Eicosanoid imbalance correlates in vitro with the pattern of clinical symptoms of Samter´s triad*

Ulrike Förster[1], Silke Strathmann[1], Dirk Schäfer[2], Agnieszka J. Szczepek[3], Heidi Olze[1,3]

[1] Department of Otorhinolaryngology, Campus Virchow, Charité-Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany

[2] Medical Clinic III, Friedrich Alexander University Erlangen-Nuremberg, Ulmenweg 18, 91054 Erlangen, Germany

[3] Department of Otorhinolaryngology, Campus Mitte, Charité-Universitätsmedizin Berlin, Charitéplatz 1, 13353 Berlin, Germany

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Summary

Background: Hypersensitivity to non-steroidal anti-inflammatory drugs (NSAIDs) is often associated with chronic rhinosinusitis (CRS), nasal polyps (CRSwNP) and asthma, together known as Samter´s triad. The disease is characterised by eicosanoid imbalance. In our study, we determined clinical and laboratory parameters in respect of three groups of patients: 1) CRSwNP, 2) CRSwNP and asthma (CRSwNP-A), and 3) CRSwNP with asthma and NSAID-triggered hypersensitivity (CRSwNP-AA). Our main goal was to improve the characterisation of the stages of development in Samter´s triad, pointing to the homogeneous or heterogeneous course of disease.

Methodology: Forty-three patients (10 CRSwNP, 14 CRSwNP-A, 19 CRSwNP-AA) and 10 control subjects were included in the study. Nasal assessment using the CRS visual analogue score, endoscopy- and computer tomography scores, allergy tests, analysis of sinus surgeries, asthma severity and in vitro functional eicosanoid tests (FET) with peripheral blood leucocytes were performed.

Results: The scores reflecting CRS symptoms such as nasal congestion, nasal discharge and smell impairment differed between the patients groups reflecting the severity of disease (CRSwNP-AA > CRSwNP-A > CRSwNP). Eicosanoid imbalance correlated with nasal congestion, nasal discharge and loss of smell.

Conclusion: The data presented support the hypothesis of the continuous development of NSAID-triggered hypersensitivity, culminating in Samter's triad.

Key words: nasal polyps, Samter´s triad, aspirin-exacerbated respiratory disease, eicosanoids, functional eicosanoid test

Introduction

In recent European multi-centre studies of more than 5000 subjects, Hastan et al. revealed that 10.9% of tested subjects demonstrated chronic rhinosinusitis (CRS) [(1)] whilst Jarvis et al. documented a significant association of CRS and asthma [(2)]. CRS with nasal polyps (NP) and co-morbid asthma (CRSwNP-A) is associated with non-steroidal anti-inflammatory drug (NSAID)-triggered hypersensitivity, a syndrome referred to as Samter´s triad [(3)]. The hypersensitivity triggered by NSAID is determined with a standard in vivo nasal, bronchial or oral challenge [(4,5)]. Respiratory reactions induced by NSAIDs may occur at any point in the course of the disease, but are usually observed following the

onset of asthma and pansinusitis. However, a subset of patients may develop aspirin-sensitive rhinitis without asthma. Other manifestations of NSAID-triggered hypersensitivity include angioedemas [3].

In 36 to 96% of cases, patients with NSAID-triggered hypersensitivity have nasal polyps (NP) and up to 96% have pathological changes in the paranasal sinuses, a fact confirmed by computer tomography. In addition, many patients develop nasal polyps following paranasal sinus surgery. This results in multiple consecutive surgical procedures [6-9]. Of those individuals characterised by NSAID-triggered hypersensitivity and asthma, 60-70% have NP, whilst in asthmatics without NSAID-triggered hypersensitivity, NP occurr less frequently (in fewer than 10% of cases) [10]. A high co-morbidity is observed, in which all forms of the disease may occur, such as NSAID-triggered hypersensitivity with or without asthma and/or NP. Nevertheless, not all patients with NSAID-triggered hypersensitivity suffer from NP or vice versa. Moreover, there are evidently many variants of the course of the disease.

Szczeklik and Sanak [11] postulated an ongoing development of the disease pattern, whereas Kowalski hypothesised that NP with and without NSAID-triggered hypersensitivity are separate entities [12]. Eosinophilia, or eosinophilic inflammation, is presumed to form the link between NP, asthma, and NSAID-triggered hypersensitivity [13-17]. Patients with NSAID-triggered hypersensitivity are characterised by elevated concentrations of leukotrienes in polyp tissues, nasal secretions, bronchoalveolar lavage, urine, and in supernatants of cultured peripheral blood cells, whereas levels of cyclooxygenase-2 and prostaglandin E_2 are diminished [18-23]. Groneberg et al. demonstrated a neuropeptidergic influence in nasal mucosa in cases of aspirin-sensitive rhinitis [24]. The study of altered eicosanoid metabolism by Schaefer's group resulted in the development of a functional eicosanoid test (FET) performed in vitro using peripheral blood cells. This test quantifies the balance of leukotrienes, prostaglandins and neuropeptides, revealing a sensitivity of 96% and a specificity of 89% [25].

The aim of our study was to determine possible differences in clinical (i.e. patients' questionnaire on CRS symptoms, nasal endoscopy- and computer tomography scores, allergies, past sinus surgeries and severity of asthma) and laboratory parameters (i.e. eicosanoid imbalances) in three groups of patients: 1) patients with CRS and nasal polyps (CRSwNP), 2) patients with CRSwNP and asthma (CRSwNP-A), and 3) patients with CRSwNP, asthma and NSAID-triggered hypersensitivity (CRSwNP-AA). We also scrutinised the possible connection between the degree of eicosanoid imbalance and the severity of CRS symptoms, as well as findings that could indicate a homogeneous or heterogeneous course of the disease.

Materials and methods

Patients

Forty-three patients (22 men and 21 women) admitted to the ENT Department of the Charité University Hospital in Berlin were included in the study. The patients and control subjects gave their informed consent. The study was approved by a local ethics committee. All the patients were diagnosed with chronic rhinosinusitis and nasal polyps (CRSwNP) in accordance with EPOS 2012 criteria, asthma in accordance with GINA criteria [26,27]. Ten patients were diagnosed with CRS with NP (CRSwNP) (mean age 49.3 y, median 48 y, standard deviation [SD] 13.9 y, range from 24-79 y), 14 patients were diagnosed with CRSwNP and asthma (CRSwNP-A) (mean age 48.9 y, median 53.5 y, SD 10.8 y, range from 23-63 y), and 19 patients were diagnosed with CRSwNP, asthma, and NSAID-triggered hypersensitivity (CRSwNP-AA) (mean age 54 y, median 55 y, SD 14.3 y, range from 21-78 y). NSAID-triggered hypersensitivity was confirmed by oral or nasal provocation tests. The provocation tests were performed 7 to 21 months prior to blood collection and consecutive FET. We have followed the guidelines of EAACI/GA2LEN in terms of medications withdrawal periods [4]. Nasal and oral steroids were discontinued four weeks prior to FET testing. Patients using leukotriene receptor antagonists had discontinued the medication for at least 8 weeks before FET, without compromising their condition. Inhalation of steroids or β2-agonists was allowed.

For the control group, ten persons (six men and four women) were selected based on following exclusion criteria: nasal septum deviation of clinical significance, CRS or allergies, pathology seen during nasal endoscopy, positive skin prick test or medical history. Mean age of the controls was 50.1 y, SD 9.3, range from 39 to 67y. At the time of participation in the study, none of the control subjects had acute infection or any other major condition.

Clinical findings

Patient questionnaire: the severity of CRS symptoms – nasal congestion, nasal discharge, loss of smell, and facial pain – were recorded following EPOS 2012 criteria by means of visual analogue scale (VAS) scores (0 = not troublesome – 10 = worst thinkable) [26].

Nasal endoscopy: the extent of NP was tested using the Davos endoscopy score [28].

Paranasal sinus computer tomography (CT): a CT scan of the paranasal sinuses documented opacifications using the Lund-Mackay CT score [29].

Allergy diagnostics: the skin prick test was implemented to investigate potential atopy, in accordance with GA2LEN recommendations [30].

Asthma diagnosis: the grade of asthma severity was established according to GINA criteria: I – intermittent, II – mild, III – mode-

Table 1. Number of sinus surgeries, asthma severity, endoscopy and CT scores, allergies, VAS nasal symptoms as well as eicosanoid test values of the controls and patient groups : CRSwNP - chronic rhinosinusitis and nasal polyps; CRSwNP-A - chronic rhinosinusitis, nasal polyps and asthma; CRSwNP-AA - chronic rhinosinusitis, nasal polyps, asthma and NSAID-triggered hypersensitivity.

	Control	CRSwNP	CRSwNP-A	CRSwNP-AA
Number of sinus surgeries	0	0-8 (2.50)	0-3 (1.93)	1-6 (2.68)
Grade of asthma severity (GINA score)	-	-	I Intermittitent 2 x II Mild 8x III Moderate 4x IV Severe 0x Mean value 2.07	I Intermittent 7x II Mild 4x III Moderate 8x IV Severe 0x Mean value 2.05
Endoscopy score	0	1,00-6.00 (3.10)	1.00-4.00 (2.50)	2.00-5.00 (3.79)
CT score	-	10.0-14.0 (12.60)	10.00-20.00 (14.29)	11.00-22.00 (16.21)
Allergies	0%	50%	42.9%	31.6%
Nasal congestion	0.00-5.00 (1.00)	20.00-100.00 (65.00)	25.00-100.00 (79.43)	50.00-100.00 (84.21)
Nasal discharge	0.00-10.00 (1.00)	0.00-85.00 (27.00)	0.00-95.00 (53.64)	0.00-100.00 (59.00)
Loss of smell	0.00-0.00 (0.00)	0.00-100.00 (45.50)	0.00-100.00 (74.29)	0.00-100.00 (91.95)
Facial pain/ pressure	0.00-10.00 (1.50)	0.00-80.00 (26.50)	0.00-50.00 (25.71)	0.00-50.00 (12.63)
Leukotriene (LT) value	0.00-0.10 (0.01)	0.80-2.50 (1.55)	0.30-2.00 (1.13)	0.80-2.30 (1.42)
Prostaglandin (PG) value	0.00-0.10 (0.04)	0.30-2.50 (0.95)	0.00-1.80 (0.98)	0.30-1.50 (0.97)
Neuropeptidergic (NP) impact	0.00-0.50 (0.12)	0.00-3.00 (1.96)	0.50-3.00 (1.89)	0.50-3.00 (1.73)
AIT value	0.45-0.54 (0.50)	0.90-1.70 (1.28)	1.00-2.40 (1.56)	0.90-2.40 (1.56)

Shown are minimum – maximum values and mean values.

rate, IV – severe [27].

Laboratory findings in vitro study

Functional eicosanoid test and typing (FET): Peripheral blood was collected using lithium heparin as an anticoagulant. Blood cells were analysed for eicosanoid release upon in vitro modification as published [18,19,25,31,32]. In brief, aliquots of peripheral white blood cells were incubated for 20 minutes with diluents, with and without arachidonic acid, acetylsalicylic acid or neuropeptide. Next, the supernatant was analysed for prostaglandin E_2 or peptido-leukotrienes. The basal and induced releases were calculated, revealing integrated values of the prostaglandin imbalance (PG value), leukotriene imbalance (LT value), and neuropeptidergic induced impact (NP value). A further step of integration revealed the overall imbalance of eicosanoids, termed value of the analgesic intolerance test (AIT value). The AIT value summarises the functional interplay of the parameters. These values were classified according to Schäfer [32] as normal (<0.7), mild (0.7 to <1.5), moderate (1.5 to ≤2.5), or severe (>2.5 to 3.0). The calculated normalised values, taking into account the interaction of eicosanoids, represent the degree of eicosanoid imbalance for each individual patient.

Statistical analysis

A nonparametric test of independent samples (Mann-Whitney U test) was used to test for differences between the endoscopy- and CT scores, CRS symptoms as well as eicosanoid imbalances of controls and patient groups and between patient groups. Allergies were tested with the chi-squared test. Spearman's rank correlation was used to investigate the correlation between the functional eicosanoid test values, endoscopy scores, CT scores and CRS symptoms as well ($p < 0.05$).

Results

Clinical findings

All clinical and laboratory scores obtained from the three groups of patients and from the controls are presented in Table 1. Average values of CT score, clinical signs of nasal congestion, nasal discharge and smell impairments differed from the control group and between the patients groups, the scores reflecting severity of disease (CRSwNP-AA > CRSwNP-A > CRSwNP). Facial pain decreased in reverse order (Table 1). Interestingly, the Davos endoscopy score in the CRSwNP-A (mean value 2.50) was lower than the score in CRSwNP (mean value 3.10) or in CRSwNP-AA (mean value 3.79) groups.
Comparison of the severity of CRS symptoms, endoscopy scores,

Table 2. Comparison of a number of sinus surgeries, asthma severity, allergies, endoscopy and CT scores, nasal symptoms as well as eicosanoid test values between the patient groups.

	CRSwNP / CRSwNP-A	CRSwNP / CRSwNP-AA	CRSwNP-A / CRSwNP-AA
Number of sinus surgeries	8 / 3 0.987*	8 / 6 0.250*	3 / 6 0.150*
GINA asthma severity score	-	-	2.07 / 2.05 0.985**
Endoscopy score	3.10 / 2.50 0.355*	3.10 / 3.79 0.119*	2.50 / 3.79 0.001*
CT score	12.60 / 14.29 0.321*	12.60 / 16.21 0.000*	14.29 / 16.21 0.095*
Allergies (n)	5.0 / 6.0 1.000*	5.0 / 6.0 0.432*	6.0 / 6.0 0.716*
Nasal discharge	27.00 / 53.64 0.046*	27.00 / 59.00 0.040*	53.64 / 59.00 0.486*
Loss of smell	45.0 / 74.29 0.197*	45.0 / 91.95 0.011*	74.29 / 91.95 0.123*
Nasal congestion	65.00 / 79.43 0.238*	65.00 / 84.21 0.061*	79.43 / 84.21 0.480*
Facial pain/ pressure	26.50 / 25.71 0.893*	26.50 / 12.63 0.208*	25.71 / 12.63 0.031*
Leukotriene (LT) value	1.55 / 1.13 0.153*	1.55 / 1.42 0.709*	1.13 / 1.42 0.140*
Prostaglandin (PG) value	0.95 / 0.98 0.636*	0.95 / 0.97 0.427*	0.98 / 0.97 0.893*
Neuropeptidergic (NP) value	1.96 / 1.89 0.535*	1.96 / 1.73 0.351*	1.89 / 1.73 0.508*
AIT value	1.28 / 1.56 0.112*	1.28 / 1.56 0.066*	1.56 / 1.56 0.780*

CRSwNP - chronic rhinosinusitis and nasal polyps; CRSwNP-A - chronic rhinosinusitis, nasal polyps and asthma; CRSwNP-AA - chronic rhinosinusitis, nasal polyps, asthma and NSAID-triggered hypersensitivity. Given are the means and the p values; * significance level $p < 0.017$ (Bonferroni adjustment); ** significance level $p < 0.025$ (Bonferroni adjustment).

and CT scores revealed no significant difference between the CRSwNP and CRSwNP-A patient groups.
CRSwNP-AA patients had more severe loss of smell ($p < 0.011$) and more extensive CT opacifications ($p < 0.001$) than CRSwNP patients. Patients with CRSwNP-AA demonstrated a more pronounced Davos endoscopy score than CRSwNP-A patients ($p < 0.001$) (Table 2).
The severity of endoscopy score, nasal congestion, nasal discharge and loss of smell were significantly more pronounced in all patient groups than in the controls (Table 3). CRSwNP-A group has reported significantly more facial pain as compared to the control subjects, whereas no significant differences were detected between the CRSwNP and controls as well as between CRSwNP-AA and controls. Atopy, examined by prick test, did not yield significant differences among the patient groups. There were no significant differences in the number of nasal sinus operations between the patients groups (Table 1 and 2). Similarily, there were no significant differences between CRSwNP-A and CRSwNP-AA groups regarding GINA asthma severity score. None of our patients suffered form the most severe form of asthma (Table 1).

Laboratory findings

Increased FET values were detected in all patients examined, indicating eicosanoid imbalance in all patients suffering from the symptoms investigated (Table 1). The CRSwNP group had a mild eicosanoid imbalance, whereas CRSwNP-A and CRSwNP-AA demonstrated a moderate eicosanoid imbalance (mean AIT value: 1.28, 1.56, and 1.56, respectively). The individual parameters of the FET (i.e. AIT, leukotriene-, prostaglandin-, and neuropeptidergic value) indicated no significant differences among the patient groups (Table 2). Patients with CRSwNP-A had lower leukotriene imbalance (mean value 1.13) than the patients with CRSwNP or CRSwNP-AA (mean values 1.55 and 1.42, respecti-

Table 3. Comparison of the endoscopy- and CT scores, allergies, nasal symptoms as well as eicosanoid test values between the control subjects and patient groups. CT was not performed in the control group.

	Controls / CRSwNP	Controls / CRSwNP-A	Controls / CRSwNP-AA
Number of sinus surgeries	- / 8 -	- / 3 -	- / 6 -
Endoscopy score	0 / 3.10 0.000*	0 / 2.50 0.000*	0 / 3.79 0.000*
CT score	-	-	-
Allergies (n)	0 / 5 0.033*	0 / 6 0.024*	0 / 6 0.068*
Nasal discharge	1.00 / 27.00 0.002*	1.00 / 53.64 0.000*	1.00 / 59.00 0.000*
Nasal congestion	1.00 / 65.00 0.000*	1.00 / 79.43 0.000*	1.00 / 84.21 0.000*
Loss of smell	10 / 45.50 0.005*	10 / 74.29 0.001*	10 / 91.95 0.000*
Facial pain/ pressure	1.50 / 26.50 0.025*	1.50 / 25.71 0.000*	1.50 / 12.63 0.211*
Leukotriene (LT) value	0.01 / 1.55 0.000*	.01 / 1.13 0.000*	0.01/ 1.42 0.000*
Prostaglandin (PG) value	0.40 / 0.95 0.000*	0.40 / 0.98 0.000*	0.40 / 0.97 0.000*
Neuropeptidergic (NP) value	0.12 / 1.96 0.000*	0.12 / 1.89 0.000*	0.12 / 1.73 0.000*
AIT value	0.50 / 1.28 0.000*	0.50 / 1.56 0.000*	0.50 / 1.56 0.000*

CRSwNP - chronic rhinosinusitis and nasal polyps; CRSwNP-A - chronic rhinosinusitis, nasal polyps and asthma; CRSwNP-AA - chronic rhinosinusitis, nasal polyps, asthma and NSAID-triggered hypersensitivity. * Significance level $p < 0.017$ (Bonferroni adjustment).

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No eicosanoid imbalance was found in the control subjects (mean AIT value 0.50) (Table 1). In addition, the individual values of the FET were significantly lower in the controls than in the patient subgroups (Table 3).

Correlation of laboratory and clinical findings

The more pronounced the imbalance of eicosanoids was (measured as AIT, prostaglandin value and neuropeptidergic impact as well), the higher were the VAS scores indicating loss of smell,nasal discharge and nasal congestion;

- *AIT*: AIT and loss of smell $p < 0.001$, $r = 0.462$; AIT and nasal discharge $p < 0.001$, $r = 0.446$; AIT and nasal congestion $p < 0.05$, $r = 0.424$;
- *Prostaglandins*: PG and loss of smell $p < 0.001$, $r = 0.466$; PG and nasal discharge $p < 0.05$, $r = 0.414$; PG and nasal congestion $p < 0.05$, $r = 0.419$;
- *Neuropeptides*: NP and loss of smell $p < 0.001$, $r = 0.431$; NP and nasal discharge $p < 0.05$, $r = 0.339$; NP and nasal congestion $p < 0.001$, $r = 0.474$;

The imbalance of leukotrienes (leukotriene value) correlated positively with the severity of loss of smell ($p < 0.05$, $r = 0.336$) and nasal congestion ($p < 0.001$; $r = 0.445$). Moreover, there was a significant positive correlation between the AIT value and the type of CT finding ($p < 0.001$, $r = 0.492$). Finally, the Davos nasal endoscopy score did not correlate with FET, but did correlate with the CT score ($p < 0.05$; $r = 0.392$) and with nasal congestion ($p < 0.05$; $r = 0.322$).

Lastly, the number of sinus surgeries correlated with specific parameters of FET only in two cases: first, there was a correlation between number of sinus surgeries and AIT value in the CRSwNP-A group ($p < 0.05$) and second, a correlation between number of sinus surgeries and prostaglandin concentration in the CRSwNP-AA group ($p < 0.01$).

Discussion

In our present work, average values of CT scores, clinical signs of nasal congestion, nasal discharge and smell impairment differed between the study groups. The scores reflected severity of disease (CRSwNP-AA > CRSwNP-A > CRSwNP), confirming these cardinal CRS symptoms according to EPOS 2012 guidelines [26]. Facial pain decreased in reverse order (Table 1) also reflecting EPOS 2012 guidelines [26] where the importance of facial pain as

Table 4. Correlation between the functional eicosanoid test values, endoscopy scores, CT scores and CRS symptoms.

		1	2	3	4	5	6	7	8	9	10
CLINICAL DATA											
1 CT Score	r	1.000	**.392**	**.303**	**.380**	.175	-.218	.295	.163	.116	**.492**
	p		**.009**	**.048**	**.012**	.262	.160	.055	.295	.460	**.001**
2 Endoscopy	r	**.392**	1.000	**.322**	.160	.018	-.019	.258	.215	-.099	-.017
	p	**.009**		**.035**	.306	.908	.903	.095	.166	.529	.912
3 Nasal congestion	r	**.303**	**.322**	1.000	**.626**	**.505**	.236	**.445**	**.419**	**.474**	**.424**
	p	**.048**	**.035**		**.000**	**.000**	.090	**.001**	**.002**	**.000**	**.002**
4 Nasal discharge	r	**.380**	.160	**.626**	1.000	**.366**	**.391**	.264	**.414**	**.339**	**.446**
	p	**.012**	.306	**.000**		**.007**	**.004**	.056	**.002**	**.013**	**.001**
5 Loss of smell	r	-.175	-.018	**-.505**	**-.366**	1.000	.086	**-.336**	**-.466**	**-.431**	**-.462**
	p	.262	.908	**.000**	**.007**		.540	**.014**	**.000**	**.001**	**.000**
6 Facial pain	r	-.218	-.019	.236	**.391**	.086	1.000	.032	.218	.075	.168
	p	.160	.903	.090	**.004**	.540		.819	.117	.592	.229
LABORATORY DATA											
7 Leukotriene value	r	.295	.258	**.445**	.264	**.336**	.032	1.000	**.410**	**.552**	**.673**
	p	.055	.095	**.001**	.056	**.014**	.819		**.002**	**.000**	**.000**
8 Prostaglandin value	r	.163	.215	**.419**	**.414**	**.466**	.218	**.410**	1.000	**.480**	**.504**
	p	.295	.166	**.002**	**.002**	**.000**	.117	**.002**		**.000**	**.000**
9 Neuropeptidergic value	r	.116	-.099	**.474**	**.339**	**.431**	.075	**.552**	**.480**	1.000	**.743**
	p	.460	.529	**.000**	**.013**	**.001**	.592	**.000**	**.000**		**.000**
10 AIT	r	**.492**	-.017	**.424**	**.446**	**.462**	.168	**.673**	**.504**	**.743**	1.000
	p	**.001**	.912	**.002**	**.001**	**.000**	.229	**.000**	**.000**	**.000**	

P values less than 0.05 were considered significant and are highlighted in bold.

a cardinal symptom in the final assessment of CRS is questioned. Interestingly, the CRSwNP-A group of patients had the lowest score of nasal polyps (mean value 2.50), followed by CRSwNP (mean value 3.10) and CRSwNP-AA (mean value 3.79).

Patients with complete Samter's triad were characterised by more severe smell impairment as well as significantly elevated CT scores, as compared to CRSwNP patients. Moreover, patients suffering from Samter's triad scored significantly higher in nasal endoscopy than patients with CRSwNP and asthma. In contrast, there were no significant differences between CRSwNP and CRSwNP-A (Table 2).

Our results were similar to those of Dufour et al. and Hox et al., who detected no significant differences in the VAS score for nasal congestion, nasal discharge and smell impairment among CRSwNP, CRSwNP-A, and CRSwNP-AA patients [33,34]. In contrast to our data, Alobid et al. could not find significant differences between aspirin-tolerant and intolerant NP patients with asthma, in terms of nasal polyp size [35].

The FET indicated eicosanoid imbalances in all patients. At the same time, the patient subgroups revealed no significant differences in any FET value (Tables 1 and 2). Using the FET, we have demonstrated for the first time that the degree of eicosanoid imbalance correlated positively with clinical symptoms, such as AIT, prostaglandin values and neuropeptidergic values as well with symptoms of smell impairment, nasal discharge, nasal congestion and the leukotriene values with smell impairment and nasal congestion (Table 4).

Eicosanoid imbalances are considered to be the cause of aspirin-exacerbated respiratory disease (AERD) with increased leukotrienes and decreased prostaglandins in the nasal tissue of patients with AERD [12,36,37]. Pavord et al. described the association of neuropeptides with prostaglandins PGE_2 [38]. The cyclooxygenase COX-2 and PGE2 decreased significantly with the severity of the disease. More recent studies determined that enterotoxins derived from *Staphylococcus aureus* can regulate eicosanoids [39]. Less PGE2, decreased apoptosis, a distinct profile of infiltrating cells as well as more extensive CT findings were detected in NSAID-intolerant but not in NSAID-tolerant patients [40]. Based on this observations, it was concluded by Kowalski that NSAID-

intolerant and NSAID-tolerant patients with CRSwNP could represent separate CRS entities, at least from the point of view of pathogenesis [11].The same group later summarised the differences between NSAID-intolerant and NSAID-tolerant CRSwNP as being quantitative rather than qualitative, and secondary to the intensity of local inflammatory reactions rather than reflecting true pathophysiological abnormalities in the subpopulations of patients [41]. Gosepath et al. reported a normalisation of eicosanoid imbalances (PGE_2/ pLT) in the course of desensitisation with acetylsalicylic acid [42]. Forer et al. have described an improvement of the CRS symptoms (nasal congestion and discharge) following desensitisation with acetylsalicylic acid [43]. Also Grundmann et al. have reported a beneficial effect of leukotriene receptor antagonists on nasal symptoms [44].

Eicosanoid imbalances can be confirmed in peripheral blood leucocytes with the aid of the in vitro FET developed by Schaefer and Baenkler, featuring a sensitivity of 96% and specificity of 89% [25]. Using the FET, the imbalance of prostaglandins and leukotrienes, as well as the influence of neuropeptides, can be measured, and the individual results obtained are combined in the value of the analgesic intolerance test (AIT) to yield a graduated classification. Using the FET, all the control subjects demonstrated normal AIT values, but all the patients were characterised by mild to moderate eicosanoid imbalances (mean AIT value: controls: 0.50; CRSwNP: 1.28; CRSwNP-A: 1.56, CRSwNP-AA: 1.56) (Table 1). The AIT value, the imbalances of prostaglandins (PG values), leukotrienes (LT values), and neuropeptidergic influences (NP values) in all patient groups (i.e. CRSwNP, CRSwNP-A and CRSwNP-AA) differed significantly from the controls (Table 1 and 3).
On the other hand, there were no significant differences in any FET parameters among the individual patient groups (Table 2). This is consistent with the continuous development of the disease, as postulated by Szczeklik and Sanak [11]: The respiratory form of AERD consists of a cluster of symptoms, typically developing continuously from initial nasal discharge, nasal polyps, bronchial asthma and NSAID-triggered hypersensitivity and culminating in Samter's triad. Nevertheless, to resolve the outstanding issue of statistical significance, the FET needs to be performed on a larger sample of NSAID-tolerant and NSAID-intolerant patients.

Gosepath et al. studied the FET in patients with CRSwNP, recurrent CRSwNP, CRSWNP-A and CRSwNP-AA. They demonstrated that a positive AIT result was frequently accompanied by fully developed Samter's triad. Evaluation was based on frequency of symptoms expressed as a percentage [45]. In our study, all the patients (10 out of 10) suffering from CRSwNP demonstrated a mild AIT value, 50% of those suffering from CRSwNP-A (7 out of 14) revealed a mild AIT value and 50% a moderate AIT value; 32% of those suffering from CRSwNP-AA (6 out of 19) revealed a mild AIT value, while 68% (13 out of 19) demonstrated a moderate AIT value. Our results therefore indicated a trend towards higher AIT values in patients suffering from symptoms of Samter's triad, although no significant levels were reached.

To our knowledge, no study has yet examined the correlation of CRS symptoms – nasal congestion, nasal discharge, smell impairment and facial pain – with the eicosanoids prostaglandins and leukotrienes in patients suffering from CRSwNP/ CRSwNP-A or CRSwNP-AA. In our study, the correlation of FET values with CRS symptoms, CT and endoscopy results was significantly higher for smell impairment, nasal congestion and nasal discharge whenever the AIT value, prostaglandin imbalance, and neuropeptide influences were more pronounced. Leukotriene imbalance correlated significantly with loss of smell and nasal congestion. It may be possible that increasing nasal congestion and smell impairment are directly triggered by increased eicosanoid imbalances. To corroborate this hypothesis, Chiba et al. have observed during experiments on rats, that elevated leukotriene levels were involved in the development of vasodilation, leading to nasal congestion and smell impairment [46]. Furthermore, Lane et al. [47] described an accumulation of inflammatory cells in the olfactory epithelium of mice. Such an accumulation could potentially be a mechanism causing smell impairment in patients with CRS.

The correlation of values revealed by the FET with the CT score was significant only in relation to the AIT value. In other words, the severity of eicosanoid imbalance – the AIT value – correlated with the severity of the disease as revealed by the CT scan. There was no significant correlation between individual FET values and Davos endoscopy scores, which was studied for the first time. The correlation of the CT score with the VAS scores was significant in case of nasal discharge and nasal congestion. Holbrook et al. found a poor correlation between CT findings and RSOM-31 symptom scores [48]. We have also demonstrated that the endoscopy score has correlated with the CT score, confirming the observation of Ryan et al. or Toros et al. [49,50]. In addition, we have observed that the endoscopy score correlates with nasal obstruction (Table 4).

In summary, eicosanoid imbalance was detected in peripheral blood cells of patients with chronic rhinosinsusitis (CRS) with nasal polyps with and without asthma/ NSAID-triggered hypersensitivity via the in vitro functional eicosanoid test (FET). Moreover, there was a correlation between the severity of the eicosanoid imbalance and the extent of clinical findings in the paranasal sinus CT as well as the extent of CRS symptoms. This could support an ongoing development of the disease pattern with nasal polyps, asthma, and NSAID-triggered hypersensitivity, although

it is still not clear what factor mediates full development of Samter's triad in some patients, but not in others.

Conclusion

Eicosanoid imbalance measured by the FET correlates with the pattern of clinical symptoms of Samter's disease. There are indications of a continuous development of clinical symptoms of NSAID-triggered hypersensitivity culminating in Samter's triad. Future studies should determine additional factors and triggering mechanisms involved in this process. In clinical practice, the in vitro measurement of eicosanoid imbalance in patients with nasal polyps and/ or asthma/ NSAID-triggered hypersensitivity seems to be an attractive marker, permitting the grading of disease severity.

Authorship contribution

UF: conceived the study, performed patients examinations, collected and interpreted the data and wrote the manuscript
SS: performed patients examinations and collected the data
DS: performed the laboratory tests and collected the data
AJ.S: interpreted the data and wrote the manuscript
HO: conceived the study, supervised the project and wrote the manuscript.

Conflict of interest

All authors certify that there is no conflict of interest with any financial organization regarding the data or material discussed in the manuscript.

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Ulrike Förster
Department of Otorhinolaryngology
Charité-Universitätsmedizin Berlin
Augustenburger Platz 1
13353 Berlin
Germany

Tel: +49 30-450-555002
Fax: +49 30-450-555900
E-mail: ulrike.foerster@charite.de