TAB y del CAST
Conclusiones: Esta técnica parece estar indicada especialmente en pacientes con historia clínica de intolerancia a AINES en los que el test de provocación no es aconsejable por razones éticas, clínicas u otras. Los resultados positivos en esta técnica confirman la historia de hipersensibilidad a AINES, pero los resultados negativos no la excluyen.

Palabras clave: Síndrome de hipersensibilidad a AINES. Hallazgos clínicos. Diagnóstico in vitro. Test de activación de basófilos. Citometría de flujo.

Introduction

Hypersensitivity to acetylsalicylic acid (ASA) and other nonsteroidal inflammatory drugs (NSAIDs) is a well-known condition that was first described as a syndrome by Widal [1] in 1922, and popularized by Samter and Beers [2] in the late 1960s. This syndrome presents with respiratory manifestations (recurrent rhinitis associated with nasal polyposis and followed by asthma attacks—the classic ASA triad) or with cutaneous manifestations such as urticaria and angioedema. Both types can coexist, but this is more uncommon. The prevalence of hypersensitivity to ASA and NSAIDs has been shown in several studies to be about 10% to 20% in adult asthmatics and 0.6% to 2.5% in the general population [3,4]. A recent meta-analysis of available data indicates a higher prevalence of hypersensitivity in asthmatics after evaluation using oral challenge (adults 14% to 29%, children 0% to 14%) than by history alone (adults 2% to 4%, children 1% to 3%) [5]. It has been reported that untoward reactions to NSAIDs constitute 20% to 25% of all hypersensitivity reactions to drugs [6]. The main features, clinical manifestations, pathogenesis, diagnosis, treatment, and prevention of NSAID hypersensitivity syndrome are well documented [3,7-10]. With the possible exception of rare cases of anaphylactic shock, which may be associated with specific immunoglobulin (Ig) E [11,12], hypersensitivity to ASA and NSAIDs is widely believed not to be associated with a mechanism other than Ig. Many years ago, it was proposed [13-15] that the reaction was generated by inhibition of cyclooxygenase (COX) by ASA-like drugs in the airways or in the skin of hypersensitive patients. This theory has recently been restricted to inhibition of the COX-1 enzyme, which diminishes the production of prostaglandin E_2 (PGE_2) that normally acts as a "brake" on the production of the sulfidoleukotrienes (sLT) LTC_4, LTD_4, and LTE_4, and on the release of other mediators by mast cells [3,16,17]. In addition, there is evidence that patients who are hypersensitive to COX-1 inhibitors tend to produce higher levels of sLTs even before exposure to ASA/NSAIDs [3,18]. The role of sLTs as major mediators of clinical symptoms in hypersensitivity reactions to ASA/NSAIDs is well recognized [2,18,19]. A key enzyme, leukotriene C4 synthase, is overexpressed in the bronchial mucosa of patients who are hypersensitive to NSAIDs [20], with the result that this condition has lately been considered a pharmacogenetic disorder [21,22]. The search for a single genetic polymorphism associated with NSAID hypersensitivity, however, has yielded somewhat contradictory results to date [23-27].

For the last 20 years, most leading allergologists have maintained that there is no in vitro diagnostic test for the NSAID hypersensitivity syndrome [3,7,28]. Indeed, traditional cell-based allergy tests (eg, the histamine release test), have yielded mostly negative results in the case of ASA/NSAID hypersensitivity [29,30]. However, the advent of the cellular allergen stimulation test (CAST), which is based on release of sLTs by activated blood basophils [31-33], presents a challenge to this opinion. Several rather anecdotal reports [31-35] and a few well-controlled and validated studies [33,36] have clearly shown that ASA/NSAIDs can induce basophil activation and sLT release in vitro, at least in a sizeable number of ASA/NSAID–hypersensitive patients. Other authors, however, have not confirmed the phenomenon [37], and a superficial analysis of the literature reveals a rather confusing and contradictory picture. However, as discussed elsewhere [38], these apparent contradictions seem to arise from technical differences and differences in the interpretation of results.

Another recently developed diagnostic technique is based on the flowcytometric evaluation of basophil activation induced in vitro by ASA [11,39] and other allergens (flowcytometric allergen stimulation test) [40]. The method has been successfully applied to the study of NSAID hypersensitivity

syndrome [38,41] and has opened new perspectives for understanding its pathophysiology. The diagnostic value of the flowcytometric assay in allergy has recently been reviewed [40,42-46].

In this paper, we present the results of the largest series of in vitro diagnostic tests reported to date in patients with clinically validated NSAID hypersensitivity compared with different categories of controls who tolerate NSAIDs. This multicenter study, which was performed within the framework of the European Network for Drug Allergy (ENDA) group, confirmed that the basophil activation test (BAT, in this study the commercially available Flow CAST), particularly when used with the 3 NSAIDs ASA, diclofenac (DIC), and naproxen (NAP) at 2 concentrations, enables us to confirm the diagnosis of NSAID hypersensitivity syndrome. The results of CAST frequently correlate with those of BAT, although not always. Our results have been presented elsewhere [44,45,47,52], and an unexpected finding was that basophil activation by NSAIDs is not an all-or-nothing phenomenon restricted to clinically hypersensitive patients, but that it also occurs in a dose-related manner in some NSAID-tolerant control participants [49]. Therefore, NSAID hypersensitivity appears as a shift in the normal pharmacological response to NSAIDs. These findings make it possible to formulate a new, rational hypothesis about the mechanism of NSAID hypersensitivity [44,47,52], a mechanism that most authors continue to describe as "unknown" [9].

Methods

Patients

A total of 152 patients with a history of hypersensitivity to NSAIDs were enrolled in 11 different centers between spring 2003 and spring 2006. Complete clinical and laboratory data according to the ad hoc ENDA protocol were obtained for 144 patients and are evaluated here. There were 71 (46.7%) men and 81 (53.3%) women aged between 16 and 71 years (mean 44 years). Most patients were in the third or fourth decade of life.

Detailed clinical information was obtained on atopic status (39/144, 27.0%), history of allergic reactions to NSAIDs, culprit drug(s), presence of symptoms related to other drugs at the time of testing, as well as time elapsed since the last clinical reaction to NSAIDs. When appropriate, the results of re-exposure and provocation tests were also given. The history was considered as validated when the clinical reaction had been documented by a physician and reproduced with classic symptoms after a provocation test within 24 hours of administration of ASA at a dose of at least 500 mg. The history was considered as likely when the clinical reaction had been documented by a physician and when more than 1 clinical event had been recorded following exposure to 1 or more NSAIDs

Among the patients with a provocation-validated or likely history of NSAID hypersensitivity, 39 (27%) presented with airway symptoms (rhinitis, asthma) only, 97 (67%) presented with skin symptoms (urticaria, angioedema) only, and 8 (6%) presented with both.

Similar data were obtained from a total of 136 control patients investigated in 8 groups. Seventy-six had no history of allergic reactions, 5 had a history of allergic reactions to other drugs, and 62 were atopic (45.5%) with a corresponding history, and positive skin test and specific IgE results to some inhalant allergens. They had all tolerated at least 500 mg of ASA.

An additional population of 29 healthy blood donors was used as controls by one investigation group, although their status in terms of clinical tolerance to NSAIDs is not known.

Flowcytometric BAT (Flow CAST)

All the reagents used in this study for BAT and all the NSAIDs were provided by the manufacturer (Flow CAST, Bühlmann Laboratories, Allschwil, Switzerland). The technique followed the manufacturer's instructions and has been fully described elsewhere [41,42,53]. Briefly, blood was collected in EDTA tubes and stored at 4°C; the test was then carried out within 24 hours of blood sampling. One milliliter of EDTA blood allows up to 2 allergens to be tested. The tubes were centrifuged at 200*g* for 5 minutes at 4°C. The supernatant (plasma leukocytes) was pipetted and recentrifuged at 500*g* for 10 minutes at 4°C. When the supernatant was decanted, the cell pellet was resuspended in 100 µL of stimulation buffer (HEPES 20 mM, NaCl 133 mM, KCl 5 mM, CaCl2 7 mM, MgCl2 3.5 mM, HAS 1 mg/mL, pH 7.4, containing 10 ng/mL of interleukin [IL] 3) per milliliter of blood. In 2 groups (PAM, LIM), the technique used for cell isolation was slightly different, that is, whole EDTA blood was first centrifuged at 500*g* for 10 minutes, yielding a buffy coat layer that was pipetted, washed, and centrifuged, before being reconstituted in the IL 3–containing buffer described above.

Subsequently, 50 µL of reconstituted solution of ASA (final concentrations, 2 and 0.4 mg/mL), paracetamol (final concentrations, 0.025 and 0.005 mg/mL), DIC (final concentrations, 0.5 and 0.1 mg/mL), NAP (final concentrations, 1.2 and 0.25 mg/mL), and metamizole (final concentrations, 1.2 and 0.25 mg/mL) were added to 50 µL of cell suspension in microplate wells. Patients with reactions to other NSAIDs were also tested with the culprit drug at several final concentrations, of which the maximum value was usually 2 mg/mL. These final concentrations were chosen following preliminary assays and analysis of dose response-curves (data not shown). A monoclonal anti-IgE receptor antibody (Bühlmann Laboratories) at 1 µg/mL was used as a positive control.

In order to evaluate baseline values without stimulation, 50 µL of stimulation buffer was added to another well and 50 µL of cell suspension was added to all wells. The microplate covered with an adhesive plastic sheet was then incubated for 40 minutes at 37°C. One group (LIM) used disposable 5m tubes instead of microplates. The reaction was stopped by adding 100 µL of HEPES buffer pH 7.3 containing EDTA (HEPES 20 mM, NaCl 133 mM, KCl 5 mM, EDTA 0.27 mM) as a stopping buffer. Plates were then centrifuged at 500*g* for 5 minutes at 4°C, and 100 µL of supernatant was pipetted and saved for sLT analysis by CAST-enzyme-linked immunosorbent assay (ELISA) in a polycarbonate tube, with the LTs binding spontaneously to polystyrene (see below). Basophils from the cell pellet

were double labeled by adding 20 µL of staining reagent containing prediluted anti-CD63 phycoerythrin (PE)-labeled and anti-IgE fluorescein isothiocyanate (FITC)—labeled antibody. After light-protected incubation for 30 minutes at 4°C, 3.5 mL of erythrolytic reagent (Ortho-Mune lysing reagent, Ortho Diagnostic Systems, San Fernando de Henares, Madrid, Spain) were added to each tube and left at room temperature for 5 minutes. Cell lysis was stopped with 1 mL of washing buffer. After centrifuging for another 5 minutes at 1000*g*, the supernatants were decanted and 500 µL of stopping buffer (or the sheath buffer used for the cytometer) was added to each tube, which was then gently shaken before flowcytometric analysis.

Flowcytometric analysis was performed at 488 nm on a FACScan flow cytometer (Becton Dickinson, Madrid, Spain) or similar instrument equipped with 1 or more argon lasers. The results were analyzed using CellQuest (Becton Dickinson) or an equivalent application. On the histogram of forward scatter and side scatter, a first cell gate was defined by a bit map around the lymphocytes. A second gate was defined around cells showing high-density fluorescence with anti-IgE FITC. These were identified as basophils. At least 500 basophils were counted in each assay. The other parameter analyzed on the identified basophils was the CD63 activation marker, as described elsewhere [41,42,53].

sLT Assay (CAST-ELISA)

The sLT assay measures the amount of sLT (LTC_4, LTD_4, LTE_4) produced by blood leukocytes after in vitro stimulation by allergens [32,33]. Following isolation of leukocytes and incubation with various NSAIDs as described above, 100 µL of supernatant was collected from all wells and frozen at –20°C until analysis. Within 1 month, the supernatants were analyzed for sLTs using ELISA according to the manufacturer's instructions (CAST-ELISA; Bühlmann Laboratories).

Statistical Analysis

The means of non-normally distributed variables were compared using the Mann-Whitney test. Qualitative data were compared using the χ^2 test with a Yates correction when necessary. All *P* values were 2-tailed, and statistical significance was set at a *P* value of .05. The specificity and sensitivity values were obtained by analysis of different cut points on receiver operating characteristic (ROC) curves. Sensitivity was calculated using the number of positive cases detected by the respective techniques in the study group, and specificity using the number of negative cases detected by the same techniques in the control group. The statistical analysis was performed using SPSS version 10.0 (SPSS Inc, Chicago, Illinois, USA).

Results

As indicated in Table 1, the multicenter study organized under ENDA sponsorship included 152 case reports from 10 groups; 2 groups reported a single case each, and these were not included in the final evaluation. Similarly, cases in which there was insufficient information or incomplete performance of the investigation protocol were also excluded, leaving 140 evaluable cases. The groups provided 136 clinically evaluable controls (patients who were tolerant of NSAIDs). In addition, 1 group provided 29 healthy blood donors with unknown NSAID tolerance.

Table 1. Total Number of Cases Investigated in the NSAID ENDA Multicenter Study

Group	Patients	Controls	Cell Isolation
AAChen	3	0	a
CRAcow	4	9	a
GRAz	7	12	a
HANover	1	0	
LIMoges	9	11	b
LODz	11	10	a
MALaga	8	8	a
NANcy	1	0	
PAMplona	78	50	a and b
SETubal	1	7	a
WROclaw	29	29	a and b
BASel		29	a and b
Total	152	165	

[a] Plasma leukocytes
[b] Buffy coat leukocytes

Several patterns of in vitro reactivity and basophil activation detected by BAT (Flow CAST) and CAST were observed. Examples of such patterns are given in Table 2. In a first evaluation, the positivity cutoff for BAT was established at 5% basophil activation and a stimulation index (SI=test value/basal value) >2. These values had been established in a first study [38,64] by ROC curves. As shown below, they retained their operational value in this ENDA multicenter study. Considering the results obtained with 5 NSAIDs, but particularly the more frequently positive ASA, DIC, and NAP with BAT and CAST performed on the same blood samples, the main patterns observed were as follows:

a) Positive results with BAT and CAST to several NSAIDs at 1 or 2 concentrations (38 [27%] of the 140 fully evaluable patients, 9 [6%] of the 136 evaluable controls).
b) Positive results with BAT only to several NSAIDs at 1 or 2 concentrations, CAST being mostly negative or unknown (42 [30%] of the 140 fully evaluable patients, 27 [20%] of the 136 evaluable controls).
c) Positive results with CAST only to several NSAIDs at 1 or 2 concentrations, BAT being mostly negative (6 [4%] of the 140 fully evaluable patients, 6 [4%] of the 136 evaluable controls).
d) Positive results with BAT (with CAST positive or negative) to a single NSAID at 1 or 2 concentrations (23 [16%] of the 140 fully evaluable patients, 8 [6%] of the 136 evaluable controls).
e) Positive results with CAST only (with BAT negative) to a single NSAID at 1 or 2 concentrations (5 [4%] of the 140 fully evaluable patients, 5 [4%] of the 136 evaluable controls).

Table 2. Individual Examples of Patients and Controls Grouped According to Results of BAT and CAST

Patients Category	ENDA No.	Events NSAIDs	Date of Test	Time Elapsed	Provocation	BAT % CAST, pg Baseline	Positive Control	ASA 1	ASA 0,2	PAR 1	PAR 0.2	DIC 0.3	DIC 0,06	NAP 1	NAP 0,2	MET 5	MET 0,5
a) Multiple BAT-positive	112	URT AE ASA	Feb-05	12 mo	ASA 150	3.2	4.3	15.9	16.8	13.1	14.6	14.6	19.0	12.1	5.2	8.3	11.6
and CAST-positive		Nov-02			pos URT AE	35	*3151*	*354*	*247*	*257*	*195*	*178*	*311*	*514*	*344*	*381*	244
	63	AA MET	Nov-02	15 d	ASA 400	2.3	98.7	55.1	41.8	30.8	36.2	22.8	34.5	31.2	41.7	10.7	21.1
		Nov-02			pos 1d	189	*7240*	*450*	*311*	152	35	23	142	45	*830*	69	57
	81	URT MET	Feb-03	1 mo	ASA 500	1.8	77.7	16.1	12.3	5.3	9.1	15.3	18.3	20.1	9	4.2	3.2
		Jan-03			pos 1d	331	*2725*	136	120	120	145	144	97	157	149	135	41
	60	AA MET DIC ASA	Oct-02	28 mo	nd	10	64.6	28.5	38.1	2.5	7.4	5.4	7.4	12.7	30.4	3.4	10.7
		Feb-99, Jul-99, Jan-00				157	*3045*	*998*	*335*	45	26	138	90	188	*899*	173	153
b) Multiple BAT-positive	122	AA, ASA PAR KET	Oct-04	1 y	ASA 180	4.4	33.3	81.8	36.3			30.9	26.1	56.2	40.0		
and CAST-negative		2001(2x), 2003, 2004			pos URT 6h	82	*3200*	145	15			27	37	148	109		
	63	AA MET	Nov-02	15 d	ASA 400	2.3	98.7	55.1	41.8	30.8	36.2	22.8	34.5	31.2	41.7	10.7	21.1
		Nov-02			pos 1d	189	*7240*	*450*	*311*	152	35	23	142	45	*830*	69	57
c) Multiple CAST-positive	22	AE IBU	Mar-05	7 mo	ASA 500 neg	3.8	10.9	5.0	2.1	1.3	1.1	3.2	1.1	0.8	1.0	3.2	1.9
and FLOW-negative		Sep-04			IBU 100 pos	849	*6218*	*8184*	1295	*1678*	*3655*	*5436*	1282	*9607*	*1854*	1104	1160
d) BAT positive to single	88	AA IBU	Apr-03	12 mo	nd	8.6	32.3	12.1	9.9	4.4	4.4	0	4.6	1.5	2.1	0	4.5
NSAID, CAST-positive or -negative		Apr-02				178	*3200*	270	280	135	145	180	256	218	*356*	140	164
e) CAST positive to single	5	URT AE AS DIC	Dec-03	4 mo	DIC 25	0.7	41.4	2.6	1.3	nd	nd	0.7	2,3	0	1.3	nd	nd
NSAID, BAT-negative		Jul-03			pos URT	0	*7045*	7	0			20	0	*188*	*131*		
f) BAT and CAST	75	URT PAR MET ASA	Nov-02	24 mo	NIM; 100	0.5	*45.1*	0.6	0.7	1.1	0	0.6	0.8	0-3	0.8	0	0.3
all negative		Oct-84, Aug-99, Dec-00			pos 1d	0	*807*	62	0	0	0	90	0	0	102	89	75

Controls Group	ENDA No.	Events NSAIDs	Date Test	Time Elapsed	Provocation	BAT CAST Baseline	Positive Control	ASA 1	ASA 0.2	PAR 1	PAR 0.2	DIC 0.3	DIC 0.06	NAP 1	NAP 0.2	MET 5	MET 0.5
f) All negative	65	No	Dec-02		ASA 500	2.7	87.7	4.7	3.3	0.5	8.4	2.6	4.9	6.1	4.7	3.9	1.3
					neg	151	*6100*	54	106	62	78	0	128	98	162	34	98
	72	No	Apr-04		ASA 500	11.7	56.8	2.1	0.8	0	1.6	4.7	2.2	5.4	3.6	0.4	0.4
					neg	76	5172	129	142	125	77	86	98	115	91	77	77
d) BAT-positive to	82	No	May-04		ASA 500	0.7	18.9	0.6	0	0	0.3	9.3	1.5	1.1	0.4	0	0.4
single NSAID					neg	177	1643	70	66	0	60	49	28	39	45	102	83
c) Multiple CAST-positive	61	No	Dec-02		MET 575	2.7	38.1	2.4	2.4	0.5	0.6	53.9	0.6	0.2	22.7	0	0
					neg	52	*5485*	*221*	*202*	*168*	75	*233*	*163*	*209*	*262*	*172*	*160*
b) Multiple BAT-positive	104	No	Sep-03		ASA 1000	2.5	20.4	10.6		8.0		11.5		16.8		5.8	
					neg	170	*3200*	240		175		*450*		210		245	

Abbreviations: AA, asthma; AE, angioneurotic ediema; AS, anaphylactic shock; ASA, acetylsalicylic acid; BAT, basophil activation test; CAST, cellular antigen stimulation test; DIC, diclofenac; ENDA, European Network for Drug Allergy; metamizole; IBU, ibuprofen; KET, ketotifen; NAP, naproxen; neg, negative; NSAID, nonsteroidal anti-inflammatory drug; PAR, paracetamol; pos, positive; URT, urticaria

BAT-positive
CAST-positive
Provocation-positive
Provocation-negative

Table 3. Results of BAT and CAST in Patients With Hypersensitivity to NSAIDs (140 Evaluable Patients)

A. Validated (provocation-positive) n=107	No.	Sensitivity
BAT-positive[a]	81	76%
BAT-negative	26	
CAST-positive only	7/79	9%
CAST- and BAT-positive	27/79	34%
B. Not validated n=33		
BAT-positive[a]	25	76%
BAT-negative	8	
CAST-positive only	3/22	14%
CAST- and BAT-positive	9/22	41%

Abbreviations: BAT, basophil activation test; CAST, cellular antigen stimulation test; NSAID, nonsteroidal anti-inflammatory drug.
[a] To at least one concentration of ASA, DIC, and/or NAP

Table 4. Reproducibility of Results in Various Groups of NSAID-Hypersensitive Patients (125 Evaluable Patients)

Group		BAT-positive	%
GRA	n=7	3	43
CRA	n=4	4	100
LIM	n=7	3	43
LOD	n=10	9	90
MAL	n=8	4	50
PAM	n=78	59	75
SET	n=1	1	100
WRO	n=10	9	90

Abbreviations: BAT, basophil activation test; CRA, Cracow; GRA, Graz; LIM, Limoges; LOD, Lodz; MAL, Málaga; NSAID; nonsteroidal anti-inflammatory drug; PAM, Pamplona; SET, Setubal; WRO, Wroclaw.

Table 5. Reproducibility of Controls

Group		BAT Positive	%
GRA	n=12	4	33
CRA	n=9	8[a]	89
MAL	n=8	5	63
LIM	n=11	0	0
LOD	n=9	1	12
PAM	n=50	5	10
SET	n=7	3[b]	43
WRO	n=7	3	43

Abbreviations: BAT, basophil activation test; CRA, Cracow; GRA, Graz; LIM, Limoges; LOD, Lodz; MAL, Málaga; NSAID; nonsteroidal anti-inflammatory drug; PAM, Pamplona; SET, Setubal; WRO, Wroclaw.
[a] 7 symptomatic instrinsic asthmatics
[b] Double NSAID concentration

Table 6. Calculation of Sensitivity and Specificity[a]

	ASA c1	SE %	ASA c2	SE %	PAR c1	SE %	PAR c2	SE %	DIC c1	SE %	DIC c2	SE %	NAP c1	SE %	NAP c2	SE %	MET c1	SE %	MET c2	SE %
Sensitivity SE (Patients)																				
Group 1[b]	17/44	38.6	9/41	21.9	3/27	11.1	2/24	8,3	24/46	52.2	17/43	39.5	18/47	38.3	9/44	20.5	3/27	11.1	2/24	8.3
Group 2[c]	33/83	39.7	12/83	14.4	3/65	4.6	6/65	9.2	26/71	39.5	21/71	29.5	23/51	45.0	16/51	31.3	6/70	8.6	8/70	11.4
Combined	50/127	39.4	21/124	16.9	6/92	10.8	8/89	8.9	50/117	42.7	38/114	33.3	41/98	41.8	25/95	26.3	9/97	9.3	10/94	10.6
Specificity SP (Positive Controls)																				
	ASA c1	SE %	ASA c2	SP %	PAR c1	SP %	PAR c2	SP %	DIC c1	SP %	DIC c2	SP %	NAP c1	SP %	NAP c2	SP %	MET c1	SP %	MET c2	SP %
Group 1[b]	17/53	68	7/49	86	2/31	93	2/35	94	16/47	66	8/43	77	14/47	70	3/43	93	3/31	90	5/27	82
Group 2[c]	2/60	96	0/60	100	0/51	100	0/51	100	3/49	94	1/49	98	4/40	90	1/40	97	0/49	100	0/49	100
Combined	19/113	83	7/109	93	2/82	93	2/86	93	19/96	80	9/92	90	18/87	79	4/83	95	4/80	95	5/76	93

Abbreviations: ASA, acetylsalicylic acid; c, concentration; DIC, diclofenac; MET, metamizole; NAP, naproxen; PAR, paracetamol; SE, sensitivity; SP, specificity.
[a] Cutoff positivity (according to ROC curve on 60 patients and 30 controls): >5% activation; SI>2
[b] Plasma leukocytes (Graz, Cracow, Lodz, Malaga, Setubal, Wroclaw)
[c] Buffy coat leukocytes (Pamplona, Limoges)
[d] BAT-positive/No. tested

f) Totally negative BAT and CAST results to all 5 NSAIDs tested (22 [16%] of the 140 fully evaluable patients, 81 [60%] of the 136 evaluable controls).

The overall results of BAT and CAST in 140 evaluable patients are summarized in Table 3. Patients validated by a positive provocation (n=64), mostly to ASA 500 mg or more, were first considered separately from those who did not undergo provocation testing but had a convincing history with at least 2 classic clinical episodes following exposure to 1 or more NSAIDs (n=43). In the positive population that underwent provocation testing, 40/64 (63%) showed multiple positive BAT results (categories A or B). In the population with a convincing history (2 or more episodes), 28/43 (65%) showed multiple positive BAT results (categories A or B). Entirely negative BAT results were obtained in 6 (10%) and 9 (21%) patients from the former and latter populations, respectively. therefore, both populations were considered validated (Table 3). An additional population of 33 cases included patients with a single clinical manifestation or a less clear-cut history. One or several positive BAT results were observed in about 75% of both populations. A positive CAST result was observed in about 30% together with a positive BAT result and in about 10% in the absence of a positive BAT result.

Since about half of the patients in this multicenter study were from a single group (PAM), it was important to assess whether this introduced a significant bias and whether the percentage of positive patients differed between the groups. As can be seen from Table 4, this does not seem to be the case, and groups providing 10 patients or more have a similar percentage of positive patients.

Very strikingly, however, this does not appear to be the case with controls (Table 5). While in 3 groups (PAM, LIM, LOD), the number of control cases with positive BAT and/or CAST results was very low, resulting in high specificity, positive control cases in most other groups were relatively frequent, resulting in poor sensitivity. This was initially unexpected and not readily understood, since all groups had allegedly used the same protocol and the same reagents. However, further enquiry revealed that the 2 groups with low control positivity had used a different method for cell isolation and preparation (buffy coat) than the technique recommended by the manufacturer and used by all the other groups (plasma leukocytes). The reasons for this discrepancy are analyzed in more detail below and elsewhere [54].

Sensitivity and specificity were calculated for each of the 5 NSAIDs tested, either by separating the groups using the buffy coat or the plasma leukocyte cell isolation technique, or by combining them (Table 6). Paracetamol and metamizole contributed little to positive results, while ASA, DIC, and NAP mostly resulted in parallel positive results. In fact, when ASA, DIC, and NAP were considered together, a sensitivity of 70%-75% was obtained, with specificity varying from 47% to 91% depending on the cell isolation method used. It appeared that the best results were obtained using both concentrations 1 and 2, whereas concentration 2 alone was definitely less sensitive (Table 7).

All the results presented above were obtained using 5% basophil activation and an SI >2. This cutoff was based on the first series of patients reported [38,41]. A study of the ROC

Table 7. Combined BAT Analysis for ASA, DIC, and NAP[a]

Sensitivity (patients)			Specificity (controls)		
		SE %			SP %
Group 1, c1 only	[b]28/41	68.3	Group 1, c1 only	24/45	46.7
Group 2, c1 only	53/73	79.4	Group 2, c1 only	6/68	91.2
Groups 1+2, c1 only	81/114	71.0	Group 1+2, c1 only	30/113	70.0
Group 1, c2 only	18/41	43.9	Group 1, c2 only	10/45	73.5
Group 2, c2 only	29/73	34.5	Group 2, c2 only	1/68	98.6
Group 1+1, c2 only	47/114	41.2	Group 1+1, c2 only	11/113	90.3
Group 1, c1 + c2	31/41	75.6	Group 1, c1 + c2	24 /45	46.7
Group 2, c1 + c2	55/73	75.3	Group 2, c1 + c2	6/68	91.2
Group 1+2, c1+c2	86/114	75.4	Group 1+2, c1+c2	30/113	73.5

Abbreviations: ASA, acetylsalicylic acid; BAT, basophil activation test; DIC, diclofenac; NAP, naproxen; SE, sensitivity; SP, specificity.
[a] Any or all of the 3 positive
[b] BAT-positive/no. tested

Table 8. BAT Sensitivity in NSAID-Sensitive Patients According to Positivity Criteria (ENDA All Groups)

Group	Positivity Criterion	BAT % Baseline	Positive Control	ASA 1	ASA 0.2	DIC 0.3	DIC 0.06	NAP 1	NAP 0.2	ADN Sum	ADN Positivity Criterion
Mean		4.16	44.89	10.63	7.91	12.24	8.94	12.49	7.213	50.61	
Pos/no.	> 5%			50/127	22/124	50/116	38/113	41/97	20/93	62/95	>25 SI>2
SE				*39*	*18*	*43*	*34*	*42*	*22*	*65*	
Pos/no.	> 8 %			41/127	19/124	46/116	26/113	35/97	16/93	43/95	>50 SI>2
SE				*32*	*15*	*40*	*23*	*36*	*17*	*45*	
Pos/no.	> 10%			31/127	19/124	39/116	25/113	34/97	12/93		
SE				*24*	*15*	*34*	*22*	*35*	*13*		

Buffy coat leukocyte method

Group	Positivity Criterion	BAT % Baseline	Positive Control	ASA 1	ASA 0.2	DIC 0.3	DIC 0.06	NAP 1	NAP 0,2	ADN Sum	ADN Positivity Criterion
Mean		3.46	51.93	9.69	6.81	9.7	6.6	11.88	5.72	40.18	
Pos/no.	> 5%			33/84	14/84	26 /73	22/73	23 /53	10/53	37/58	>25 SI>2
SE				*39*	*17*	*36*	*30*	*43*	*19*	*64*	
Pos/no.	> 8 %			26/84	10/84	23/73	13/73	20/53	6/53	21/58	>50 SI>2
SE				*31*	*12*	*32*	*18*	*38*	*11*	*36*	
Pos/no.	> 19%			19/84	10/84	19/73	12/73	19/53	4/53		
SE				*23*	*12*	*26*	*16*	*36*	*8*		

Plasma leukocyte method

Group	Positivity Criterion	BAT % Baseline	Positive Control	ASA 1	ASA 0.2	DIC 0.3	DIC 0.06	NAP 1	NAP 0.2	ADN Sum	ADN Positivity Criterion
Mean		5.84	33.34	12.84	10.11	17.36	12.96	13.72	8.95	74.59	
Pos/no.	> 5%			15/42	9/42	23/42	17/42	17/42	10/42	27/42	>25 SI>2
SE				*36*	*21*	*54*	*40*	*40*	*24*	*64*	
Pos/no.	> 8 %			14/42	9/42	22/42	13/42	14/42	10/42	19/42	>50 SI>2
SE				*33*	*21*	*52*	*31*	*33*	*24*	*45*	
Pos/no.	> 19%			11/42	9/42	20/42	13/42	14/42	10/42		
SE				*26*	*21*	*48*	*31*	*33*	*24*		

Abbreviations: ADN, acetylsalicylic acid-diclofenac-naproxen; ASA, acetylsalicylic acid; BAT, basophil activation test; ENDA, European Network for Drug Allergy; NAP, naproxen; pos, positive.

curves including the cases of the multicenter study yielded essentially the same results (results not shown). The effect of choosing a higher cutoff of 8% or 10% for basophil activation is shown in Table 8. As expected, increasing the cutoff slightly diminishes sensitivity but increases specificity, thus making it less dependent on the cell isolation method used (Table 8). It also shows that the plasma leukocyte technique yields higher BAT activation values to NSAIDs, increasing sensitivity but decreasing specificity. For some unknown reason, BAT activation values to anti-IgE receptor antibody are higher with buffy coat leukocytes than with plasma leukocytes (Table 9).

If we examine the results for individual patients, it quickly becomes clear that most NSAID-hypersensitive patients, when BAT positive, react to ASA, DIC, and NAP (Figure 1), often with a quantitative correlation of the activation values obtained (ASA/DIC, r=0.67; ASA/NAP, r=0.77; DIC/NAP, r=0.69). In order to facilitate clinical evaluation of the results, we combined the values obtained in a so-called ADN index (ASA/DIC/NAP). This index is calculated by adding the BAT values (as a percentage) for 2 concentrations of ASA, DIC,

Table 9. BAT Specificity in Controls According to Positivity Criteria in Control

Controls all groups

	Positivity Criterion	BAT % Baseline	Positive Control	ASA 1	ASA 0.2	DIC 0.3	DIC 0.06	NAP 1	NAP 0.2	ADN Sum	ADN Positivity Criterion
Mean		3	37.08	3.81	2.6	6.34	3.78	4.26	3.03	19.25	criterion
Pos/no.	> 5%			14/111	6/111	20/97	10/97	15/89	4/89	17/85	
SP				*87*	*95*	*79*	*89*	*83*	*95*	*80*	>25 SI>2
Pos/no.	> 8 %			9/111	2/111	17/97	7/97	10/89	2/89	9/87	
SP				*92*	*98*	*82*	*92*	*88*	*97*	*90*	>50 SI>2
Pos/no.	> 10%			5/111	1/111	15/97	6/97	6/89	1/89		
SP				*95*	*99*	*84*	*94*	*93*	*99*		

Buffy coat leukocyte method

	Positivity Criterion	BAT % Baseline	Positive Control	ASA 1	ASA 0,2	DIC 0,3	DIC 0.06	NAP 1	NAP 0.2	ADN Sum	ADN Positivity Criterion
Mean		2.61	42,26	2.5	2.05	3.71	2.83	2.08	2.55	12.4	criterion
Pos/no.	> 5%			2 /61	0/61	3 /50	1/50	4/41	1/41	2 /50	
SP				*97*	*100*	*94*	*98*	*90*	*98*	*96*	>25 SI>2
Pos/no.	> 8%			0/61	0/61	2/50	1/50	0/41	1/41	1/50	
SP				*100*	*100*	*96*	*98*	*100*	*98*	*98*	>50 SI>2
Pos/no.	> 10%			0/61	0/61	1/50	1/50	0/41	1/41		
SP				*100*	*100*	*98*	*98*	*100*	*98*		

Plasma leukocyte method

	Positivity Criterion	BAT % Baseline	Positive Control	ASA 1	ASA 0,2	DIC 0.3	DIC 0.06	NAP 1	NAP 0.2	ADN Sum	ADN Positivity Criterion
Mean		3.44	31.21	5.42	3.28	8.64	4.87	6.08	3.38	27.1	criterion
Pos/no.	>5%			12/50	6/49	16/46	8/43	11/46	3/43	15 /43	
SP				*76*	*88*	*65*	*81*	*76*	*93*	*65*	>25 SI>2
Pos/no.	> 8%			8/50	2/49	14/46	5/43	10/46	1/43	8/43	
SP				*84*	*96*	*70*	*88*	*78*	*98*	*81*	>50 SI>2
Pos/no.	> 10%			5/50	1/49	11/46	4/43	6/46	0/43		
SP				*90*	*98*	*76*	*91*	*87*	*100*		

Abbreviations: ADN, acetylsalicylic acid-diclofenac-naproxen; ASA, acetylsalicylic acid; BAT, basophil activation test; DIC, diclofenac; NAP, naproxen; pos, positive; SP, specificity.

Table 10. Combined ASA, DIC, and NAP Using the ADN Index

	ADN Index	Sensitivity, %	Specificity, %
ENDA all cases	SI>25	65	80
	SI>50	45	3
ENDA Buffy coat	SI>25	64	96
	SI>50	36	98
ENDA Leukocytes	SI>25	64	65
	SI>50	45	81

Abbreviations: ADN, acetylsalicylic acid-diclofenac-naproxen; ASA, acetylsalicylic acid; DIC, diclofenac; ENDA, European Network for Drug Allergy; NAP, naproxen; SI, stimulation index.

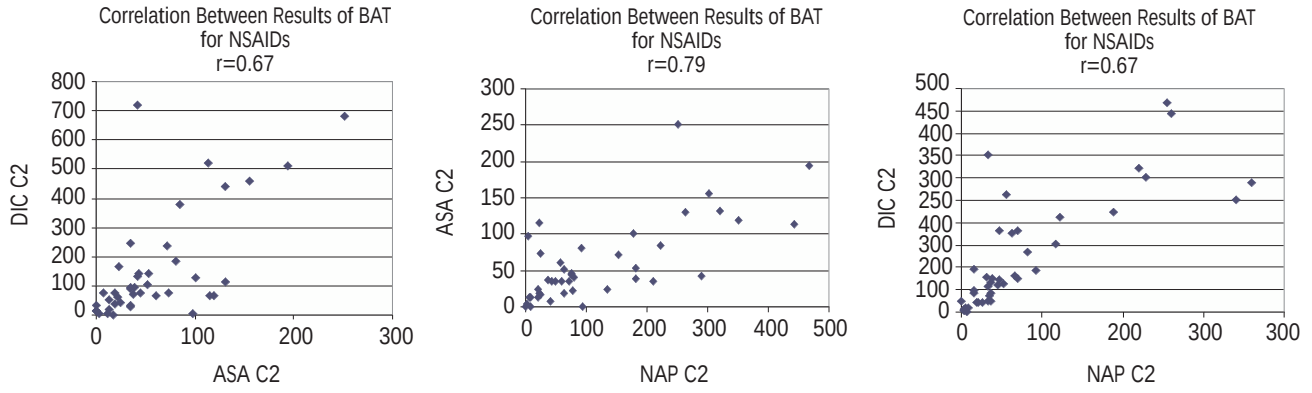

Figure 1. Correlation of basophil activation test results between acetylsalicylic acid, diclofenac, and naproxen among patients with NSAID hypersensitivity syndrome. NSAID indicates nonsteroidal anti-inflammatory drug; C, concentration.

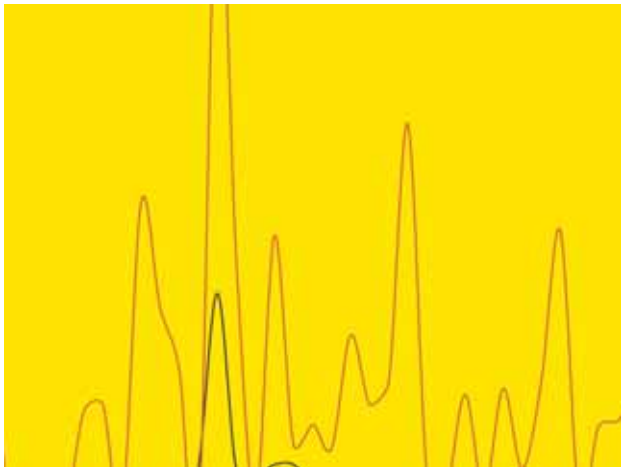

Figure 2. Net value of ADN index in patients and controls. ADN indicates acetylsalicylic acid-diclofenac-naproxen.

and NAP and subtracting 6 times the negative control baseline value from the sum obtained. As shown in Table 10, such a calculation does not markedly affect sensitivity when the ADN index is >25, since evaluation with a positive criterion of 5% basophil activation to any of the 3 NSAIDs has already identified a maximum of BAT-positive patients. However, the ADN index increases specificity by magnifying the difference between clinically positive patients and negative controls with an occasional positive BAT result. This is also clear from Figure 2. The ADN index also enables a quantitative evaluation to be made. In Tables 8 and 9, the highest sensitivity manifested by the plasma leukocyte cell isolation technique is also manifested by a higher ADN index.

As for CAST, there is clearly a correlation between the results of BAT and CAST in a sizeable number of patients. With a cutoff point for CAST of 100 pg/mL greater than the baseline value and an SI >2, 46 patients of 101 tested (45.5%)

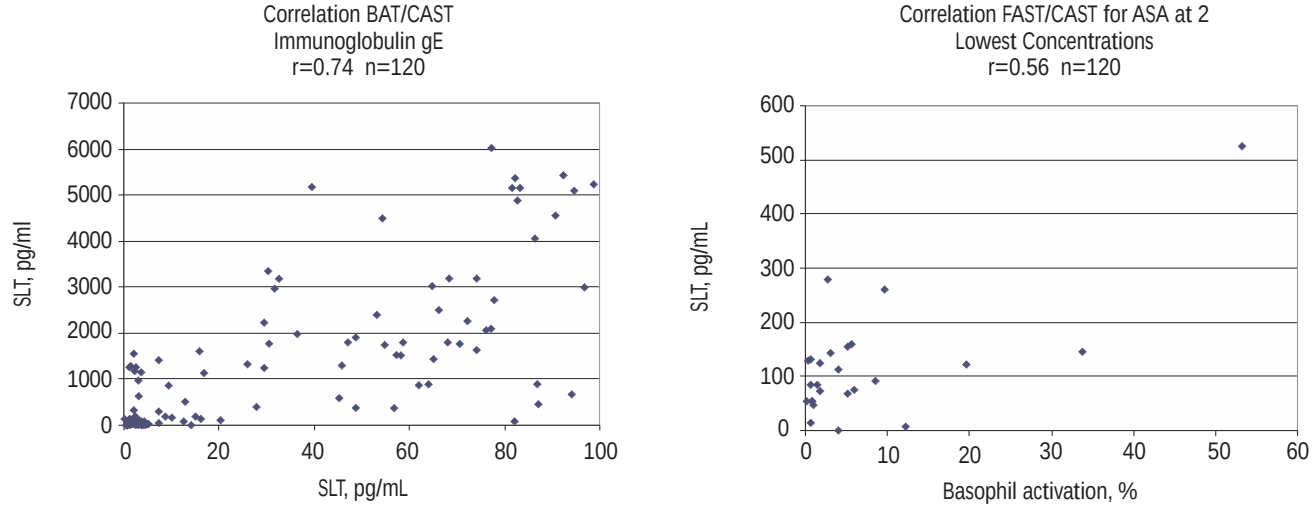

Figure 3. Correlation between the basophil activation test and cell allergen stimulation test using anti–immunoglobulin E and acetylsalicylic acid.

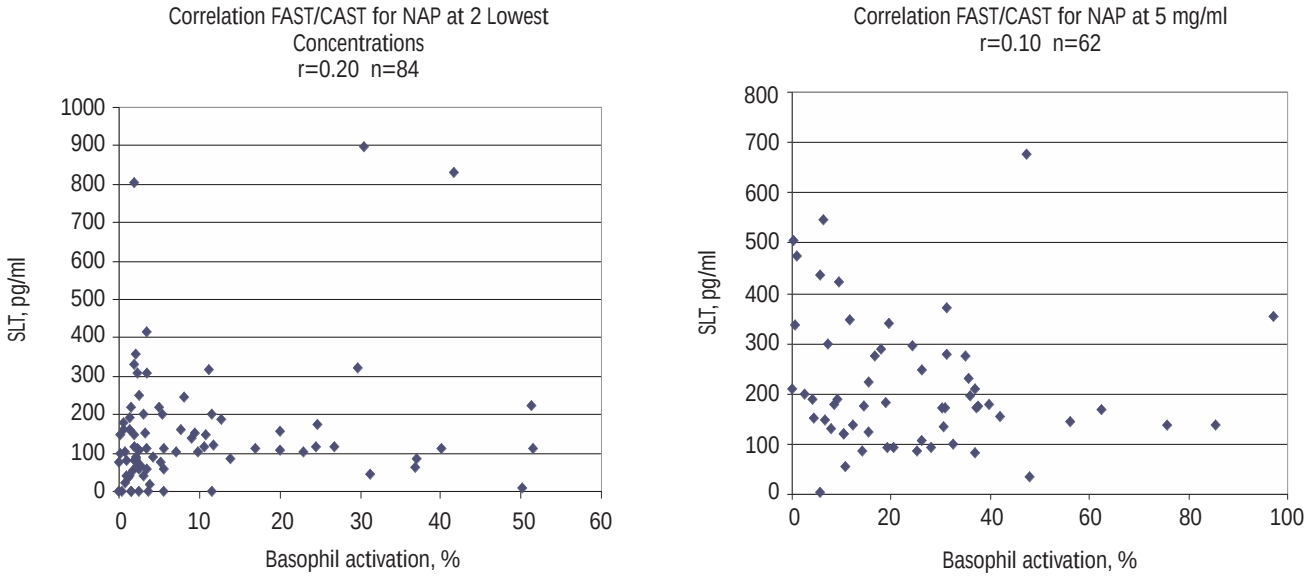

Figure 4. Correlation between the basophil activation test and cell allergen stimulation test using naproxen. sLT indicates sulfidoleukotriene.

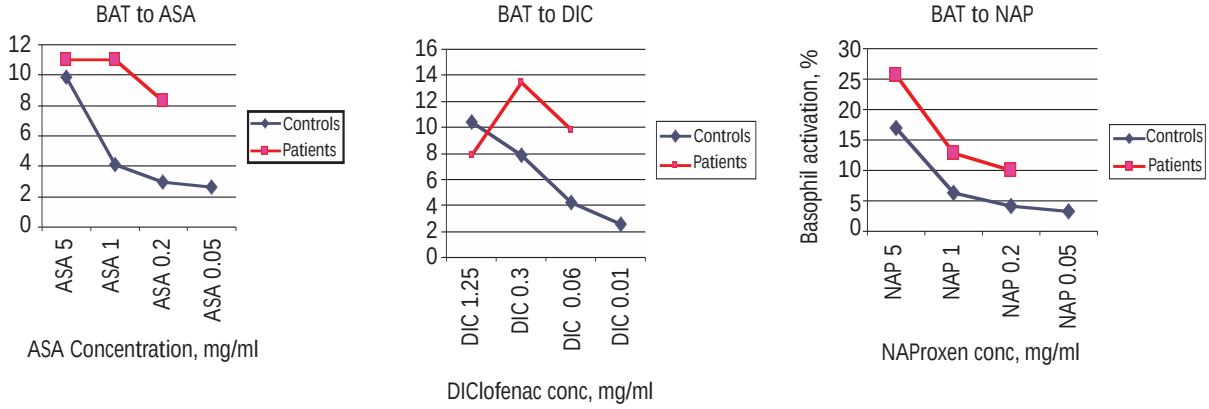

Figure 5. Dose-response curves for acetylsalicylic acid, diclofenac, and naproxen in hypersensitive patients and controls. The number of test points are given for both patients and controls.

were CAST-positive (Table 3); 36 of 101 (35.6%) were positive to both BAT and CAST. The correlation is not only qualitative (pos/neg) but also quantitative (Figures 3 and 4). It is, however, markedly higher for basophil activation induced by anti-IgE receptor antibody than for stimulation induced by ASA (Figure 3) or NAP (Figure 4). A number of patients have a positive BAT result but negative CAST results to several NSAIDs and vice versa.

It is also obvious that the BAT activation response to NSAIDs is dose-dependent, both in clinically hypersensitive patients and in controls. This is already apparent with the 2 concentrations used in the ENDA multicenter study (concentrations 1 and 2 in Tables 6, 8, and 9), although it became even more evident when 2 additional concentrations, one higher than concentration 1 and one lower than concentration 2 were used in a number of patients and controls (Figure 5) (detailed results not shown). The difference between controls and patients in their BAT response to NSAIDs in vitro is represented by a shift in their dose-response curve.

Several authors highlight the problem of so-called BAT nonresponders, which makes it impossible to interpret the results in as many as 8%-10% of cases [43,53]. In this multicenter study, 14 patients of the 152 tested (10%) were nonresponders to the BAT-positive control (anti-IgE receptor antibody). However, they were all positive to CAST performed on the same sample, thus qualifying them as false nonresponders, an artifact that has been elucidated elsewhere [53] and no longer occurs in later phases of the study. Accordingly, true nonresponders amounted in this study to 0/152 (0%) in patients and 1/152 (0.6%) in controls.

Discussion

The characteristics of our study population correspond to the classic descriptions of NSAID hypersensitivity syndrome [3,7-10]. For most patients, the syndrome started in the third to fourth decade of life and manifested in the airways or on skin. It was often preceded by episodes of rhinitis and asthma or urticaria before the ability of NSAIDs to elicit symptoms became evident. The preponderance of patients with skin symptoms in this collective is probably due to the fact that most groups participating in the study were from allergy or dermatology departments, and very few were from internal medicine or pneumology departments. When analyzed separately, however, patients with cutaneous or respiratory symptoms had the same percentage (64%-65%) of strongly positive BAT results, thus enabling them to be considered together. Other authors have shown a similar mechanism between these 2 categories of patients [55].

Most of the study patients (107/140 evaluated) can be considered as validated, that is, they had experienced NSAID hypersensitivity syndrome, since they had positive results to provocation testing with ASA or presented a history of at least 2 clinical events with 1 or more NSAIDs. As shown, both groups yielded a similar percentage of multiple positive BAT results (63%-65%). Even in the 33 patients with a less clear-cut clinical validation, it appears that the clinical history was reliable, since the percentage of strongly positive BAT results was quite similar, although with a slightly higher percentage of negative test results.

NSAID hypersensitivity syndrome does not appear to be related to atopy, since most patients (about 75%) have neither a history of nor positive test results (IgE, skin tests) for atopy. In controls, the proportion of atopic patients was markedly higher (about 45%), probably reflecting the population of patients easily available in an allergy department. However, all of the controls were shown by provocation to be clinically tolerant to ASA.

As can be seen from Table 2 and the results presented above, 57% of 140 patients presented very clear-cut positive BAT results (categories A and B) to multiple NSAIDs and 16% were entirely negative, making interpretation easy. In about 27% of cases, positive results were obtained with 1 or 2 concentrations of a single NSAID.

In patients with a validated history, the BAT results were relatively homogeneous between the groups, particularly when a larger number of patients (7 or more) were studied (Table 3). However, extensive heterogeneity was observed in controls (Table 4). This could be due to the clinical status of patients taken as controls. For example, in most groups, a sizeable number of controls were asymptomatic individuals with no signs or history of allergic reactions. In 1 group (CRA), however, all controls had perennially active asthma and were under treatment at the time of the BAT, although they showed negative results to a provocation test with ASA. In this group, 7 out of 9 presented multiple positive BAT results. However, in another group (LOD) of 10 controls also afflicted by perennial asthma and rhinitis but negative ASA asthma provocation, none presented a positive BAT result. Therefore, the question remains open as to whether patients with respiratory symptoms (asthma, rhinitis) or cutaneous symptoms (urticaria) are more prone to basophil activation in vitro by NSAIDs, even if they tolerate them clinically. Other studies [56] have demonstrated that patients who are symptomatic at the time of basophil-based tests are more prone to basophil activation.

Another major factor in the heterogeneity of controls seems to be the cell isolation technique used. This study revealed, apparently for the first time, that a number of control patients, even those who tolerated ASA, clearly show dose-dependent basophil activation in vitro by NSAIDs. Furthermore, this activation appears more pronounced and more frequent when plasma leukocytes rather than buffy coat cells are used for the BAT (Tables 6, 8, and 9). This phenomenon is further analyzed and discussed elsewhere [54].

Our results clearly show that NSAID hypersensitivity in vitro is not an all-or-nothing qualitative phenomenon, but rather a shift in the dose-response curve that appears to occur in parallel for various NSAIDs. Indeed, reactivity to ASA, DIC, and NAP appears to be quantitatively correlated (Figure 1). It is also obvious that the BAT tests are much easier to interpret when performed with several NSAIDs (ASA, DIC, and NAP), at least at 2 concentrations. This makes it possible to interpret results using the corresponding combined ADN index (Figure 2). Some previous negative or less favorable reports in the literature on the results of basophil-based in vitro tests in NSAID-hypersensitive patients [30] included only 1 NSAID, sometimes at only 1 markedly lower concentration [37], and are therefore easily explainable. Furthermore, reviewers should no longer consider that such reports contradict other positive findings obtained under different technical conditions. It is always important to consider the details.

The finding that clinical hypersensitivity to NSAIDs is often accompanied by dose-dependent in vitro basophil hypersensitivity is not revolutionary. It corresponds rather well with the hypothesis that NSAID hypersensitivity is not an immunological but a pharmacological phenomenon related to the inhibitory effect of these drugs on prostaglandin synthesis [4]. The fact that some controls that are clinically tolerant to NSAIDs show positive BAT results and that some clinically hypersensitive patients show negative BAT results does not invalidate the hypothesis. It is well documented that the minimum NSAID dose needed to elicit clinical symptoms can vary by a factor of as much as 100 (eg, 5-500 mg) in hypersensitive patients. Conditions of in vitro and in vivo administration are also very different. It would be interesting, although cumbersome, to evaluate by progressive provocation in vivo whether the dose-response curve in vivo parallels the BAT dose-response curve in vitro.

Although BAT with NSAIDs does not correlate completely with clinical provocation in qualitative terms—about a quarter of provocation-positive patients have negative results in BAT—there is no doubt that patients with a clinical history of allergy to NSAIDs supported by positive provocation test results have significantly more positive BAT results than individuals with no history who tolerate NSAIDs. The BAT with several NSAIDs at 2 appropriate concentrations appears to have confirmatory diagnostic value when positive. It should no longer be legitimate to state that no in vitro tests exist for that condition [3]. In addition, these findings add new

elements to the discussion on the pathogenesis of NSAID hypersensitivity syndrome, as discussed in greater detail elsewhere [44,47,52].

There also appears to be an association between the results of CAST (based on production of sLTs by NSAIDs from basophils in vitro) and clinical NSAID hypersensitivity syndrome, as shown elsewhere [33,34,36] and as confirmed by this multicenter study. Some negative reports [37] appear to be due to suboptimal or inappropriate technical conditions, as discussed elsewhere [53]. Nevertheless, in practice, there is no absolute parallelism between BAT and CAST results (as we show here), since CAST shows positive results in only about half of the BAT-positive cases. CAST is positive alone in only about 6% of the cases with a clinical history and positive NSAID provocation results. The reasons for such discrepancies are manifold. BAT and CAST detect different steps in basophil activation, and these steps may be influenced by various factors on an individual basis. For example, we have clearly established that BAT is more dependent than CAST on the external Ca^{+} concentration [53]. In practical terms, it seems that the BAT has greater diagnostic value than CAST under the conditions used in that study.

BAT seems particularly indicated in patients with a clinical history of NSAID intolerance in whom a provocation test is not advisable for ethical, clinical, or other reasons. Clear-cut positive results can be considered as confirming a history of NSAID hypersensitivity, while negative results may not exclude it. This situation is similar to that of many in vitro tests in drug hypersensitivity (eg, lymphocyte transformation test).

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References

1. Widal MF, Abrani P, Lermoyez J. Anaphylaxie et idiosyncrasie. Presse Méd. 1922;30:189-92. Reprinted in: Allergy Proc. 1993;14:373-6.
2. Samter M, Beers RF. Concerning the nature of intolerance to aspirin. J Allergy. 1967;40:281-93.
3. Szczeklik A, Stevenson D. Aspirin-induced asthma: Advances in pathogenesis, diagnosis and management. J Allergy Clin Immunol. 2003;111:913-21.
4. Kasper L, Sladek K, Duplaga M, Bochenek G, Liebhard J, Gladysz U, Malolepszy J, Szeklik A. Prevalence of asthma with aspirin hypersensitivity in the adult population of Poland. Allergy. 2003;58:1064-6.
5. Jenkins C, Costello J, Hodge L. Systematic review of prevalence of aspirin induced asthma and implications for clinical practice. BMJ. 2004;328(7437):434.
6. Faich GA. Adverse drug reaction monitoring. N Engl J Med. 1986;314:1589-92.
7. Szczeklik A, Stevenson DD. Aspirin-induced asthma: Advances in pathogenesis and management. J Allergy Clin Immunol. 1999;104:5-13.
8. Stevenson DD. Aspirin and NSAID sensitivity. Immunol Allergy Clin North Am. 2004;24:491-505.
9. Bush RK, Asbury DW. Aspirin-sensitive asthma. In: Busse WW, Holgate ST, editors. Asthma and Rhinitis. Oxford: Blackwell Scientific 2nd ed, 2000. p. 1315-25.
10. Hamad AM, Sutcliffe AM, Knoy AJ. Aspirin-induced asthma: clinical aspects, pathogenesis and management. Drugs. 2004;64:2417-32.
11. Sainte-Laudy J. Nine cases of suspected IgE-mediated anaphylaxis induced by aspirin. ACI International. 2002;14:220-2.
12. Blanca M, Pérez E, García JJ, Miranda A, Terrados S, Vega JM, Suau R. Angioedema and IgE antibodies to aspirin: a case report. Ann Allergy.1989;62:295-8.
13. Szczeklik A, Gryglewski RJ, Czerniawska-Mysik G. Relationship of inhibition of prostaglandin biosynthesis by analgesics to asthma attacks in aspirin-sensitive patients. Br Med J. 1975;1:67-9.
14. Szczeklik A. The cyclooxygenase theory of aspirin-induced asthma. Eur Respir J. 1990;3:588-93.
15. Stevenson DD, Lewis RA. Proposed mechanism of aspirin sensitivity reactions. J Allergy Clin Immunol. 1977;80:788-90.
16. Szczeklik A, Sanak M. Molecular mechanisms in aspirin-induced asthma. ACI International. 2000;12:171-8.
17. Fischer AR, Rosenberg MA, Lilly CM, Callery JC, Rubin P, Cohn J, White MV, Igarashi Y, Kaliner MA, Drazen JM, Israel E. Direct evidence for a role of the mast cell in the nasal response to aspirin in aspirin-sensitive asthma. J Allergy Clin Immunol. 1994;94:1046-56.
18. Nasser SM, Lee TH. Leukotrienes in aspirin-sensitive asthma. In: Szczeklik A, Gryglewski RJ, Vane J, editors. Eicosanoids, aspirin and asthma. New York: Marcel Dekker, 1998. p. 317-36.
19. Lee TH, Smith CM, Arm JP, Christie PE. Mediator release in aspirin-induced reactions. J Allergy Clin Immunol. 1991;88:827-9.
20. Cowburn AS, Sladek K, Soja J, Adamek L, Nizankowska E, Szczeklik A, Lam BK, Penrose JF, Austen KF, Holgate ST, Sampson AP. Overexpression of leukotriene C4 synthase in bronchial biopsies from patients with aspirin-intolerant asthma. J Clin Invest. 1998;101:834-46.
21. Sanak M, Simon H-U, Szczeklik A. Leukotriene C4 synthase promoter polymorphism and risk of aspirin-induced asthma. Lancet. 1997;350:1599-2000.
22. Sanak M, Szczeklik A. Genetics of aspirin induced asthma. Thorax. 2000;55(Suppl 2):S45-S47.
23. Van Sambeck R, Stevenson DD, Baldasaro M, Lam BK, Zhao JL, Yoshida S, Yandora C, Dos JM, Penrose JF. 5' Flanking region polymorphism of the gene encoding leukotriene C4 synthase does not correlate with the aspirin-intolerant asthma phenotype in the United States. J Allergy Clin Immunol. 2000;106:72-6.
24. Torres-Galvan MJ, Ortega N, Sanchez-Garcia F, Blanco C, Carrillo T, Quiralte J. LTC4-synthase A-444C polymorphism: lack of association with NSAID-induced isolated periorbital angioedema in a Spanish population. Ann Allergy Asthma Immunol. 2001;87:506-10.
25. Kawagishi Y, Mita H, Taniguchi M, Maruyama M, Ooosaki

R, Higashi N, Kashii T, Kobayashi M, Akyama K. Leukotriene C4 synthase promoter polymorphism in Japanese patients with aspirin-induced asthma J Allergy Clin Immunol. 2002;109:936-42.
26. Kedda MA, Shi J, Duffy D, Phelps S, Yang L, O'Harn K, Fong K, Thompson PJ. Characterization of two polymorphisms in the leukotriene C-4 synthase gene in an Australian population in subjects with mild, moderate and severe asthma. J Allergy Clin Immunol. 2004;113:889-95.
27. Choi JH, Park HS, Oh HB, Lee JH, Suh YJ, Park CS, Shin HD. Leukotriene-related gene polymorphism in ASA-intolerant asthma: an association with a haplotype of 5 lipoxygenase. Hum Genet. 2004;114:337-44
28. Stevenson DD, Simon RA. Sensitivity to aspirin and nonsteroidal anti-inflammatory drugs. In "Allergy: Principles and practice" (Middleton E, Reed CE, Ellis EF, Adkinson NF, Yunginger JW, Busse WW, editors) St. Louis, Mosby, 1998, p. 1225-34.
29. Czech W, Kapp A, Kownatzki E, Schöpf E. Die Bedeutung der in vitro-induzierten Histaminfreisetzung aus isolierten Leukozyten für die Diagnostik pseudo-allergischer Reaktionen auf Azetylsalizylsäure. Allergologie. 1990;13:201-4.
30. Lebel B, Messaad B, Kvedariene V, Rongier M, Bousquet J, Demoly P. Cysteinyl-leukotriene release test (CAST) in the diagnosis of immediate drug reactions. Allergy. 2001;56:688-92.
31. De Weck AL, Stadler BH, Urwyler A, Wechsner HU, Bühlmann RP. Cellular antigen stimulation test (CAST): a new dimension in allergy diagnosis. Allergy Clin Immunol News. 1993;5:9-14.
32. De Weck AL. Zellulärer Allergen De -Stimulierungs-Test (CAST). Eine Uebersicht und kritische Auswertung der klinischen Anwendung in der Allergiediagnose. Allergologie. 1997;20:487-502.
33. De Weck AL. Cellular Allergen Stimulation Test (CAST) 2003: a review. J Invest Allergol Clin Immunol. 2004;14:253-73.
34. Kubota Y, Imayama S, Toshitani A, Miyahara H, Tanahasshi T, Uemura Y, Koga T, Sugawara S, Kurimoto F, Hata K. Sulfidoleukotriene release test (CAST) in hypersensitivity to nonsteroidal anti-inflammatory drugs. Int. Arch. Allergy Immunol. 1997;114:361-6.
35. Mewes T, Riechelmann H, Klimek L. Increased in vitro cysteinyl leukotriene release from blood leukocytes in patients with asthma, nasal polyps and aspirin intolerance. Allergy. 1996;51:506-10.
36. May A, Weber A, Gall H, Kaufmann R, Zollner TM. Means of increasing sensitivity of an in vitro diagnostic test for aspirin intolerance. Clin Exp Allergy. 1999;29:1402-11.
37. Pierzchalska M, Mastalerz L, Sanak M, Zalula M, Szczeklik A. A moderate and unspecific release of cysteinyl leukotrienes by aspirin from peripheral blood leukocytes precludes its value for aspirin sensitivity testing in asthma. Clin Exp Allergy. 2000;30;1785-91.
38. Sanz ML, Gamboa P, de Weck AL. A new combined test with flowcytometric basophil activation and determination of sulfidoleukotrienes is useful for in vitro diagnosis of hypersensitivity to aspirin and other nonsteroidal anti-inflammatory drugs? Int Arch Allergy. 2005;136:58-72.
39. Sabbah A, Drouet M, Sainte-Laudy J, Lauret MG, Loiry M. Apport de la cytométrie en flux dans le diagnostic allergologique. Allergie & Immunologie. 1997;29:15-21.
40. Sanz ML, Maselli JP, Gamboa PM, Garcia-Aviles MC, Oehling A, Diéguez I, de Weck AL. Flow-cytometric basophil activation test. A review. J Invest Allergol Clin Immunol. 2002;12:143-54.
41. Gamboa P, Sanz ML, Caballero MR, Urrutia I, Antepara I, Esparza I, de Weck AL. The flow-cytometric determination of basophil activation induced by aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) is useful for in vitro diagnosis of the NSAID hypersensitivity syndrome. Clin Exp Allergy. 2004;34:1448-57.
42. De Weck AL, Sanz ML. Flow cytometric Cellular Allergen Stimulation Test (FAST / Flow CAST): technical and clinical evaluation of a new diagnostic test in allergy and pseudo-allergy. ACI International. 2002;14:204-15.
43. Ebo DG, Hagendorens MM, Bridts CH, ScheurweghAJ, De Clerck LS, Stevens WJ. In vitro allergy diagnosis: should we follow the flow? Clin Exp Allergy. 2004;34:332-9.
44. De Weck AL, Gamboa PM, Esparza R, Sanz ML. Hypersensitivity to aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs). Curr Pharm Des. 2006;12(26):3347-58.
45. De Weck AL, Sanz ML, Gamboa PM, Aberer W, Bienvenu J, Blanca M, Demoly P, Ebo DG, Mayorga L, Monneret G, Sainte-Laudy J. Diagnostic tests based on human basophils: more potentials and perspectives than pitfalls. I. Clinical issues. Int Arch Allergy Immunol. 2008;146(3):177-89.
46. Sanz ML, Gamboa PM, de Weck AL. Cellular tests in the diagnosis of drug hypersensitivity. Curr Pharm Design. 2008;14:2803-8.
47. De Weck AL, Sanz ML, Gamboa PM. Hypersensitivity to NSAID Drugs – A new integrated approach to its pathophysiological understanding and diagnosis . 25 th CIA Symposium Bornholm 2003 – ACI International J World Allergy Org, suppl 2, 2005; 203-8.
48. De Weck AL, Sanz ML, Gamboa PM, Aberer W, Sturm G., Blanca M, Torres M, Mayorga L, Correia S, Kowalski M, Medrala W, Szczeklik A, and ENDA group. Diagnosis of hypersensitivity to non-steroidal anti-inflammatory drugs (NSAIDs) in vitro by flowcytometry (Flow-CAST) and sulfidoleukotriene assay. A multicentric study. EAACI Vienna 2006 (Abstr 654)
49. De Weck AL, Sanz ML, Gamboa PM. Further considerations on the mechanism of NSAID hypersensitivity. In "Cellular and Molecular Targets in Allergy and Clinical Immunology" 26th CIA Symposium, Malta, 2006 (S Holgate, G Marone, J Ring editors) J World Allergy Org, Göttingen: Hogrefe; 2007. Suppl 2, p. 297-300.
50. Sanz ML, Gamboa PM. De Weck AL. In vitro tests: Basophil activation tests. In Pichler W (editor). Drug Hypersensitivity. Basel: Karger; 2007. p. 389-400.
51. De Weck AL, Sanz ML. Basophil activation tests in allergy diagnosis In "Allergy Frontiers" (Pawankar R, Holgate S, editors) Springer Japan. 2009 (in press).
52. De Weck AL, Sanz ML, Gamboa P. New pathophysiological concepts on aspirin hypersensitivity (Widal syndrome); diagnostic and therapeutic consequences. Bull Natl Acad Med (Paris). 2005;189:1201-18.
53. De Weck AL, Sanz ML, Gamboa PM, Aberer W, Bienvenu J, Blanca M, Demoly P, Ebo DG, Mayorga L, Monneret G, Sainte Laudy J. Diagnostic tests based on human basophils: more potentials and perspectives than pitfalls. II. Technical issues. J Invest Allergol Clin Immunol. 2008;18:143-55.
54. De Weck AL, Sanz ML, Gamboa PM, Jermann JM, Medrala W, Sainte-Laudy J, Schneider MS, Weber JM. A multicentric study on the NSAID hypersensitivity syndrome. II. Basophil activation by NSAIDs and its impact on the pathogenesis of the syndrome. J Invest Allergol Clin Immunol. 2009 (in press).

55. Mastalerz L, Setkowicz M, Sanak M, Szczeklik A. Hypersensitivity to aspirin: common eicosanoid alterations in urticaria and asthma. J Allergy Clin Immunol. 2004,113:771-5.
56. Czech W, Schöpf E, Kapp A. Release of sulfidoleukotrienes in vitro: Its relevance in the diagnosis of pseudoallergy to acetylsalicylic acid. Inflamm Res. 1995;44:291-5.

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Dr. María L. Sanz

Department of Allergology and Clinical Immunology
University Clinic of Navarra
Apartado 4209
31008 Pamplona, Spain
E-mail: mlsanzlar@unav.es