

Original article

Aspirin-triggered 15-HETE generation in peripheral blood leukocytes is a specific and sensitive Aspirin-Sensitive Patients Identification Test (ASPITest)*

Background: We have previously demonstrated that aspirin triggers specific generation of 15-hydroxyeicosatetraenoic acid (15-HETE) from nasal polyp epithelial cells and peripheral blood leukocytes (PBL) from aspirin-sensitive (AS) but not aspirin-tolerant (AT) patients with asthma/rhinosinusitis. The goal of this study was to assess the diagnostic value of ASA-induced 15-HETE generation measurement to identify AS patients.

Methods: PBL were obtained from 43 AS patients with asthma and rhinosinusitis, 35 AT asthmatics and 17 healthy control (HC) subjects. PBL were incubated with 2–200 μ M aspirin (ASA) and 15-HETE release was measured in cell supernatants with competitive ELISA.

Results: Unstimulated PBL from all three groups of patients generated similar amount of 15-HETE. Incubation with 200 μ M ASA resulted in an increase in an 15-HETE generation (mean increase +421%) in AS-asthmatics but small and nonsignificant response in AT-asthmatics or control subjects. Receiver operating curve (ROC) analysis revealed that the sensitivity of the test for confirmation of ASA-sensitivity was 83% and the specificity 82%. Positive predictive value was 0.79 and negative predictive value was 0.86. Naproxen induced a significant increase in 15-HETE only in some AS-asthmatics, but not in AT-asthmatics.

Conclusion: Our data demonstrate that ASA-induced 15-HETE generation by PBL is a specific and sensitive aspirin-sensitive patients identification test (ASPITest).

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Aspirin may induce respiratory symptoms in 5–21% of patients with chronic asthma and the prevalence of sensitivity seems to be higher among patients with more severe asthma (1–3). These patients suffer usually from severe hyperplastic rhinosinusitis with nasal polyps, thus sensitivity to aspirin may be considered as a hallmark of a chronic inflammatory disease of the upper and lower airways (4–6). In addition cross-reactivity to other nonsteroidal anti-inflammatory drugs (NSAIDs) is a common feature of AS-asthmatics (7).

The mechanism of aspirin-sensitivity associated with severe asthma and chronic rhinosinusitis with nasal polyposis is not immunological, but is related to cyclooxygenase-1 (COX-1) inhibition by aspirin and subsequent activation of inflammatory cells including mast cells and eosinophils (4, 7–9). Aspirin challenge *in vivo* is accompanied by release of leukotriene metabolites into urine and cysteinyl leukotrienes, mast cell tryptase and ECP into nasal washes (10–15).

Diagnosis of aspirin sensitivity in clinical setting is usually based on medical history, which is quite often not reliable. Although sensitivity to ASA can be confirmed or

excluded by oral, bronchial or intranasal challenge these procedures are time consuming, require special expertise and equipment and thus are not suitable for use in general practice (16–19).

In vitro release of mast cell or eosinophil-specific mediators induced by aspirin could not be demonstrated, and so far there is no commercially available *in vitro* test which could distinguish aspirin sensitive from insensitive individuals (4, 20). Following our previous observation in nasal polyp epithelial cells (21), we have recently reported, that aspirin could specifically trigger *in vitro* generation of arachidonic acid metabolite 15-hydroxyeicosatetraenoic acid (15-HETE) from peripheral blood leukocytes (PBL) in AS-asthmatics, not affecting 15-HETE release in AT-asthmatics or healthy subjects (22). Aspirin-triggered 15-HETE release from PBL seemed to mimic hypersensitivity reaction to aspirin observed *in vivo*: release of 15-HETE could be also triggered by another nonselective cyclooxygenase inhibitors naproxen, but COX-2 selective NSAIDs did not affect the release. Furthermore, misoprostol – a synthetic PGE₁ analogue, which was shown to prevent aspirin-induced asthmatic

reactions, inhibited 15-HETE generation triggered by aspirin *in vitro*.

In this study we determined ASA-induced 15-HETE release from PBLs of well-characterized AS-asthmatics and AT-asthmatics in order to assess diagnostic value of 15-HETE measurement to identify aspirin-sensitive (AS) patients.

Patients

Forty-three aspirin sensitive (AS) patients with asthma and rhinosinusitis and 35 patients with asthma with history of good tolerance of ASA and other NSAIDs (AT) were studied. They were recruited from the database of patients with bronchial asthma treated at the Lodz University Asthma Centre. The diagnosis of aspirin hypersensitivity was based on a positive history of bronchial and nasal reaction to aspirin and in 18 patients in whom the history was not clear cut, the diagnosis was confirmed by positive oral challenge with aspirin or nasal/bronchial challenge with lysine aspirin (23). Atopic status was defined by the presence of positive skin prick test to at least one of the battery of 14 inhalant allergens. Clinical characteristics of patients is shown in Table 1. All AS-asthmatics and majority of AT-asthmatics were treated with fixed doses of inhaled steroids (corresponding to 200–1600 µg of budesonide) and some also used long acting β₂-agonists. Inhaled medications were withheld for at least 12 h before the venipuncture. Patients did not receive any oral medications for at least 48 h before the study and anti-leukotriene drugs were not used at least 4 weeks prior to venipuncture. A control group included 17 healthy persons with history of good tolerance of aspirin, recruited from medical students and department staff (6 males and 11 females). The mean age of this group was 29 ± 2 (age range 26–50 years). They did not take any medications. The procedures (ASA-challenges and

venipunctures) have been approved by the Local Ethical Committee.

Methods

Leukocyte culture and experimental design

Leukocytes were obtained from anti-coagulated venous blood samples. In brief, 50 ml of venous blood were collected into a syringe containing 3.8% sodium citrate and leukocytes were separated by centrifugation on Gradisol G (AquaMedica, Poland). To minimize platelet contamination, the cells were washed with phosphate-buffered saline (PBS) and resuspended in PBS with Mg²⁺ and Ca²⁺. Cell viability determined by trypan blue exclusion test was always over 90%. Cells (3 × 10⁶ cells/ml) were incubated in separate tubes with different concentration of lysine aspirin (ASA; Bayer AG, Leverkusen, Germany), naproxen (Polfa, Pabianice, Poland) or medium. Based on previous time-course experiments, a 60 min time point was chosen as a standard incubation time with ASA (22). Following incubation cells were centrifuged, supernatants collected and stored at –70°C for 15-HETE analysis.

Measurement of eicosanoids

Enzyme-linked immunoassays were used for 15-HETE quantification (Assay Designs, USA). Kits were used following the original manuals and the measurements were performed in duplicates. The results were expressed in picograms per ml (3 millions of cells) of cell suspension. Sensitivity of the immunoassay for 15-HETE detection was 69.2 pg/ml and cross-reactivity with other eicosanoids was negligible.

Statistical analysis

Results of eicosanoids measurement were expressed as medians (25–75%). Wilcoxon-matched pairs test was used for analysis of differences within the group and Mann–Whitney U-test was used to compare data between groups of patients. The ROC curves were prepared and analyzed using GraphROC for Windows. Data for eicosanoids measurements are shown as medians and 25–75%. Results with *P* < 0.05 were considered as statistically significant.

Results

There was no statistical differences in the number of PBLs or eosinophils in AS and AT-asthmatics. Preincubation of PBLs from AS-asthmatics (*n* = 18) with 2, 20 and 200 µM of ASA increased generation of 15-HETE by mean 35, 197, and 360%, respectively (Fig. 1). In AT-asthmatics (*n* = 14) no significant change in the mean 15-HETE release was noticed for the whole range of ASA concentration. Based on these results 200 µM of ASA was used in further experiments.

When leukocytes from the whole group of AS-patients (*n* = 43) were incubated with 200 µM of ASA generation of 15 HETE was significantly increased [from baseline: median 170; range 87–369 pg/ml to median 450 (range 271–982) pg/ml; *P* < 0.001], and the mean increase was 421%. In 38 patients the increase in 15-HETE generation

Table 1. Characteristics of aspirin-sensitive (AS) and aspirin-tolerant (AT) patients with bronchial asthma (differences between groups are not significant except for nasal polyps; **P* < 0.01; Chi-square test)

	AS-asthmatics	AT-asthmatics
N	43	35
Sex (F/M)	32/11	21/14
Age (years)	45 ± 2.1	42 ± 2.6
Age range (years)	23–77	21–74
Patients taking steroids:		
Inhaled (<i>n</i>)	38	16
Mean daily dose (µg)	893 ± 102	667 ± 91
Oral (<i>n</i>)	6	4
Mean daily dose (mg)	0.86 ± 0.35	1.0 ± 0.31
Patients with nasal polyps	21	2*
Patients with positive skin prick test	28	18
Blood eosinophilia (mean ± SD)	4.3 ± 2.8	3.0 ± 1.5

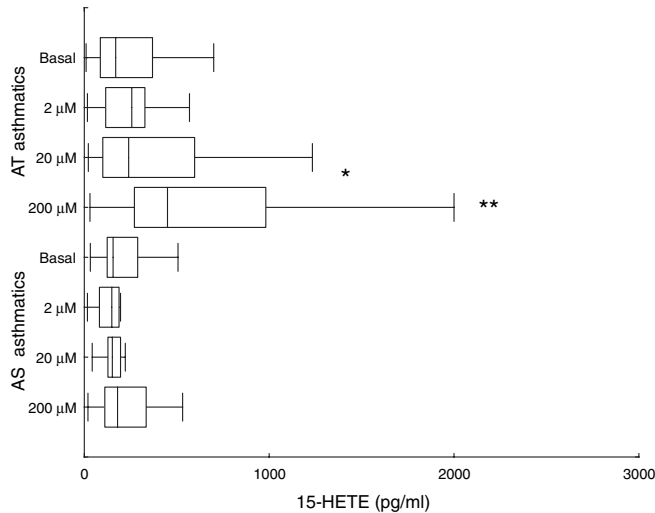


Figure 1. The effect of three concentrations of aspirin on 15-HETE generation in PBLs from AS-asthmatic ($n = 18$) and AT-asthmatic ($n = 14$); * $P < 0.05$ and ** $P < 0.01$ as compared to medium). Data shown as Box-and-Whisker Plot (medians represented by horizontal bars, 25–75% by boxes).

by 13–2800% was observed (mean increase was 482%) and in five patients 15-HETE generation decreased (between –6 and –56%; mean decrease –28%). 15-HETE release was increased by more than 50% in 35 of 43 AS-asthmatic. In contrast in 35 AT-asthmatics exposure to 200 μM of ASA did not change 15-HETE generation (median 156; range 124–289 pg/ml at baseline vs median 180 (111–335) pg/ml after aspirin; NS). In five patients 15-HETE release remained unchanged, in 15 decreased, in 11 patients the increase was less than 50% and in four patients the increase exceeded 50%. No effect of aspirin on 15-HETE release from PBLs was observed in healthy patients with history of good tolerance of aspirin [before aspirin: median 175 (110.5–279) pg/ml; after aspirin: median 196 (131–298) pg/ml; NS) (Fig. 2).

Calculation of sensitivity and specificity

In order to establish sensitivity and specificity of the ASA-triggered 15-HETE release to confirm/exclude ASA-sensitivity. Receiver Operating Curve (ROC) analysis was performed (Fig. 3). When the test was used to obtain the cut-off giving maximal diagnostic efficiency (100%) to confirm the diagnosis (+59% increase in 15-HETE release was calculated as the cut off point) the sensitivity was 82% and the specificity was 83%; the area under the curve was 0.9. Positive predictive value was 0.79 and negative predictive value was 0.86. When the diagnostic efficiency level was set up at 75% the sensitivity was 99% and the specificity was 94%. Similar ROC curve analysis for 20 μM of aspirin yielded sensitivity and specificity of the test 47 and 52%, respectively.

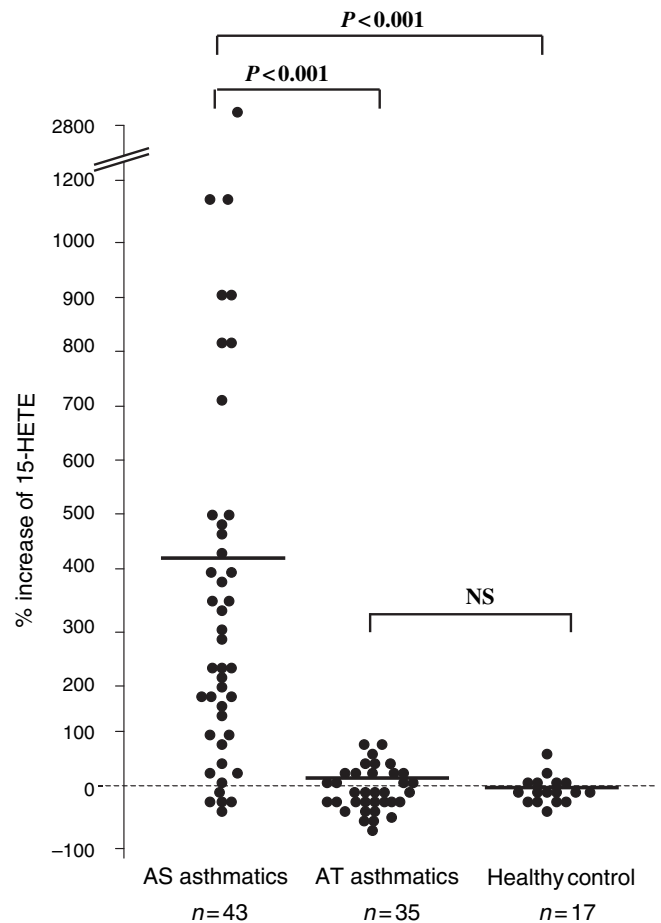


Figure 2. Individual values for percentage change in 15-HETE generation by PBLs after incubation with 200 μM ASA in AS-asthmatics, AT-asthmatics and healthy control subjects. Horizontal bars represent mean percentage of 15-HETE increase.

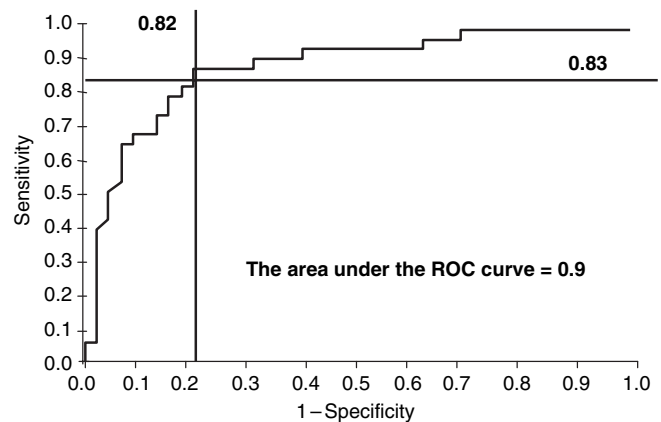


Figure 3. The ROC curve analysis for percentage increase in 15-HETE generation between unstimulated and ASA (200 μM) stimulated PBLs from AS-asthmatic and AT-asthmatic. The sensitivity was 82% and the specificity was 83%; area under the curve was 0.9. The positive predictive value was 0.79 and the negative predictive value was 0.86.

Table 2. Reproducibility of the ASPITest

Patient	15-HETE generation (pg/ml)								
	First test			After 4 weeks			After 8 weeks		
	Basal	200 μ M ASA	Increase (%)	Basal	200 μ M ASA	Increase (%)	Basal	200 μ M ASA	Increase (%)
E K	1200	2000	67	1500	2400	60			
I K	429	1394	225	950	1500	59			
G Z	300	950	217	254	830	227			
K A	51	817	1502	106	1193	1025	73	1243	1603
S C	289	593	105	161	345	114	158	377	139
B G	900	500	-44	750	500	-33	739	642	-13
J S	230	300	30	300	450	50	198	290	46
M W	136	111	-18	173	155	-10	154	125	-19

Reproducibility of ASPITest

In order to determine the reproducibility of responses, the test was repeated in eight patients (three times in five patients and two times in three patients) over the period of 2–8 weeks (Table 2). Except for one patient (I.K.) in whom the two measurements differed substantially, in the remaining seven patients the degree of response to aspirin expressed as percentage of baseline were very similar on different occasions. The coefficient of variation for basal 15-HETE release ranged from 0.11 to 0.36 and for aspirin-induced 15-HETE generation ranged from 0.15 to 0.3 in individual patients.

The correlation of 15-HETE release with clinical characteristics of patients

There was no correlation between the magnitude of aspirin triggered 15-HETE increase *in vitro* and the presence or absence of nasal polyps ($r = 0.09$, $P = 0.64$); atopic status ($r = 0.54$, $P = 0.55$); daily doses of oral glucocorticosteroids ($r = 0.13$, $P = 0.38$) or inhaled glucocorticosteroids ($r = -0.13$, $P = 0.39$); baseline FEV1 ($r = -0.016$, $P = 0.91$) or blood eosinophilia ($r = -0.09$, $P = 0.57$).

Naproxen-induced 15-HETE release

Since AS-asthmatics usually cross-react *in vivo* with other than aspirin NSAIDs, we aimed to determine *in vitro* cross-reactivity to naproxen, a nonselective COX-1/COX-2 inhibitor. Dose response studies with naproxen (1, 10 and 50 μ M) demonstrated that 50 μ M concentration was the most effective in triggering 15-HETE release in AS-patients and this concentration was used for further studies. Concentrations above 50 μ M were toxic. Incubation of PBL from 22 AS-asthmatics (some of them had history of adverse reactions to other NSAIDs) with 50 μ M of naproxen triggered a mean 49% increase in 15-HETE generation [from median 128 (87–395) pg/ml to median 190 (140–436) pg/ml; $P < 0.05$]. In three AS patients the increase was below 10%, in three between 10

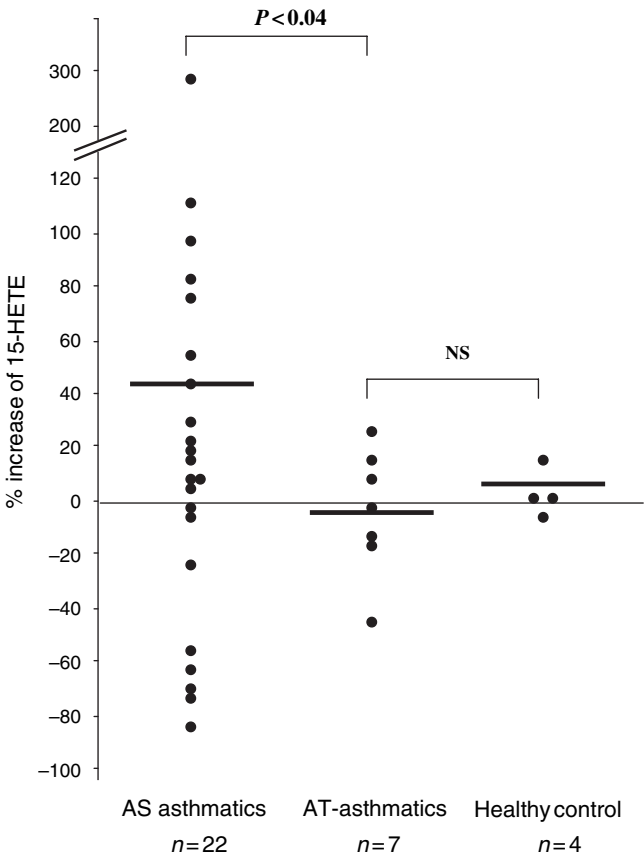


Figure 4. Individual values for percentage change in 15-HETE generation by PBLs after incubation with naproxen (50 μ M) in AS-asthmatics ($n = 22$), AT-asthmatics ($n = 7$) and healthy control subjects ($n = 4$).

and 20% and in eight patients the increase exceeded 23% (i.e. the highest increase observed in control AT-asthmatics). In eight patients 15-HETE decreased following 50 μ M of naproxen (Fig. 4). In AT-asthmatics naproxen did not change the mean 15-HETE generation [median 150 (123–173) pg/ml before and median 134 (90–238) pg/ml after naproxen]. In three AT patients only a small increase in 15-HETE generation was observed

(between +9 and +23%) and in four patients 15-HETE a small decrease was noticed.

Discussion

Our study involving relatively large groups of subjects demonstrated that measurement of aspirin-induced 15-HETE generation by PBLs is highly sensitive and specific to confirm/exclude the history of aspirin sensitivity in asthmatic patients. Analysis of the ROC curve allowed to determine the positive and negative predictive value of ASPITest in patients with history of aspirin-induced asthmatic reaction which (0.79 and 0.86, respectively) approached figures reported previously for bronchial or nasal inhalation challenge with aspirin (17). Our groups of AS and AT asthmatics were similar with respect to clinical features (e.g. severity of asthma) and blood eosinophilia, strongly implying the sensitivity to aspirin as the critical differential trait. The majority of patients had a clear history of at least one (and usually two or more) episodes of asthmatic attacks provoked by aspirin. In those patients with equivocal history, sensitivity to aspirin was confirmed by oral, nasal or bronchial challenge. However, it has to be stressed that the time interval between clinical episodes of reactions or ASA-challenges and *in vitro* study varied from several weeks to a few years, thus the true aspirin sensitivity status of some patients was difficult to determine.

Although a significant range of the *in vitro* responses was observed among different patients (ranging from a decrease in 15-HETE production to a 27-fold increase) the response was quite reproducible over the period of several weeks in individual patients. Moreover, the degree of 15-HETE response to aspirin was not related to any other factor than a history of ASA-induced reaction features, including asthma severity or blood eosinophilia, suggesting a causal relationship of positive 15-HETE release test to the clinical diagnosis of aspirin hypersensitivity.

Aspirin sensitive asthmatics are likely to cross-react to other NSAIDs which are strong inhibitors of cyclooxygenase-1, and tolerate well selective cyclooxygenase-2 inhibitors (e.g. celecoxib and rofecoxib) (24, 25). The rate of cross sensitivity to nonselective cyclooxygenase inhibitors varies among patients and it cannot be predicted if a particular ASA-sensitive patient reacts to oral challenge with an alternative NSAID. In our previous study it has been demonstrated, that also other nonselective cyclooxygenase inhibitor (naproxen), but not selective COX-2 inhibitors (celecoxib and NS398) triggered 15-HETE generation in some AS-patients (22). The present study demonstrated that naproxen which did not affect 15-HETE release in AT-patients, induced significant release of 15-HETE into supernatants of PBL in 8 of 22 sensitive asthmatics. Unfortunately, *in vitro* responses

to naproxen could not be referred to clinical sensitivity, since only a few of our patients reported asthmatic symptoms after NSAIDs other than aspirin.

15-HETE is a potential mediator of the airway inflammation although the mechanism leading to increased generation of 15-HETE following *in vitro* incubation with aspirin is not known. The major source of 15-hydroxyeicosatetraenoic acid in this reaction seems to be 15-lipoxygenase (15-LOX) and we have postulated that the activity of 15-LOX in AS-asthmatics is controlled by endogenous COX-1 derived PGE₂, and removal of PGE₂ production by aspirin results in activation of 15-LOX and 15-HETE production (22). Inhibitory effect of PGE₁ synthetic analogues misoprostol and sulprostone on 15-HETE generation strongly supports the involvement of prostaglandin EP receptors and confirms relationship between observed 15-HETE triggering and the mechanisms of ASA-induced reactivity (26).

The gold standard for the diagnosis of ASA-sensitivity is at present the combination of positive history and placebo controlled challenge test. The history is not always clear-cut in patients with severe and often unstable disease, which could be easily exacerbated by difficult to detect environmental factors. On the other hand, oral challenge test is a time-consuming procedure usually requiring well-trained personnel and availability of emergency care unit. Bronchial or nasal instillation tests, although in experienced hands may be very efficient to confirm or exclude ASA-sensitivity, require specialist equipment (dosimeter, rhinomanometer) and cannot be recommended as routine procedure for allergy or pulmonary unit (16). Although several *in vitro* assays have been proposed to confirm sensitivity to aspirin, their practical usefulness turn out to be limited. Earlier studies employing platelets, basophiles or leukocytes have never been reproduced (27–30). More recently some studies demonstrated aspirin-triggered release of LTC₄ from peripheral blood leukocytes but the differences between AS and AT-asthmatics were only quantitative and the test's sensitivity to detect ASA-intolerance rarely exceeded 50% (31–35). Furthermore, two recent studies either found nonspecific and not reproducible responses in both sensitive and tolerant patients (36) or did not find any significant effect of aspirin on release of LTC₄ from PBLs (22). In this study 15-hydroxyeicosatetraenoic acid was shown to be specifically released *in vitro* by aspirin and naproxen in AS asthmatics.

We believe that further studies are needed before the measurement of 15-HETE release from PBLs might be used as Aspirin Sensitive Patients Identification Test (ASPITest) for detection of ASA-sensitivity in asthmatic patients.

Acknowledgment

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References

- Samter M, Beers MF. Intolerance to aspirin: clinical studies and consideration of its pathogenesis. *Ann Intern Med* 1968;**68**:975–983.
- Kasper L, Sladek K, Duplaga M, Bochenek G, Liebhart J, Gladysz U et al. Prevalence of asthma with aspirin hypersensitivity in the adult population of Poland. *Allergy* 2003;**58**:1064–1066.
- Jenkins C, Costello J, Hodge L. Systematic review of prevalence of aspirin induced asthma and its implications for clinical practice. *BMJ* 2004;**328**:434.
- Szczeklik A, Stevenson DD. Aspirin-induced asthma: advances in pathogenesis, diagnosis, and management. *J Allergy Clin Immunol* 2003;**111**:913–921.
- Kowalski ML, Bienkiewicz B, Kordek P, Pawliczak R. Nasal polyposis in aspirin-hypersensitive patients with asthma (Aspirin Triad) and aspirin-tolerant patients. *Allergy Clin Immunol Int – World Allergy J* 2003;**6**:1–5.
- Perez-Novo CA, Kowalski ML, Kuna P, Ptasińska A, Holtappels G, Van Cauwenberge P et al. Aspirin sensitivity and IgE antibodies to *Staphylococcus aureus* enterotoxins in nasal polyposis: studies on the relationship. *Int Arch Allergy Immunol* 2004;**133**:255–260.
- Szczeklik A, Gryglewski RJ, Czerniawska-Mysik G. Relationship of inhibition of prostaglandins biosynthesis by analogues to asthma attacks in aspirin-sensitive patients. *Br Med J* 1975;**1**:67–69.
- Pods R, Ross D, Van Hulst S, Rudack C, Maune S. Rantes, eotaxin and eotaxin-2 expression and production in patients with aspirin triad. *Allergy* 2003;**58**:1165–1170.
- Rolla G, Di Emanuele A, Dutto L, Marsico P, Nebiolo F, Corradi F et al. Effect of inhalation aspirin challenge on exhaled nitric oxide in patients with aspirin-inducible asthma. *Allergy* 2004;**59**:827–832.
- Fischer AR, Rosenberg MA, Lilly CM, Callery JC, Rubin P, Cohn J et al. Direct evidence for role of the mast cell in the nasal response to aspirin in aspirin-sensitive. *J Allergy Clin Immunol* 1994;**84**:1045–1056.
- Christie PE, Tagari P, Ford-Hutchinson AW, Black C, Markendorf A, Schmitz-Schumann M et al. Urinary leukotriene E4 after lysine-aspirin inhalation in asthmatic subjects. *Am Rev Respir Dis* 1992;**146**:1531–1534.
- Kowalski ML, Grzegorzczak J, Wojciechowska B, Poniatowska M. Intranasal challenge with aspirin induces cell influx and activation of eosinophils and mast cells in nasal secretions of ASA-sensitive patients. *Clin Exp Allergy* 1996;**26**:807–814.
- Mita H, Endoh S, Kudoh M, Kawagishi Y, Kobayashi M, Taniguchi M et al. Possible involvement of mast-cell activation in aspirin provocation of aspirin-induced asthma. *Allergy* 2001;**56**:1061–1067.
- Picado C, Ramis I, Rosello J, Prat J, Bulbena O, Plaza V. Release of peptide leukotrienes into nasal secretions after local instillation of aspirin in sensitive asthmatic patients. *Am Rev Respir Dis* 1992;**145**:65–69.
- Kowalski ML, Sliwinska-Kowalska M, Igarashi Y, White MV, Wojciechowska B, Brayton P et al. Nasal secretions in response to acetylsalicylic acid. *J Allergy Clin Immunol* 1993;**91**:580–598.
- Melillo G, Balzano G, Bianco S, Dahlen B, Godard P, Kowalski ML et al. Report of the INTERASMA working group on standardization of inhalation provocation tests in Aspirin-induced Asthma. Oral and inhalation provocation tests for the diagnosis of aspirin-induced asthma. *Allergy* 2001;**56**:899–911.
- Nizankowska E, Bestynska-Krypel A, Cmiel A, Szczeklik A. Oral and bronchial provocation tests with aspirin for diagnosis of aspirin-induced asthma. *Eur Respir J* 2000;**15**:863–869.
- Milewski M, Mastalerz L, Nizankowska E, Szczeklik A. Nasal provocation test with lysine-aspirin for diagnosis of aspirin-sensitive asthma. *J Allergy Clin Immunol* 1998;**101**:581–586.
- Alonso-Llamazares A, Martinez-Cocera C, Dominguez-Ortega J, Robledo-Echarren T, Cimarra-Alvarez M, Mesa Del Castillo M. Nasal provocation test (NPT) with aspirin: a sensitive and safe method to diagnose aspirin-induced asthma (AIA). *Allergy* 2002;**57**:632–635.
- Lebel B, Messaad D, Kvedariene V, Rongier M, Bousquet J, Demoly P. Cysteinyl-leukotriene release test (CAST) in the diagnosis of immediate drug reactions. *Allergy* 2001;**56**:688–692.
- Kowalski ML, Pawliczak R, Wozniak J, Siuda K, Poniatowska M, Iwaszkiewicz J et al. Differential metabolism of arachidonic acid in nasal polyp epithelial cells cultured from aspirin-sensitive and aspirin-tolerant patients. *Am J Respir Crit Care Med* 2000;**161**:391–398.
- Kowalski ML, Ptasińska A, Bienkiewicz B, Pawliczak R, Dubuske L. Differential effects of aspirin and misoprostol on 15-hydroxyeicosatetraenoic acid generation by leukocytes from aspirin-sensitive asthmatic patients. *J Allergy Clin Immunol* 2003;**112**:505–512.
- Dahlen B, Zetterstrom O. Comparison of bronchial and per oral provocation with aspirin in aspirin-sensitive asthmatics. *Eur Respir J* 1990;**3**:527–534.
- Szczeklik A, Nizankowska E, Bochenek G, Nagraba K, Mejza F, Swierczynska M. Safety of a specific COX-2 inhibitor in aspirin-induced asthma. *Clin Exp Allergy* 2001;**31**:219–225.
- Dahlen B, Szczeklik A, Murray JJ. Celecoxib in Aspirin-intolerant Asthma study group. celecoxib in patients with asthma and aspirin intolerance. The celecoxib in Aspirin-intolerant Asthma study group. *N Engl J Med* 2001;**344**:142.
- Kowalski ML, Ptasińska A, Jedrzejczak M, Bienkiewicz B, Borowiec M, Dubuske L. Prostaglandin EP receptor agonists differentially modulate aspirin-induced generation of 15-HETE in aspirin-sensitive asthmatics. *J Allergy Clin Immunol* 2004;**113** suppl:266.
- Ameisen JC, Capron A, Joseph M, Maclouf J, Vorng H, Pancre V et al. Aspirin-sensitive asthma: abnormal platelet response to drugs inducing asthmatic attacks. Diagnostic and physiopathological implications. *Int Archs Allergy Appl Immunol* 1985;**78**:438–448.
- Nizankowska E, Michalska Z, Wandzilak M, Radomski M, Marcinkiewicz F, Gryglewski RJ et al. An abnormality of arachidonic acid metabolism is not a generalized phenomenon in patients with aspirin-induced asthma. *Eicosanoids* 1988;**1**:45–48.
- Okuda Y, Hattori H, Takashima T, Miyatake A, Yamatodani A, Tsuyuguchi I et al. Basophil histamine release by platelet-activating factor in aspirin-sensitive subjects with asthma. *J Allergy Clin Immunol* 1990;**86**:548–553.
- Goetzl EJ, Valacer DJ, Payan DG, Wong MY. Abnormal responses to aspirin of leukocyte oxygenation of arachidonic acid in adults with aspirin intolerance. *J Allergy Clin Immunol* 1986;**77**:693–698.
- Czech W, Schopf E, Kapp A. Release of sulfidoleukotrienes in vitro: its relevance in the diagnosis of pseudoallergy to acetylsalicylic acid. *Inflamm Res* 1995;**44**:291–295.

32. Celik G, Bavbek S, Misirligil Z, Melli M. Release of cysteinyl leukotrienes with aspirin stimulation and the effect of prostaglandin E(2) on this release from peripheral blood leucocytes in aspirin-induced asthmatic patients. *Clin Exp Allergy* 2001;**31**:1615–1622.
33. 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**:506–510.
34. 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**:1402–1411.
35. Abrahamsen O, Haas H, Schreiber J, Schlaak M. Differential mediator release from basophils of allergic and non-allergic asthmatic patients after stimulation with anti-IgE and C5a. *Clin Exp Allergy* 2001;**31**:368–378.
36. Pierzchalska M, Mastalerz L, Sanak M, Zazula M, Szczeklik A. A moderate and unspecific release of cysteinyl leukotrienes by aspirin from peripheral blood leucocytes precludes its value for aspirin sensitivity testing in asthma. *Clin Exp Allergy* 2000;**30**:1785–1791.