

F.W. VELTEN¹*, C. BAYERL², H.W. BAENKLER³, D. SCHAEFER³

FUNCTIONAL EICOSANOID TEST AND TYPING (FET) IN ACETYLSALICYLIC ACID INTOLERANT PATIENTS WITH URTICARIA

¹Department of Dermatology and Allergology, University Medical Center Mannheim,
Ruprecht-Karls-University of Heidelberg, Mannheim, Germany

²Department of Dermatology and Allergology, Dr. Horst-Schmidt Clinic Wiesbaden, Germany

³Department of Medicine III, University Hospital Erlangen, Germany

One of the common side effects of acetylsalicylic acid (ASA) is the induction of pseudoallergic reactions that range from urticarial wheals to anaphylactic shock. At present there is no reliable detection method available for the diagnosis of ASA-hypersensitivity and its relation to clinical symptoms. The purpose of the present study was to evaluate the functional eicosanoid typing (FET) score taking into account several parameters of the equilibrium between prostaglandins (PG) and peptido-leukotriens (pLT). A total eicosanoid pattern score (TEP) ranging from 0.0 to 3.0, was defined that exhibited significant differences ($p \leq 0.001$) between ASA-intolerant patients and healthy subjects. In addition to the differentiation of both groups at a TEP cut-off value of 1.0, the increasing TEP values correlated with an increasing severity of clinical symptoms in ASA-intolerant patients. We conclude that the FET has the potential for the safe and reliable detection of ASA-intolerance and, probably, other eicosanoid-related pseudoallergic reactions.

Key words: *aspirin-intolerance, urticaria, prostaglandine – leukotriene balance, eicosanoid pattern score*

Abbreviations: *ASA – acetylsalicylic acid, CAD – cardiovascular disease, COX – cyclooxygenase, Cys-LT - cysteinyl-leukotriene*

INTRODUCTION

Since its introduction more than a century ago, Aspirin® (acetylsalicylic acid, ASA) is one of the most commonly used medications. Its primary indication as analgesic was expanded during the following decades. Thus, life-long ASA-treatment is mandatory for secondary prevention of coronary artery diseases such as unstable angina, chronic stable angina, after myocardial infarction, and after coronary angioplasty and stent implantation (1). In addition, ASA is used for the prevention of non-fatal ischemic stroke, cerebral ischemia, intestinal cancer, and with its derivative 5-aminosalicylic acid – a component of sulfasalazine - in Crohn's disease and ulcerative colitis (2). Soon after its introduction adverse side effects have been observed after ASA-treatment (3). Clinical symptoms range from urticaria, angioedema, acute bronchospasm to anaphylactic shock. The prevalence of ASA-intolerance in the general population ranges from 0.6% to 2.5% and in asthmatics from 4.3% to 11.0% (4).

Under physiological conditions, ASA functions as an inhibitor of prostaglandin formation from arachidonic acid by inhibition of the cyclooxygenase (COX) 1 and 2 isoforms. Whereas COX-1 is constitutively expressed in most tissues, COX-2 expression is controlled by TNF- α and IL-2 during inflammation. COX-1 inhibition results in a decrease of the formation of prostanoids like prostaglandins and thromboxanes. Since prostaglandins are potent inhibitors of the key enzyme of leukotriene synthesis, namely the 5-lipoxygenase (5-LO), COX-inhibition results in an increase of peptido-leukotrienes (pLTs) (5). The ASA-based inhibition of the COX enzymes is combined with a shift of the prostaglandin – leukotriene balance in favor of peptido-leukotrienes, namely LTC₄, LTD₄, and LTE₄ (2, 6, 7). This is tolerated in healthy individuals without the occurrence of adverse side effects. Recently, several polymorphisms of key enzymes involved in arachidonate metabolism have been described contributing to the development of ASA-intolerance (8). All of them cause elevated levels of pLTs and subsequently vasoconstriction. Since prostaglandins, especially PGE₂ and thromboxane A₂, lead to bronchodilatation and vasoconstriction, respectively, an imbalance of eicosanoids in favor of leukotrienes is related to the occurrence of urticaria and asthma (9).

At present, detection of ASA-hypersensitivity is mainly based upon clinical history. Skin tests are unsuitable to detect ASA-sensitization due to false negative results and the induction of anaphylactic reactions which may cause myocardial ischemia and subsequent heart infarction (10). Cellular tests such as the cellular allergen stimulation test (CAST) are available for many years for the detection of IgE-mediated and some non-IgE-mediated hypersensitivity reactions. The latter are solely based on the determination of pLTs from isolated leukocytes. However, since ASA-induced intolerance is rather related to an imbalance of the prostaglandin – leukotriene pathway than to the absolute pLT concentration (2, 4, 6, 7), a reliable testing system for ASA-intolerance should

take into account the relative proportion of prostaglandins and leukotrienes in addition to their absolute amount.

For this study we applied the previously described functional eicosanoid typing (FET) (6, 7) taking into account the quantitative relation of prostaglandin (PGE_2) and peptido-leukotrienes (i.e. LTC_4 , LTD_4 , LTE_4) concentrations in patients suffering from ASA-induced urticaria.

MATERIALS AND METHODS

Patients and control subjects

The study included 28 patients with allergic skin reactions within 24 hours after oral application of 1000 mg ASA (22 females, mean age 59.0 years, range 25 to 77 years; 6 males, mean age 44.8 years, range 27 to 76 years) and 30 healthy controls (15 females and 15 males, mean age 52.1 ± 2.5 years, range 28 to 71 years).

The clinical symptoms of the 28 patients ranged from urticarial wheals (11 patients), angioedema with or without urticarial wheals (14 patients) to anaphylaxis with loss of consciousness (with either urticarial wheals or angioedema) (3 patients). Ten of twenty-eight patients were atopic (Erlangen atopy score ≥ 10 points, 8/28 with allergic rhinitis, total IgE 457.4 ± 85.5 kU/L, eosinophil count 2.1 ± 1.2 % and 181.3 ± 75.9 eosinophils/nL). Thirteen of twenty-eight had at least one immediate-type hypersensitivity (7 against aeroallergens, 4 against food, 5 against food-additives, 5 against non-steroidal anti-inflammatory drugs (NSAID) different from ASA, 6 against drugs different from NSAIDs) and 3 of 27 had at least one delayed-type hypersensitivity. At the time of testing all individuals did not receive antihistaminics for at least 1 week and glucocorticoids for at least 3 weeks.

Thirty healthy individuals (15 females, mean age 55.5 years, range 29 to 71 years; and 15 males, mean age 46.4 years, range 28 to 67 years) served as controls. They had neither a history of hypersensitivity or allergy to aero- or food-allergens, or food-additives, nor a history of drug hypersensitivity or intolerance to NSAIDs. Allergic diseases were ruled out by prick and specific IgE testing. Antihistaminics, steroids or other regular medication were not taken.

All subjects gave their written consent before participating in this study, which was approved by the Institutional Ethical Committee of Erlangen-Nuremberg-University. The study was performed in accordance with the ethical standards of the Helsinki Declaration 1975 (revised 2004).

Collection of blood samples

Five mL of venous blood was collected during routine examination using a S-monovette® (Sarstedt, Hannover, Germany), including heparin to prevent clotting. Thereafter blood was further processed using the LiPiDoC®-AIT (SIAT, Bad Essen, Germany) according to the instruction of the manufacturer. Briefly, blood samples were diluted in accordance to the provided standard protocol. Subsequently, the aliquots were incubated with diluent, arachidonic acid, acetylsalicylic acid, and neuropeptide (substance P) for 20 minutes at room temperature. The reaction was stopped by immediately storing the samples at -20°C for up to two weeks until further processing for eicosanoid analysis.

Quantification of the in vitro leukotriene and prostaglandin release

Both eicosanoids, PGE_2 and pLT, were analyzed in parallel using highly specific and sensitive competitive enzyme-immuno-assays (SIAT, Bad Essen, Germany; SPI-bio, Paris, France),

specifically produced and validated for use in combination with the LiPiDoC®-AIT). The analyses were performed according to the instructions of the manufacturer. In brief, samples were thawed, centrifuged (900g, 4°C, 7 min), and aliquots of the supernatants were transferred to the assay plates in duplicates. The inter- and intra-assay variance for the PGE₂ or pLT enzyme-immuno-assays were 7.8% and 9.4% or 9.2% and 12.4%, respectively.

Evaluation of data

The data were processed according to the algorithm as recently published (7) which was extended by the inclusion of the neuropeptide-modified eicosanoid ratios as presented in this volume (s. Schäfer, in this issue). In brief, the individual enzymatic capacity (EC_E) of COX and 5-LOX were calculated according equation [1] from arachidonic acid-induced and basal eicosanoid synthesis, which was combined with the individual ratio of arachidonic acid-induced eicosanoids (ER_A), of acetylsalicylic acid-mediated eicosanoids (ER_S), and of neuropeptide-modified eicosanoids (ER_N).

$$[1] \quad ER_E = EC_{Ex} + EC_{Ey} + EB_E$$

The integration of the individual values according equation [2] revealed the “total eicosanoid pattern” score (TEP) as a single value for the typing of modified eicosanoid pattern in the range of 0.0 to 3.0, with 0.0 to 0.6 for “normal”, 0.7 to 1.0 for “intermediate” and > 1.0 for an abnormal pattern.

$$[2] \quad TEP = \eta EPS + \sum \eta EC_E + \sum \eta ER_E$$

Statistical analysis

The PGE₂ and pLT data were calculated as arithmetic mean (pg/mL) ± SEM. Controls and patients were compared using the two-tailed Mann-Whitney U-test. The t-test of Satterthait was performed if homogeneity of variances of the individual values in both groups was not achieved. The linear regression model was used for controlling the integrated total eicosanoid pattern score. GraphPrism Version 4 was used for statistical analysis. P = 0.05 was represented by ‘*’, p = 0.01 by ‘**’, p = 0.001 by ‘***’, and p > 0.05 by ‘ns’ (no significant difference).

RESULTS

Eicosanoid release pattern in healthy individuals (Table 1)

Peripheral blood mononuclear cells (PBMC) of thirty healthy individuals exhibited clearly higher basal PGE₂ than pLT levels (121.0 ± 4.2 pg/mL and 81.2 ± 4.5 pg/mL, respectively; p = 0.001). After challenge with arachidonic acid, this difference was further extended (183.8 ± 8.0 and 99.8 ± 4.7 pg/mL, respectively; p = 0.01; see Fig. 2 A/B and Fig. 3 A/B). Challenge with ASA and neuropeptide diminished PGE₂ secretion (119.0 ± 3.3 and 94.7 ± 3.6 pg/mL, respectively) below the basal PGE₂ secretion values. ASA-challenge slightly elevated pLT secretion (93.6 ± 5.3 pg/mL), whereas neuropeptide-challenge diminished pLT secretion (75.8 ± 4.9 pg/mL) of PBMC from healthy individuals. The increase of

Table 1. PGE₂, pLT and eight score values of 30 healthy controls after ASA-challenge (B - basal eicosanoid release, AA - arachidonic acid-induced eicosanoid release, ASA - acetylsalicylic acid-induced eicosanoid release, NP - neuropeptide-induced eicosanoid release).

control	gender	age years	total eicosanoid pattern Group score: 0,0 - 3,0	PGE ₂ B	PGE ₂ AA	PGE ₂ ASA	PGE ₂ NP	pLT B	pLT AA	pLT ASA	pLT NP
				pg / mL							
1	f	40	0.7	83.7	160.8	76.3	58.7	63.6	62.1	64.5	47.3
2	m	28	0.8	87.3	149.5	106.2	80.2	61.5	77.1	78.6	69.0
3	f	65	0.5	125.5	233.6	121.5	97.3	77.7	84.5	78.2	79.1
4	f	58	0.7	128.3	258.5	105.0	112.6	64.7	97.6	71.3	54.4
5	m	38	0.8	107.2	126.0	97.7	90.4	78.3	91.8	88.9	83.1
6	m	43	0.6	91.6	213.3	124.1	88.4	49.6	69.1	58.9	54.2
7	f	29	0.5	118.8	264.5	116.1	78.1	52.3	59.8	53.2	49.4
8	m	35	0.5	107.3	153.8	109.2	86.7	97.0	106.7	104.9	89.6
9	f	56	0.6	131.2	161.9	121.2	87.9	89.8	91.2	97.0	82.7
10	f	48	0.7	105.6	226.8	109.2	117.4	76.1	102.3	97.6	78.2
11	f	59	0.8	134.0	161.0	129.0	111.4	146.5	159.2	157.7	140.2
12	f	68	0.8	102.9	218.9	119.8	64.2	56.3	146.9	49.8	25.2
13	m	65	0.7	97.4	158.2	110.5	85.0	60.6	96.5	78.2	55.1
14	m	62	1.0	94.7	147.8	87.3	77.1	37.2	72.6	42.9	30.8
15	f	67	0.6	136.9	208.6	134.1	71.0	58.2	66.3	55.6	42.0
16	m	41	0.6	96.4	169.8	91.4	65.3	62.4	85.8	64.1	62.2
17	f	54	0.7	149.7	162.6	120.2	80.1	79.3	90.5	86.4	79.9
18	f	71	0.8	158.5	253.3	137.6	88.4	74.9	79.2	98.0	78.9
19	m	33	0.6	155.3	263.7	159.0	99.1	113.5	132.0	129.2	82.4
20	f	69	0.8	139.7	203.7	133.3	121.1	94.5	111.5	126.0	102.4
21	f	66	0.8	119.9	149.1	121.2	92.2	88.9	104.1	116.9	68.2
22	m	28	0.8	119.9	149.1	121.2	92.2	88.9	104.1	116.9	68.2
23	f	64	0.8	154.8	236.5	143.9	114.4	117.9	142.4	128.5	94.7
24	m	42	0.8	119.9	149.1	121.2	92.2	88.9	104.1	116.9	68.2
25	f	53	0.9	162.6	191.8	143.1	116.6	81.5	92.6	102.5	95.4
26	m	67	0.9	138.4	143.4	140.3	127.5	102.6	121.5	128.2	116.2
27	m	48	0.6	91.2	127.8	104.4	91.0	85.1	101.9	94.5	88.1
28	m	56	0.5	145.2	188.3	138.0	127.4	137.2	145.3	140.9	136.8
29	m	45	0.9	113.7	140.3	106.2	93.7	69.7	113.1	97.3	79.2
30	m	65	0.5	113.5	141.0	123.2	132.1	80.0	82.8	83.4	73.0
mean (total) (n=30)				121.0	183.8	119.0	94.7	81.2	99.8	93.6	75.8
SEM				4.2	8.0	3.3	3.6	4.5	4.7	5.3	4.9
min.		28	0.5	83.7	126.0	76.3	58.7	37.2	59.8	42.9	25.2
max.		71	1.0	162.6	264.5	159.0	132.1	146.5	159.2	157.7	140.2
mean females (n=15)				130.2	206.1	122.1	94.1	81.5	99.3	92.2	74.5
SEM				5.7	10.3	4.4	5.4	6.4	7.8	8.1	7.4
min.				83.7	149.1	76.3	58.7	52.3	59.8	49.8	25.2
max.				162.6	264.5	143.9	121.1	146.5	159.2	157.7	140.2
mean males (n=15)				111.9	161.4	116.0	95.2	80.8	100.3	94.9	77.1
SEM				5.4	9.3	5.0	5.0	6.6	5.6	7.3	6.6
min.				87.3	126.0	87.3	65.3	37.2	69.1	42.9	30.8
max.				155.3	263.7	159.0	132.1	137.2	145.3	140.9	136.8

pLTs after ASA-challenge and the decrease of PGE₂ and pLTs after ASA- and neuropeptide-challenge yielded a partial compensation of both, the basal and the arachidonic acid-induced imbalance of PGE₂ and pLTs (~27% and 25% higher PGE₂ levels after ASA- and neuropeptide challenge, respectively).

The TEP value of 0.7 ± 0.0 was within the lower third on a scale between 0.0 and 3.0 (range of the results between 0.5 and 1.0). The TEP value for the fifteen males was the same than for the fifteen females (0.7 ± 0.0).

Table 2. PGE₂, pLT and eight score values of 28 ASA-intolerant patients after ASA-challenge (B - basal eicosanoid release, AA - arachidonic acid-induced eicosanoid release, ASA - acetylsalicylic acid-induced eicosanoid release, NP - neuropeptide-induced eicosanoid release). Clinical symptoms after ASA-intake are classified into urticarial wheals (U), angioedema (AE), shortness of breath (SB), and anaphylactic shock (AS). AE* indicates two subjects with exercise-induced angioedem after ASA-intake.

symptoms	gender	age years	total eicosanoid pattern Group score: 0,0 - 3,0	PGE ₂ B	PGE ₂ AA	PGE ₂ ASA	PGE ₂ NP	pLT B	pLT AA	pLT ASA	pLT NP
				pg / mL							
AE*	m	27	0.6	95.8	191.2	92.6	113.3	115.7	164.2	107.2	89.8
U	f	58	1.0	136.9	225.6	101.2	100.5	53.6	72.7	70.3	46.4
AE*	f	63	1.0	146.1	167.7	129.4	138.7	119.9	73.0	87.6	137.8
U	m	29	1.2	84.6	88.3	107.3	95.3	130.9	141.9	179.2	93.2
U	f	59	1.2	159.9	207.1	225.1	124.5	83.7	126.5	135.0	125.3
U	f	65	1.3	209.6	232.1	172.4	121.3	341.2	368.3	262.7	201.9
U	f	43	1.3	287.2	349.5	360.9	257.5	147.1	200.7	274.4	216.6
AE	f	63	1.3	81.3	198.6	219.4	127.8	149.9	295.2	342.8	130.5
U	f	35	1.3	174.3	176.4	88.5	105.8	152.3	106.6	60.6	87.9
U	f	51	1.4	180.2	160.2	195.9	124.1	56.7	67.5	85.5	65.3
U	f	76	1.4	109.0	142.5	135.0	102.5	40.6	105.4	52.5	91.1
U	f	25	1.4	104.2	99.7	118.8	89.4	83.7	87.2	140.1	129.2
AE SB	f	73	1.4	131.4	181.4	85.9	82.4	20.3	55.6	61.2	14.4
U	f	71	1.4	158.7	215.8	108.8	158.1	87.1	157.3	18.9	124.7
AE	f	60	1.5	196.3	225.6	137.2	119.9	65.0	148.1	56.6	114.9
AE	f	77	1.5	295.3	335.3	196.2	202.8	382.2	369.9	341.5	336.6
AE SB	f	49	1.6	227.7	372.7	215.0	187.4	331.2	326.2	331.3	328.3
AE SB	m	48	1.6	122.5	346.3	341.5	235.7	264.1	290.2	422.1	307.0
U	f	73	1.6	174.3	199.4	140.6	117.9	89.7	72.8	23.0	70.3
AE SB	f	68	1.7	113.2	140.3	86.2	55.2	124.9	169.3	150.2	88.6
AE	m	76	1.9	162.7	176.1	107.6	89.8	21.0	20.7	34.0	10.4
AE SB	f	50	2.1	118.4	78.6	46.0	74.1	149.8	149.6	178.8	118.0
AE	f	49	2.1	37.6	142.6	43.4	92.2	177.6	216.2	175.3	134.4
AS	m	50	2.1	79.4	148.2	52.3	44.2	146.8	159.1	149.5	116.6
AE SB	f	76	2.4	111.9	194.5	157.5	98.7	19.8	186.2	73.2	8.2
AE SB	m	39	2.4	51.9	156.8	85.0	33.7	159.1	179.8	203.8	105.0
AS	f	55	2.5	146.0	199.5	132.1	111.8	7.6	121.5	10.5	21.4
AS	f	58	2.8	112.8	155.4	79.3	92.7	10.0	12.0	20.1	10.2
mean (total) (n=27)		55.9	1.6	143.2	196.7	141.5	117.8	126.1	158.7	144.6	118.7
SEM		2.9	0.1	11.6	14.2	14.8	9.7	18.8	18.3	21.6	16.9
min.		25	0.6	37.6	78.6	43.4	33.7	7.6	12.0	10.5	8.2
max.		77	2.8	295.3	372.7	360.9	257.5	382.2	369.9	422.1	336.6
mean females (n=22)		59.0	1.6	155.1	200.0	144.3	122.1	122.5	158.5	134.2	118.3
SEM		2.9	0.1	13.2	15.7	15.3	9.7	22.7	21.9	23.7	19.1
min.		25	1.0	37.6	78.6	43.4	55.2	7.6	12.0	10.5	8.2
max.		77	2.8	295.3	372.7	360.9	257.5	382.2	369.9	342.8	336.6
mean males (n=6)		44.8	1.6	99.5	184.5	131.0	102.0	139.6	159.3	182.7	120.3
SEM		7.3	0.3	15.7	35.4	42.9	29.6	32.0	35.2	53.8	40.4
min.		27	0.6	51.9	88.3	52.3	33.7	21.0	20.7	34.0	10.4
max.		76	2.4	162.7	346.3	341.5	235.7	264.1	290.2	422.1	307.0
mean < 1,0 (n = 1)		27.0	0.6	95.8	191.2	92.6	113.3	115.7	164.2	107.2	89.8
mean ? 1,0 (n = 28)		57.0	1.6	144.9	196.9	143.3	117.9	126.5	158.5	146.0	119.8
SEM		2.8	0.1	11.9	14.7	15.2	10.1	19.6	19.2	22.6	17.6
mean ? 1,5 (n = 14)		59.1	2.0	139.3	205.1	130.0	111.2	139.2	173.0	155.0	126.4
SEM		3.2	0.1	18.4	23.3	21.6	15.9	32.0	28.3	35.9	31.2
mean ? 2,0 (n = 7)		53.9	2.3	94.0	153.6	85.1	78.2	95.8	146.3	115.9	73.4
SEM		4.3	0.1	14.8	15.1	16.8	11.0	30.5	26.6	31.8	22.3

Eicosanoid release pattern in ASA-intolerant patients (Table 2)

Challenge of PBMCs of 28 ASA-intolerant patients with ASA produced about equal amounts of PGE₂ and pLTs (141.5 ± 14.8 pg/mL and 144.6 ± 21.6 pg/mL, respectively). Both, the basal and the dynamic of eicosanoid synthesis after challenge with arachidonic acid showed slightly higher PGE₂ than pLTs values (PGE₂: 143.2 ± 11.6 pg/mL and 126.1 ± 18.8 pg/mL, respectively; pLTs: 196.7 ± 14.2 pg/mL and 158.7 ± 18.3 pg/mL, respectively) ($p \leq 0.01$ and $p > 0.05$, respectively; see Fig. 1 A and B). For ASA-intolerant patients a TEP value of 1.6 ± 0.1 was calculated. The mean values of the TEP were the same for females (1.6 ± 0.1) and males (1.6 ± 0.3).

Comparison of ASA-intolerant and healthy individuals

Contrary to our assumption, the amount of pLT secretion from ASA-intolerant patients did not exceed the PGE₂ secretion. Furthermore, the PGE₂ secretion did not differ significantly between ASA-intolerant and healthy subjects in all experimental set-ups (basal, arachidonic acid-, ASA-, and neuropeptide-induced secretion) (Fig. 1A, 1B, and 1C, Table 1 and 2). In contrast to the almost identical amount of PGE₂ secretion and as expected, the pLT secretion was significantly ($p \leq 0.001$) increased in all four experimental set-ups (Fig. 2A, 2B, and 2C, Table 1 and 2). The TEP showed highly significant differences ($p \leq 0.001$) between healthy (0.7) and ASA-intolerant (1.6) subjects (Fig. 3).

Linking clinical symptoms and TEP

The TEP of healthy individuals were in the range of 0.5 and 1.0 (Table 1). Except for one patient who developed exercise-induced urticarial wheals and angioedema after intake of ASA, all TEP values of ASA-intolerant patients were in the range of 1.0 and 2.8 (Table 2). ASA-intolerant patients revealing TEP values between 1.0 and 1.4 after ASA-intake suffered from urticarial wheals (11

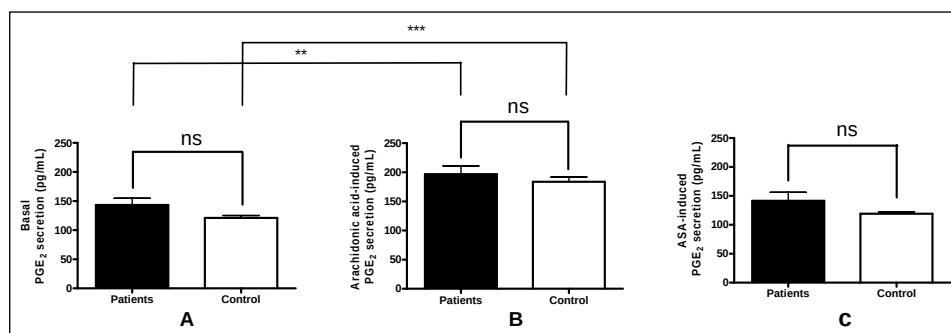


Fig. 1. Mean $\pm$ SEM of the basal, arachidonic acid, and ASA related PGE₂ release in ASA-intolerant patients (filled bars) and healthy controls (empty bars).

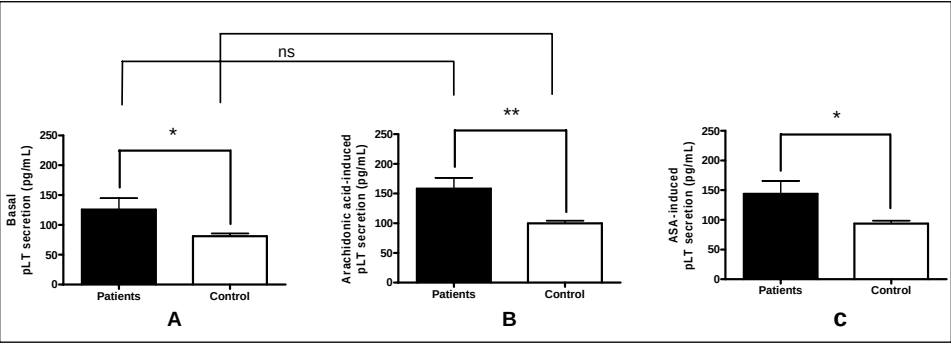


Fig. 2. Mean $\pm$ SEM of the basal, arachidonic acid, and ASA related pLTs release in ASA-intolerant patients (filled bars) and healthy controls (empty bars).

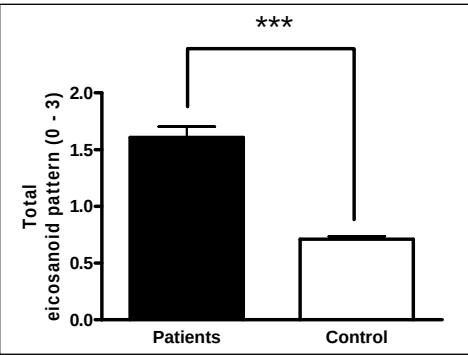


Fig 3. Mean score values $\pm$ SEM for the total eicosanoid pattern (TEP) in ASA-intolerant patients (filled bars) and healthy controls (empty bars).

from 13 patients) exclusively. One of the 14 patients with a TEP value of 1.0 suffered from urticarial wheals and exercise-induced angioedema. Another patient with a TEP value of 1.3 suffered from exercise-independent angioedema. Except for one patient with a TEP value of 1.6, all ASA-intolerant patients with TEP values above 1.4 (14 patients) suffered from either angioedema (with or without shortness of breath) or anaphylaxis. TEP values above 2.1 (four patients) were always related to signs of pronounced ASA-intolerance (either anaphylaxis or shortness of breath).

DISCUSSION

Proper diagnosis of uncertain ASA-intolerance is essential for early referral of patients with a history of urticaria, angioedema or anaphylactic shock upon ASA-treatment to an allergy department. Since ASA-intolerance is not based on humoral immune mechanisms and since appropriate diagnostic systems different from the determination of specific IgE antibodies are limited, most of these

patients are not tested and ASA is withheld without further investigations. In particular, patients suffering from coronary heart disease are further endangered from a rapid synthesis of thromboxane A_2 through the recovery of COX by fresh platelets after ASA-withdrawal (11). Subsequently, new coronary events cause a higher mortality than in patients that continue ASA-treatment (12). Apparently, potentially harmful challenge tests have to be avoided (13), particularly in patients with a history of coronary heart disease.

In the present study, we analyzed the quantitative interaction of PGE_2 and pLTs under different experimental conditions in healthy individuals as well as in patients suffering from urticaria, angioedema, and anaphylactic shock after ASA-treatment.

Since the absolute amount and the balance of PG and pLT is related to the dynamic capacity of the underlying enzymatic system, which shows individual variations, the experimental set-up included the definition of individual responder ranges under steady physiologic conditions and under induced conditions. The steady physiologic conditions were defined by the unmodified "basal" eicosanoid release and after challenge with arachidonic acid as the natural substrate for the COX- and LOX-mediated synthesis of eicosanoids. Induced conditions were defined by ASA- and neuropeptide stimulation. These allowed us to determine a total eicosanoid pattern score, namely TEP.

Challenge with arachidonic acid resulted in a noticeable increase of the PGE_2 values, which were almost equal in healthy and ASA-intolerant patients under basal conditions. In contrast, the basal pLT values of ASA-intolerant patients exceeded those of healthy individuals. Since the increase of eicosanoids after challenge with arachidonic acid was almost equal in ASA-intolerant and healthy individuals, the eicosanoid dynamic did not differ significantly between both groups. However, the separate consideration of the absolute eicosanoid values did not allow distinguishing between healthy individuals and ASA-intolerant patients since the individual PGE_2 and pLT values showed a wide distribution overlapping both groups (see *Table 1* and *2*).

Comparable difficulties of the available monoparametric tests for ASA-intolerance might be responsible for their limited value in routine diagnostic procedures. Thus, the determination of basophile histamine release by platelet-activating factor (14), the determination of platelet hydrogen peroxide metabolism (15) and the urine LTE_4 determination (16) is not routinely used since these approaches show a lack of discriminatory sensitivity. Newer tests such as the determination of sulfidoleukotrienes after ASA-challenge revealed a reliable sensitivity only when NSAIDs different from ASA are used to perform an *in-vitro* tests (17).

As shown in the present study, the detailed examination of the complex interaction of PGE_2 and pLT not only under steady physiological conditions but also after the inclusion of specific stimulatory conditions is mandatory to define the TEP as a final score, which allows to discriminate unambiguously healthy and

ASA-intolerant patients at a defined cut-off value. Concerning the stimulation of PBMC with ASA, we found slight differences in the pLT response only, but not in the PGE₂ response. On the other hand, the amount of both eicosanoids was clearly increased after challenge with neuropeptide in ASA-intolerants compared to healthy individuals. The integration of these values and those obtained under basal conditions revealed significant differences, which were almost twice as high in ASA-intolerant than in healthy individuals. This observation emphasizes the dysregulation of the eicosanoid network by neuropeptides, especially in ASA-intolerant asthmatics. Accordingly, neuropeptides that show no chemical relationship to ASA, are not only increasingly released (18), but also might trigger the imbalance of the eicosanoid network in ASA-intolerant patients.

Since the differences of pLT were different in all four experimental set-ups, the impact of LTs in ASA-intolerant patients was significantly higher than in healthy individuals. Due to the almost identical PGE₂ values in three of the four experimental set-ups, the impact PGs did not differ between ASA-intolerant and healthy individuals. However, integration of the PG impact and the LT impact into the TEP revealed a significantly higher TEP value for ASA-intolerants than for healthy individuals.

Only the integration of all score values into the TEP led to the discrimination of healthy individuals and ASA-intolerant patients. Whereas healthy individuals showed a maximum TEP score of 1.0, the majority of ASA-intolerant patients (25 out of 28) showed significantly ($p = 0.001$) higher TEP values. Moreover, we were able to correlate the clinical symptoms in ASA-intolerants with their individual TEP values. Thus, patients suffering from minor clinical symptoms (urticarial wheals) showed TEP values between 1.0 and 1.4, whereas patients with intermediate clinical symptoms (angioedema with or without urticarial wheals) were found in the ranged between 1.4 and 2.1, and patients suffering from pronounced ASA-intolerance (either anaphylaxis or shortness of breath) had TEP values higher than 2.1.

Closer examination of two of the three ASA-intolerant patients with TEP values within the range of the control group (0.6 and 1.0, respectively) had a history of exercise-induced angioedema after ASA-consumption. Both patients consumed bread and wheat noodles before exercising. They reported no clinical history of a gluten-induced enteropathy.

It has been described, that ASA might trigger exercise-induced food-dependent anaphylaxis that is characterized by wheals, angioedema, wheezing, dyspnea and loss of consciousness after eating certain foods, especially wheat with its major allergen gluten. Since the antigenicity of gluten is increased after pepsin digestion, which occurs in the stomach, it has been speculated that food-dependent anaphylaxis is triggered by ASA due to the induction of the gastric pepsin production. (19). Correspondingly, a reliable test system should not give any evidence for an ASA-intolerance in these patients, which was the case for both patients.

We conclude that the integration of the PG and pLT release under basal and stimulatory conditions represents a sensitive approach for the diagnosis of ASA-intolerance in delimitation to healthy individuals. Moreover, the application of the functional eicosanoid typing (FET) represented here seems to be promising for statements concerning the strength of clinical symptoms before the intake of ASA. In addition, the FET might be useful not only for the diagnosis of ASA-intolerance but also for other pseudoallergic reactions.

REFERENCES

1. Gunnar RM, Passamani ER, Bourdillon PD, et al. Guidelines for the early management of patients with acute myocardial infarction. A report of the American College of Cardiology/American Heart Association Task Force on Assessment of Diagnostic and Therapeutic Cardiovascular Procedures (Subcommittee to Develop Guidelines for the Early Management of Patients with Acute Myocardial Infarction). *J Am Coll Cardiol* 1990; 16(2): 249-292.
2. Baenkler HW, Schafer D. Abnormal eicosanoid-pattern of peripheral white-blood cells in gastro-intestinal cancer. *J Physiol Pharmacol* 2005; 56(Suppl 5): 119-128.
3. Hirschberg VGS. Mitteilung über einen Fall von Nebenwirkungen des Aspirin. *Dtsch Med Wochenschr* 1902; 28416-28417.
4. Szczeklik A, Sanak M, Nizankowska-Mogilnicka E, Kielbasa B. Aspirin intolerance and the cyclooxygenase-leukotriene pathways. *Curr Opin Pulm Med* 2004; 10(1): 51-56.
5. Schafer D, Lindenthal U, Wagner M, Bolcskei PL, Baenkler HW. Effect of prostaglandin E2 on eicosanoid release by human bronchial biopsy specimens from normal and inflamed mucosa. *Thorax* 1996; 51(9): 919-923.
6. Schafer D, Schmid M, Gode UC, Baenkler HW. Dynamics of eicosanoids in peripheral blood cells during bronchial provocation in aspirin-intolerant asthmatics. *Eur Respir J* 1999; 13(3): 638-646.
7. Schafer D, Baenkler HW. Functional eicosanoid test and typing (FET) of peripheral blood cells in eicosanoids related diseases. *J Physiol Pharmacol* 2005; 56 (Suppl 5): 103-118.
8. Choi JH, Park HS, Oh HB, et al. Leukotriene-related gene polymorphisms in ASA-intolerant asthma: an association with a haplotype of 5-lipoxygenase. *Hum Genet* 2004; 114(4): 337-344. Epub 2004 Jan 29.
9. Raskovic S, Bogic M, Peric-Popadic A, Arandjelovic S, Jovcic Z, Tomic-Spiric V. The role of prostaglandins in allergic inflammation. *Srp Arh Celok Lek* 1998; 126 (9-10): 388-393.
10. Szczeklik A, Nizankowska E, Mastalerz L, Bochenek G. Myocardial ischemia possibly mediated by cysteinyl leukotrienes. *J Allergy Clin Immunol* 2002; 109(3): 572-573
11. McDonald JW, Ali M. Recovery of cyclooxygenase activity after aspirin in populations of platelets separated on stractan density gradients. *Prostaglandins Leukot Med* 1983; 12(3): 245-252.
12. Ferrari E, Benhamou M, Cerboni P, Marcel B. Coronary syndromes following aspirin withdrawal: a special risk for late stent thrombosis. *J Am Coll Cardiol* 2005; 45(3): 456-459.
13. Aberer W, Bircher A, Romano A, et al. Drug provocation testing in the diagnosis of drug hypersensitivity reactions: general considerations. *Allergy* 2003; 58(9): 854-863.
14. Okuda Y, Hattori H, Takashima T, et al. Basophil histamine release by platelet-activating factor in aspirin-sensitive subjects with asthma. *J Allergy Clin Immunol* 1990; 86(4 Pt 1): 548-553.

15. Pearson DJ, Suarez-Mendez VJ. Abnormal platelet hydrogen peroxide metabolism in aspirin hypersensitivity. *Clin Exp Allergy* 1990; 20(2): 157-163.
16. Oosaki R, Mizushima Y, Mita H, Shida T, Akiyama K, Kobayashi M. Urinary leukotriene E4 and 11-dehydrothromboxane B2 in patients with aspirin-sensitive asthma. *Allergy* 1997; 52(4): 470-473.
17. May A, Weber A, Gall H, Kaufmann R, Zollner T. M. Means of increasing sensitivity of an in vitro diagnostic test for aspirin intolerance. *Clin Exp Allergy* 1999; 29(10): 1402-1411.
18. Palgan K, Dziedziczko A. Role of neurotrophin and neuropeptides in bronchial asthma. *Pol Merkuriusz Lek* 2004; 16(92): 101-103.
19. Harada S, Horikawa T, Ashida M, Kamo T, Nishioka E, Ichihashi M. Aspirin enhances the induction of type I allergic symptoms when combined with food and exercise in patients with food-dependent exercise-induced anaphylaxis. *Br J Dermatol* 2001; 145(2): 336-339.
20. Schäfer D. Testing and typing of eicosanoid-patterns. *J Physiol Pharmacol* 2006; 57 (Suppl 12): 47-64.

Received: November 1, 2006

Accepted: December 1, 2006

Author's address: Dr. Florian W. Velten, Department of Dermatology, University Medical Center Mannheim, Ruprecht Karls University of Heidelberg, Theodor-Kutzer Ufer 1-3, 68167 Mannheim (Germany), Phone: +49 621 383 2280, Fax: +49 621 383 3815; e-mail: florian.velten@haut.ma.uni-heidelberg.de