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12-hydroxy-eicosatetraenoic acid (12-HETE): a biomarker of Churg-Strauss syndrome.

Condensed title: Elevation of 12-HETE in Churg Strauss syndrome.

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Abstract:

Background: Churg-Strauss syndrome (CSS), shares similarities with asthma and hypereosinophilic syndrome (HES). Eicosanoids - important inflammatory and signaling molecules – are present in exhaled breath condensate (EBC) and broncho-alveolar lavage fluid (BALF).

Objectives: To assess eicosanoid profile both in EBC and BALF of CSS subjects searching for a pattern characteristic for this syndrome.

Methods: EBCs from 23 CSS patients, 30 asthmatics, 12 HES patients and 54 healthy controls (HC) were assessed quantitatively for 19 eicosanoids by a high-performance liquid chromatography- tandem mass spectrometry (HPLC-MS/MS). Additionally in 21 out of 23 CSS subjects and in 9 asthmatics eicosanoids were determined in BALF.

Results: EBC from CSS patients showed markedly elevated levels of 12-HETE – as compared to other studied groups. BALF was characterized by a significant elevation of 12 HETE and its metabolite 12-tetranor HETE in CSS as compared to asthma. Clinical activity of CSS correlated with 12-HETE and its metabolites levels in BALF, but not in EBC.

Conclusion & Clinical Relevance: CSS is clearly distinguished from bronchial asthma, and HES by a marked increase in 12-HETE concentration in both EBC and BALF. This points to a possible new pathogenic mechanism in CSS and may help in future in establishing the diagnosis of CSS.

Key words: 12-HETE, Churg–Strauss syndrome, eicosanoids, exhaled breath condensate, vasculitis

Introduction

Churg-Strauss syndrome (CSS) is a rare, systemic, necrotizing, small-vessel vasculitis, characterized by blood eosinophilia, eosinophil infiltration of various tissues and bronchial asthma [1]. CSS develops in three successive phases: it usually starts as chronic rhinitis, joined by asthma, sinusitis and nasal polyposis; in the second stage peripheral blood eosinophilia prevails with eosinophil tissue infiltrations, progressing into the third phase of the life-threatening vasculitis [1]. CSS is diagnosed usually in the vasculitic phase, when the treatment may not prevent serious complications (ie. heart failure) [2]. Therefore, it is crucial to diagnose the disease as early as possible. However, this task is not easy, since CSS shares several features with other diseases, especially hypereosinophilic syndrome (HES) [3] and asthma [4].

Of hundreds of lipid mediators related to lipoxygenation and cyclooxygenation of polyunsaturated fatty acids the best recognized are prostanoids. Prostaglandin E₂ (PGE₂) exerts both pro- and anti-inflammatory actions in the lung, depending on the receptor subtype. PGD₂ is a bronchoconstrictor; it increases pulmonary oedema and attracts eosinophils. PGF_{2α} and its stereoisomere – 8-iso-PGF₂ are proinflammatory [5]. Thromboxane A₂ and prostacyclin have opposite effects on the lung vessels and bronchial muscles, but can be assessed only through the metabolites due to their rapid enzymatic inactivation. Lipoxygenation products are less characterized, the only exception being 5-lipoxygenase pathway. Its products (5-HETE, leukotrienes B₄, C₄, D₄, and E₄) were investigated in airway diseases, mostly because of therapeutic availability of both lipoxygenase inhibitors and cysteinyl leukotriens receptor antagonists. Two other lipoxygenases i.e. 12-LO and 15-LO are expressed in the lung. The 12LO/15LO metabolites exhibit a variety of biological activities; their role in diseases of cardiovascular and nervous systems has been of rapidly growing interest [6], but little is known of their possible involvement in pathophysiology of respiratory tract. A dual activity product –

lipoxin A4 is an inflammatory stop signal [7]. Recently described eoxins consist of 15-isomeres of cysteinyl leukotrienes, their pro-inflammatory action was related to asthma in children [8]. Little is known about a similar family of 12-isomeres, named hepoxilins; they affect granulocytes extravasation [9].

Eosinophil is the most abundant cell in CSS [10], HES [11], and often predominates in bronchial asthma . It is one of the effector cells responsible for cysteinyl-leukotrienes (cys-LTs) production, and it plays a major role in pathogenesis of asthma and other forms of allergic inflammation [12, 13], contributing to bronchial and parenchymal remodeling [14]. Urinary leukotriene E₄, the end metabolite of cys-LTs rises during exacerbation of both asthma and CSS [15]. Two other lipoxygenases, namely 12-LO and 15-LO, are also expressed in eosinophils [16].

The aim of the study was to evaluate the AA metabolites in the exhaled breath condensate (EBC) and bronchoalveolar lavage fluid (BALF) from patients with CSS. They were compared with EBC measurements in healthy controls, HES and asthmatic patients. To validate the findings, 12-HETE and its metabolites were also determined in BALF of a subgroup of asthmatics and the results were compared to those obtained in CSS.

Patients and Methods:

Patients

We enrolled 23 patients with CSS, 30 patients with asthma and 12 patients with HES from the Department of Medicine of the Jagiellonian University Hospital. Control group consisted of 54 healthy volunteers recruited from the hospital employees.

CSS diagnosis was established according to the American College of Rheumatology (ACR) criteria [17], which distinguishes six key features, namely: asthma, peripheral blood eosinophilia

> 10%, mono- or polyneuropathy, paranasal sinus abnormality, eosinophilic accumulation in tissues confirmed by a biopsy, and migratory infiltrates in lungs. Presence of four of these criteria makes the diagnosis highly probable. Only patients who fulfilled at least four out of six ACR criteria were included in our study. The BVAS score (*The Birmingham Vasculitis Activity Score* v.3 range: 0-63 pts. [18]) was used to assess activity of CSS; results above 4 indicated active disease. Remission of the disease was defined both on the basis of low BVAS score < 4 and on physical assessment with additional laboratory tests (ie: low and stable peripheral eosinophil count for at least 3 months, decreased acute inflammatory phase parameters and no clinically active vasculitis). Asthma diagnosis and severity were established according to the guidelines of National Heart, Lung, and Blood Institute/National Asthma Education and Prevention Program 2007 (NHLBI/NAEPP 2007) [19]. The severity of asthma was graded as follows: 1- intermittent asthma; 2 – mild asthma; 3 – moderate asthma; 4 – severe asthma. Patients with aspirin hypersensitivity had at least one episode of asthma attack after ingestion of a nonsteroidal anti-inflammatory drug in the past, and the diagnosis was confirmed by oral or inhalation provocation tests with aspirin [20], performed within one year before the study. HES was defined as chronically increased eosinophilia of ≥ 1500 cells/ μ l (≥ 6 months), accompanied by at least one organ damaged by eosinophil infiltration. The diagnosis was established after excluding other causes of hypereosinophilia, such as allergic sensitization, parasitic infestation or malignancy. Patients with diagnosed FIP1L1-PDGFR α rearrangement were excluded from the study [21]. Characteristics of patients with asthma who underwent bronchoscopy, is presented in the Results section.

All subjects studied gave an informed consent to participate. The study protocol complied with the Helsinki Declaration and was approved by the Jagiellonian University Ethics Committee.

Exhaled breath condensate collection and analysis

EBC were collected according to the ATS/ERS recommendations [22] using EcoScreen I Jaeger® condensing chamber (GmbH Hoechberg, Germany). Prior to the EBC collection participants were instructed to perform a mouth rinse. The whole amount of EBC collected (1-2ml) within 15min was immediately stored at -80 °C in polypropylene micro-centrifuge tubes.

Internal deuterated standards for the eicosanoid products were added and high-performance liquid chromatography – tandem mass spectrometry (HPLC-MS/MS- MS/MS Qtrap 4000, Applied Biosystems, Foster City, CA, USA) and gas chromatography – mass spectrometry (GC-MS Engine 5989B series II Hewlett Packard, Palo Alto, CA, USA) were used for the substances quantification with a stable isotope dilution method [23]. The detection levels for the eicosanoids were as previously described [23, 24] and the results were expressed in pg/ml. In 85% samples, the palmitic acid (PA) was analyzed, thus enabling correction of the data for EBC dilution factor expressed as eicosanoid content in picograms per microgram PA (ppm).

Bronchoalveolar lavage fluid collection and analysis

Following the EBC collection, either on the same, or the following day, BALF was obtained according to the British Thoracic Society guidelines [25]. BALF collection was performed after insertion of the bronchofiberscope (1T160; Olympus Medical Systems Corporation, Tokyo, Japan) to the right middle lobe bronchus under a mild sedation with midazolam (2.5-5 mg i.v.) and fentanyl (0.05-0.1 mg i.v.). Four 50-ml aliquots of sterile 0.9% sodium chloride at 37° C were sequentially instilled and withdrawn by gentle aspiration into a silicon-lined glass container placed on melting ice. Following centrifugation at 5 000 g for 15 minutes at 4° C supernatant aliquotes were stored in -80° C for further evaluation. The measurements generally followed the same procedure as for EBC. The difference consisted of a targeted measurement of

12-HETE, 15-HETE, and 12-LO metabolites: tetranor-12-HETE and 12-oxo-ETE, the deuterated standards of which were added to 1 mL BALF prior to organic-phase extraction.

BALF concentrations of eicosanoids were analyzed as raw data without normalization to the total protein, albumin or other markers. This strategy is consistent with the recommendation of the Bronchoalveolar Lavage Cooperative Study Group [26] and with the analytic approach of the US Severe Asthma Research Program (SARP) [27].

Lung function tests

In all patients lung function tests were performed using the standard protocols according to the ATS/ERS guidelines [28]. Forced expiratory volume in 1 s (FEV₁) and vital capacity (VC) were expressed as a percentage of predicted value. The lung function tests were performed in patients receiving a standard long-term control medications for asthma. Short-acting β_2 -agonists were stopped prior to testing.

Statistical analysis:

Statistical analysis was performed using StatSoft, Inc. (2009). STATISTICA (data analysis software system), version 9.0. www.statsoft.com, and GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA). Data are presented as the mean $\pm$ SD or median with interquartile range, when applicable. The comparisons of the eicosanoids between the groups was performed on log-transformed data if applicable, by one way Analysis of Variance (ANOVA) and post hoc Tukey test, or Kruskal-Wallis test for nonparametric distributed variables. Independent-sample t test (for unequal sized groups) was used on log-transformed data in comparisons of the eicosanoids in BALF samples. Correlation analyses were performed using Pearson's test or Spearman's test as appropriate with respect to data distribution. The diagnostic performance of 12-HETE, as a differential biomarker, was evaluated using Receiver Operating Characteristic

(ROC) curve analysis. The ROC curve was established with nonparametric approach methods, and the optimal threshold was based on the point of combined best sensitivity/specificity results. P values less than 0.05 were considered as statistically significant.

Results

Patients characteristics

We studied 23 patients with CSS (16 female, 7 male) aged 43.8 ± 9.8 years. Four patients had fulfilled all six diagnostic criteria, thirteen had five key features and in six patients four ACR criteria were present. All patients had a history of rhinitis, and asthma with elevated peripheral blood eosinophil count ($>10\%$ leukocytes with median maximal value of 5000 cells/ μL - before the initiation of treatment) and 91.3% had chronic sinusitis. Thirteen patients presented with peripheral nervous system involvement (56.5%), and 12 patients (52.2%) had histologically proven vasculitis. Positive perinuclear pattern of anti-neutrophil cytoplasmic antibodies (pANCA) was present in 5 patients (21.7%). In the course of the disease 21 patients (87%) had lung involvement, documented - by either X-ray or computed tomography. At the time of the examination fifteen patients were in remission, and the rest had exacerbation of the disease. Only 4 patients were not receiving corticosteroid therapy. In the remaining, the median dose was 5mg/per day [4-10] of methylprednisolone equivalent. Seven patients received additional immunosuppressive treatment (cyclophosphamide, azathioprine, or cyclosporine-A). The median value of the eosinophil count at the time of the study was 393 cells/ μL [128-640] in CSS patients, and did not differ from asthma 269 cells/ μL [144-484] and HES patients 425 cells/ μL [300-618]. However, in each of these groups, eosinophilia was higher when compared to healthy controls ($p<0.05$). The activity of the CSS expressed as BVAS correlated with the

lung function test parameters: FEV₁ (rho= -0.51; p=0.01), VC (rho= -0.51; p=0.015), eosinophil blood count (rho=0.82; p<0.0001) and blood platelet count (rho= 0.51; p=0.012). Patients in exacerbation of the disease, as compared to patients in remission had higher eosinophil blood count (394.5 cells/ μ L [267.5-1136.5] vs 368 cells/ μ L [22-587] respectively; p<0.0001) platelet count (315.7 $\times 10^3$ /mL ± 65.5 vs 229.3 $\times 10^3$ /mL ± 73.7 respectively; p=0.03), and lower lung function tests: FEV₁ (81.3% [74.9-92] vs 100.3 [78-105] respectively; p=0.007) and VC (94.8% [90-107] vs 104[69.5-112] respectively; p=0.02). Individual clinical data of CSS subjects are presented in Table.1.

Out of 30 patients with asthma (18 women, 12 men; aged 39.9 ± 9.6 years), seven (23.3%) had aspirin sensitive asthma (AIA), while the remaining tolerated aspirin well. The severity of asthma was graded as follows: the first grade - 21 patients, the second - 3 and the third grade - one. No patient was in exacerbation or received oral corticosteroids. Ten patients (33.3%) had chronic sinusitis. Lung function of asthmatics was: FEV₁ (90.4% ± 11) and VC (94.6% ± 10.7).

In the HES group all patients were in the remission of the disease. They were all treated with corticosteroids (median dose in methylprednisolone equivalents was 10mg/per day [4-16]). Additionally two patients received hydroxycarbamide and 5/12 (42%) were on inhaled corticosteroids. In the course of the disease 8/12 (66.7%) patients had respiratory symptoms, 4/12 (33.3%) had cardiac involvement, 8/12 (66.7) had cutaneous abnormalities, and nervous involvement was found in 3/12 (25%). Their mean FEV₁ was (92% ± 7 and VC 96% ± 8).

The control group, comprising 54 volunteers (34 female, 20 male), aged 36 ± 13.1 years, was matched by– sex and –age with CSS and asthmatic patients. It was, however, younger than the HES group (age 54.4 ± 16.8 years; p<0.05).

None of the healthy controls had a history of respiratory diseases; 5 out of 54 subjects were current smokers, and 4 had a history of previous smoking. The FEV1 for control group was $96.4\% \pm 7$ and VC 97.8 ± 8 . No differences were observed in lung function tests between studied groups and healthy controls.

EBC results

Groups comparisons

EBC differentiated CSS subjects from other participants of the study (ie. HC, asthma and HES) by a significant elevation of LTC₄, eoxin D₄, 12-HETE and PGF_{2α}. The most striking was the high concentration of 12-HETE in CSS patients (Fig.1). ROC curves for 12-HETE as a differentiating biomarker were analyzed for comparisons as shown in Fig.2. The optimal threshold of overall performance of 12-HETE as a distinguishing biomarker was chosen as the point of combined best sensitivity and specificity and established at the level above 6.16pg/ml. The combined specificity and sensitivity (in all groups in comparison to CSS patients) was above 80 %. The area under the curve was 86.2% (p<0.001) for the contrast between CSS and asthma, 96.6% (p<0.001) for HES vs CSS and 88.9% (p<0.001) between CSS and HC.

When EBC metabolites were corrected for dilution factor using palmitic acid, 12-HETE remained as the only significantly differential parameter.

The analyzed metabolites of arachidonic acid are presented in Table.2; Table 3 presents dilution-corrected data. Within the asthma group (n=30), aspirin sensitive subjects (n=7) showed no distinct differences in EBC eicosanoids.

Correlation of the clinical data and EBC eicosanoids in CSS patients.

No correlation between clinical manifestation of the CSS presentation, dose of oral corticosteroids, BVAS score, or remission/exacerbation period and EBC eicosanoids was observed. When additional immunosuppression (AIS) was taken into account, patients without corticosteroids or on corticosteroids alone as compared to patients receiving AIS had higher levels of 15 HETE (5.9 ± 2.9 vs 2.7 ± 0.8 respectively; $p=0.004$) and lower levels of eoxin D₄ (0.9 ± 0.5 vs 1.2 ± 0.19 respectively; $p=0.04$). No other differences were found within CSS group, stratified for the requirement of AIS. Lung function tests correlated with 15-HETE levels (FEV₁, $\rho = -0.61$; $p=0.002$). Increased eosinophil count correlated with lower levels of LTD₄ ($\rho = -0.46$; $p=0.027$) and elevated platelets blood count ($\rho = 0.59$; $p=0.003$).

Bronchoalveolar lavage evaluation

Subjects sampled for BALF

Out of 23 patients with CSS who underwent EBC sampling, BALF was collected in 21. Detailed characteristic of these patients is shown in Table 1.

To compare a limited set of lipoxygenases metabolites, BALF was collected from 9 asthmatic patients aged 49.6 ± 14.1 years. At the time of BALF sampling 5 asthmatic patients had severe asthma (severity grade 4) and the remaining four had moderate asthma (severity grade 3); average FEV₁ was $64.3 \pm 33.2\%$. 5 patients were treated with oral steroids (17.6 ± 3.6 mg of methylprednisolone) and all were on inhaled steroids. All patients had blood eosinophil count within the normal range (median 31 cells/ μ L).

12-HETE metabolites and 15 HETE in BALF

When compared to asthmatic subjects, CSS patients, had higher concentrations of both 12-HETE (99.6 ± 113.6 pg/ml vs 30.5 ± 20.94 respectively; $p=0.012$) and its metabolite, tetranor-12-HETE (9.7 ± 20.2 pg/ml vs 2.59 ± 3.85 respectively; $p=0.038$). These two compounds were positively intercorrelated ($\rho=0.72$; $p<0.001$). Additionally there was a negative correlation between 12-oxoETE and 12-HETE ($\rho=-.62$; $p=0.0003$) and a positive one between 12-oxoETE and tetranor-12-HETE ($\rho=0.5$; $p=0.0057$). However, 12-oxoETE levels did not differ between the CSS and asthmatic groups. 15-HETE concentration was higher in BALF from CSS when compared to asthmatics (144.2 ± 139.3 pg/ml vs 42.6 ± 29.7 respectively; $p=0.032$) and correlated inversely with FEV1 ($\rho=-0.43$; $p=0.05$) and VC ($\rho=-0.47$; $p=0.02$). No correlation between eicosanoids measured concurrently in EBC and BALF was observed.

When patients with CSS were divided according to the disease activity, exacerbation (8 patients) as contrasted to remission (13 patients) was accompanied by higher concentrations of 12-oxo-ETE (5.3 ± 8.4 pg/ml vs 1.6 ± 1.3 ; $p=0.03$) and 15-HETE (221.2 ± 170.4 pg/ml vs 96.8 ± 95 ; $p=0.048$). Trends towards higher concentrations of 12-HETE (149 ± 159.2 pg/ml vs 69.3 ± 64.1 ; $p=0.09$) and tetranor-12-HETE (19.6 ± 31.3 pg/ml vs 3.6 ± 2.7 ; $p=0.053$) in the disease exacerbation were noted.

There were no differences in BALF eicosanoids in the CSS group stratified for AIS.

Discussion

CSS is regarded as an eosinophilic vasculitic syndrome, the prognosis of which depends on systemic damage of the kidney, heart, gastrointestinal tract or nervous system [2]. The syndrome has insidious onset and differential diagnosis, particularly with asthma is difficult, until the full clinical spectrum develops. We used a non-invasive method of EBC collection, which offers an insight into pathology of respiratory tract and holds promise for clinical utility. Eicosanoids, important inflammatory and signaling molecules, are present in EBC in very low concentrations, close to the detection threshold of immunoenzymatic methods. In this study, we applied a newly developed and a highly sensitive analytical method of mass spectrometry [23] already validated in the children asthma study [8]. Concurrent measurement of palmitic acid concentration in EBC seems a convenient surrogate for a dilution factor of the condensate, although alternative methods are available [22].

We investigated the eicosanoids in EBC of CSS subjects in order to identify differences from other diseases sharing similarities to CSS, i.e. asthma and HES. The prominent finding, a distinct 12-HETE elevation in CSS, seems highly specific for the disease. Next, using the same lipidomic platform, we observed similar elevation of 12-HETE in BALF, an established material for studies of the lung pathology. 12-HETE, together with hepoxilins, are the two major arachidonic acid metabolites produced via the 12-LOX pathway. Several studies have suggested that 12-HETE may be involved in pro-inflammatory actions, such as: migration and chemotaxis of inflammatory cells and their activation, leukocyte-vascular adherence, angiogenesis, and vascular permeability [6, 29]. Mice lacking 12/15-LO did not develop intense airway inflammation after allergen challenge and did not show the consecutive airway remodeling [30]. Additionally, 12-HETE increases the cell proliferation, and has been documented as procarcinogenic factor in many neoplasms [31, 32]. It is overexpressed in the immature cancer

cells, and promotes the metastasis [33]. Hepoxilins, the chemical standards of which were not available during the current study, are represented by the best described hepoxilin A₃ and are far less abundant than 12-HETE [34]. These lipid mediators are believed to contribute to a chemotaxis of neutrophils in the inflammatory process [9]. 12-HETE is mostly produced by blood platelets. However, we did not observe concomitant increase in the levels of 11-dehydro-TXB₂, which may suggest that 12-HETE was unlikely to be derived solely from platelets. We, therefore, hypothesize that in CSS, the 12-HETE increase may originate either from eosinophils or interactions between eosinophils and platelets, as both of these blood cells intercorrelated in CSS patients. The latter hypothesis finds its rationale in many previous studies on platelet-eosinophil interaction. Activation of platelets in patients with asthma results in an increased eosinophil infiltration [35], and increase in platelet number precedes the increase in eosinophil count [36]. This finding is parallel to our observation of positive eosinophil-platelet count correlation in CSS patients. Additionally, in asthmatic patients, the platelet activating factor (PAF), produced by the platelets, is a potent mediator of airway hyperresponsiveness and inflammatory process [37] that attracts and activates the eosinophils [38]. The proinflammatory action of PAF and bradykinin has been postulated to act via the 12-LO pathway and increased production of 12-HETE [34]. Furthermore, it has been described recently that platelets activation markers may contribute to exacerbation of ANCA-associated vasculitis [39].

It is interesting that the usage and dose of oral corticosteroids did not influence the 12-HETE level, which may suggest that the 12-HETE production is steroid-insensitive.

In the present study ROC curve analysis proved 12-HETE to be a highly efficient biomarker differentiating CSS from HC, asthma and HES. The increase in 12-HETE was independent from the activity of the disease and showed a potential as a useful noninvasive hallmark in differential diagnosis. One of the reasons for the differences between the studied

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groups may be the high lung involvement in CSS in addition to asthma [40, 41] which was present in all of studied CSS patients during the course of the disease. The CSS patients had higher levels of LTC₄, and PGF_{2α} in comparison to other groups, however the statistical significance of this difference was lost after the correction for dilution using palmitic acid concentration. Analysis of EBC of CSS patients according to AIS therapy revealed that these drugs suppressed 15-HETE synthesis, a metabolite that has been reported to contribute to asthma pathogenesis, and to increase the inflammatory process [42]. 15-HETE overproduction, was also associated with tissue eosinophil accumulation, a phenomenon relevant to CSS pathogenesis [43], which may be driven by platelet activation and PAF production [44]. In the present study 15-HETE overproduction (both in EBC and BALF) correlated with the decreased FEV1, and VC (only in BALF) of CSS patients. Moreover, patients with AIS had higher levels of eoxin D₄, the novel 15-LO pro-inflammatory isomer of cysteinyl leukotrienes. This could suggest more severe phenotype of CSS, requiring an AIS medication. EBC from the CSS patients did not show differences when disease activity was taken into account, possibly due to relatively small number of patients in exacerbation. Some of these CSS specific changes in eicosanoid content of EBC lost their statistical significance, upon correction for the dilution factor using palmitic acid. Although between the groups differences in PA concentration in EBC were negligible, among adjusted eicosanoids only 12-HETE was left as a specific biomarker of CSS.

EBC content of non-volatile compounds has a great inter-individual variability. Moreover, it depends on the lung ventilation mechanics as the lung epithelial fluid is dispersed into aerosol and thereafter most particles get trapped in the airways during exhalation. Notwithstanding a possible bias resulting from differences in FEV1 between asthmatics including CSS and non-asthmatic subjects, 12-HETE remained a distinct biomarker of CSS. This conclusion is

supported by the same phenomenon observed in BALF. It seems, that beta-oxidation catabolic pathway of 12-HETE is the most important one, resulting in a parallel elevation of tetranor-12-HETE. The other downstream metabolite, 12-ETE is increased during exacerbation of the disease and probably represents a spillover metabolite of 12-HETE. Pathological implications of 12-HETE overproduction are extensively studied in cardiovascular medicine and cancer research [6], but the role of this mediator in the lung diseases remains obscure.

No correlation was found between identical eicosanoids in BALF and in EBC. This is in agreement with the results of Jackson et al. [45], who following a study specifically addressing this question, and measuring a number of biomarkers, concluded that EBC sampling cannot be directly compared with information derived from BALF.

Limitations of the study: Palmitic acid was not measured in all samples, which might have reduced the study power, although it had no impact on the distinct elevation of 12-HETE. We did not study downstream 12-LO metabolites hepoxilins. We did not measure the eicosanoid products in other than EBC and BALF biological fluids, however, most of these compounds are difficult to detect in either blood or urine, and their levels vary widely across the methodology used (e.g 12-HETE, is abundant in serum, but its levels depend on platelet activation due to sampling). Differences in BALF 12-LO eicosanoids were less spectacular because 1) no measurements of their concentrations were not corrected for the fluid recovery and 2) a limited number of asthmatics agreed to subdue the invasive procedure of bronchoscopy.

To summarize, exhaled breath condensate of subjects with Churg-Strauss syndrome is characterized by significantly elevated concentration of 12-HETE. This non-invasive assessment of the inflammatory mediator suggests a new pathogenic mechanism of this rare vasculitis syndrome and may find a place in differential diagnosis of CSS.

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Conflict of interest statement:

All authors declare no conflict of interest

Table 1. Characteristics of patients with Churg-Strauss syndrome

I.D.	1	2*	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19*	20	21	22	23
Age/Gender	47/F	56/F	42/M	30/F	36/F	44/F	50/M	58/F	37/M	57/M	47/F	39/F	33/F	55/M	31/M	32/F	46/F	61/F	33/F	53/M	36/F	52/F	41/F
ACR score total	6	4	5	5	5	5	6	6	5	5	4	5	5	5	4	4	5	4	5	5	4	6	5
EOS10%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
sinuses	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+
chest X-ray	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
biopsy	+	-	-	-	-	+	+	+	+	+	-	-	-	+	-	-	+	-	+	+	-	+	+
neuropathy	+	-	+	+	+	+	+	+	-	+	-	+	+	-	-	+	-	+	-	-	-	+	-
BVAS	0	0	0	24	0	0	37	4	0	16	0	0	0	0	4	0	6	0	14	0	0	12	14
EOS	128	267	587	4992	3	37	8424	618	268	558	393	28	454	368	1229	287	270	300	640	396	28	21622	1633
p-ANCA	+	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+
Asthma	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astma severity**	1	2	1	2	1	1	1	2	1	2	1	1	1	1	3	1	3	1	3	2	1	2	3
FEV1 (%)	78.1	90.5	101	77.5	120	68.7	73.5	72.6	94	93.7	107	79.7	89.9	100	37.2	93.5	105	76.2	77.9	83	104	82.9	52.6
FEV1/FVC	100	94.9	90	84.9	84	76.9	71.3	68.5	82.3	94.4	95.4	89.4	82.0	85.5	67.7	89.0	87.0	92.0	75.9	82.7	94.2	63.7	69.6
OCS	8	10	4	0	4	10	0	4	4	6	5	4	20	16	4	4	16	10	0	8	10	12	0
AIS	+	-	+	-	+	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	+	-
EBC																							
12-HETE	14.9	15.7	11.5	63.1	6.66	9.54	12.9	40.7	12.1	13.18	3.15	32.8	30.87	2.12	10.7	14.8	8.02	1.65	23.78	8.96	7.24	4.27	9.45
12-HETE ppm	NA	NA	39.6	NA	NA	17.8	NA	90.5	25.6	29.82	7.07	67.7	64.02	4.47	NA	NA	22.4	5.11	38.08	NA	NA	16.42	28.48
15-HETE	2.71	3.73	4.11	7.49	2.1	7.66	4.96	10.1	2.64	2.13	2.69	6.22	12.81	1.83	3.3	6.85	2.71	3.37	8.99	4.26	3.32	4.04	6.05

15-HETE ppm	NA	NA	14.2	NA	NA	14.3	NA	22.4	5.58	4.82	6.03	12.8	26.57	3.85	NA	NA	7.59	10.5	14.39	NA	NA	15.53	18.24
BALF																							
12-HETE	54.5	NA	176	152	37.9	37.5	94.6	48.6	192	18.01	12.2	50.8	11.89	13.2	525	32.4	151	24.3	NA	123.5	134	77.11	125.6
12-tetranor-HETE	2.31	NA	7.67	3.7	5.74	0.48	6.05	3.13	7.45	2.46	0.72	4.6	0.75	0.58	37.5	3.84	10.1	2.4	NA	3.31	6.99	2.68	91.34
12-OH-ETE	0.97	NA	4.46	3.08	1.34	1.11	1.38	1.58	2.38	2.08	3.23	0.36	0.14	0.21	25.9	1.69	4.85	0.28	NA	2.9	1.57	1.44	2.38
15-HETE	80.1		297	296	54.9	57.9	128	90.1	250	22.8	20.1	82.6	3.04	19.2	554	33.8	257	23.1	NA	185.8	150	110.1	311.3

Values expressed as medians. Abbreviations: ACS – asthma control score; ACR - American College of Rheumatology; AIS – additional immunosuppressants; BALF – broncho-alveolar lavage fluid; BVAS - the Birmingham Vasculitis Activity Score; EBC - exhaled breath condensate; EOS – blood eosinophilia; FEV1 - forced expiratory volume in the one second; FVC: Forced vital capacity; FEV1/FVC - the ratio of FEV1 to FVC (Tiffeneau index); NA – not available; OCS – oral corticosteroids; p-ANCA – perinuclear anti-neutrophil cytoplasmic antibodies; 12-HETE – 12-hydroxyeicosatetraenoic acid, 12-OH-ETE – 12-oxo-5,8,10,14-eicosatetraenoic acid; 15-HETE – 15-hydroxyeicosatetraenoic acid, 12-tetranor-HETE- 12-teranor- hydroxyeicosatetraenoic acid. *- indicates patients who did not have BALF studied; **- severity of asthma established according to the guidelines of National Heart, Lung, and Blood Institute/National Asthma Education and Prevention Program 2007 (NHLBI/NAEPP 2007) [19].

Table 2. Eicosanoids in exhaled breath condensate (EBC) of Churg-Strauss patients, healthy controls, asthmatics and hypereosinophilic syndrome cases.

	CSS (n=23)	Asthma (n=30)	HES (n=12)	HC (n=54)
LTB ₄	3.33 [1.72-7.55]	15.7 [5.7-36.2]***	2.98 [1.91-6.62]	8.44 [4.87-13.6]*
LTC ₄	3.49 [1.55-7.92]	0.94 [0.53-1.9]**	1.07 [0.719-1.41]**	1.63 [0.87-2.81]**
LTD ₄	1.84 [0.82-3.19]	2.2 [1.13-4.67]	0.57 [0.4-0.98]	1.5 [0.65-2.48]
LTE ₄	1.14 [0.74-1.47]	2.05 [1.43-3.28]*	1.31 [0.59-2.01]	1.18 [0.77-1.85]
Lipoxin A ₄	0.27 [0.17-0.64]	0.62 [0.4-1.17]*	1.14 [0.6-1.58]**	0.29 [0.17-0.64]
Eoxin C ₄	1.15 [0.77-2.89]	0.71 [0.36-1.38]	0.62 [0.33-0.87]	1.76 [0.45-8.87]
Eoxin D ₄	0.98 [0.7-1.15]	0.87 [0.27-2.17]*	0.8 [0.65-1.44]*	0.89 [0.54-1.55]
Eoxin E ₄	0.62 [0.5-1.25]	1.55 [0.84-4.56]	1.7 [1.44-2.48]	1.26 [0.65-2.88]
PGD ₂	0.97 [0.63-1.98]	1.55 [0.85-2.67]	0.95 [0.57-2.32]	0.67 [0.31-1.56]
PGE ₂	0.45 [0.26-0.76]	0.59 [0.45-1.01]	0.93 [0.51-2]	1 [0.56-1.88]*
tetranor-PGEM	90.3 [62.2-283.73]	38.1 [22.01-52.32]***	67.15 [34.93-91.31]	118.6 [32.9-183.8]
12-HETE	11.45 [7.24-15.68]	3.34 [1.64-5.78]***	1.59 [1.32-2.28]***	3.16 [2.22-4.32]***
11-dehydro-TXB ₂	5.07 [3.98-5.73]	6.73 [5.43-6.9]*	6.42 [5.58-8.87]	4.78 [3.96-6.7]
5-HETE	2.66 [1.99-	2.27 [1.65-	1.86 [1.1-2.47]	2.75 [1.8-5.1]

	3.25]	3.06]		
15-HETE	4.04 [2.71-6.85]	2.95 [1.8-4.04]	3.25 [2.5-7.32]	2.64 [2.13-4.4]
6-keto-PGF _{1α}	9.23 [7.8-17.44]	7.42 [7.14-8.05]	6.31 [3.25-8.4]**	9.13 [7.15-13.15]
8-iso-PGF ₂	0.29 [0.22-0.53]	0.27 [0.2-0.4]	0.3 [0.21-0.46]	0.25 [0.15-0.3.9]
9a,11b-PGF ₂	0.48 [0.24-1.53]	0.3 [0.2-0.4]	0.12 [0.035-0.42]*	0.29 [0.16-0.49]
PGF _{2α}	0.94 [0.67-1.2]	0.3 [0.22-0.4]***	0.16 [0.06-1.49]**	0.38 [0.15-0.56]***
palmitic acid	449.9 [331.8-482.3]	425 [260-585.1]	498.8 [468.1-621.8]	450.3 [250.7-693]

Median values [25-75 interquartile range] of eicosanoids presented in pg/ml and palmitic acid in ng/ml.

Abbreviations: CSS- Churg-Strauss syndrome; HES-hypereosinophilic syndrome; HC- healthy controls

*- P values compared to CSS patients:

* p<0.05; ** p<0.01; *** p<0.001

Table.3

Eicosanoids in exhaled breath condensate (EBC) of Churg-Strauss patients, healthy controls, asthmatics and hypereosinophilic syndrome cases standardized for palmitic acid.

	CSS (n=23)	Asthma (n=30)	HES (n=12)	HC (n=54)
LTB ₄	16 [8.58-23.1]	34.4 [17.9-70.2]**	4.79 [3.85-14.98]	17.53 [10.6-29.76]
LTC ₄	3.91 [3.02-7.76]	2.38 [1.44-3.74]	1.76 [1.31-2.63]	2.34 [1.23-8.67]
LTD ₄	3.37 [1.29-5.9]	5.08 [2.79-10.84]	1.26 [0.67-1.39]	2.42 [1.05-5.38]
LTE ₄	2.6 [0.95-4.3]	5.17 [3.11-8.9]	2.5 [1.02-4.73]	2.03 [1.33-5.9]
Lipoxin A4	1.09 [0.6-2.09]	1.65 [1.01-2.92]	2.21 [0.68-3.13]	0.8 [0.38-1.58]
Eoxin C ₄	3.72 [1.59-6.41]	1.43 [0.98-2.38]	1.11 [0.41-1.93]	2.99 [0.84-25.13]
Eoxin D ₄	2.24 [1.69-3.04]	2.05 [0.9-3.8]	1.83 [1-3.64]	2 [1-4.73]
Eoxin E ₄	1.58 [0.81-2.83]	3.74 [2.64-11.31]*	3.2 [2.47-4.5]	3.26 [1.47-6.75]
PGD ₂	4.02 [1.23-7.44]	3.73 [1.86-6.37]	1.93 [1-4.93]	1.53 [0.68-3.39]
PGE ₂	1.29 [0.65-1.57]	1.32 [0.8-2.92]	1.59 [0.69-3.77]	2.31 [1.19-3.52]
tetranor-PGEM	138.62 [88.2-271.37]	88.07 [47.81-122.48]	97.34 [66.93-156.21]	180.09 [54.64-603.03]
12-HETE	27.04 [16.42-39.6]	8 [3.88-14.68]*	2.79 [2.41-3.24]***	4.95 [3.51-9.55]***
11-dehydro-TXB ₂	8.7 [6.85-15.78]	15.53 [9.77-25.03]	13.55 [10.47-15.86]	9.87 [6.43-16.31]
5-HETE	4.67 [3.72-6.77]	4.62 [3.47-8.93]	3.41 [1.97-4.52]	6.78 [3.27-11.48]

15-HETE	13.52 [6.03-15.53]	6.72 [3.52-11.85]	7.05 [3.57-11.6]	5.85 [3.3-12.49]
6-keto-PGF _{1α}	18.51 [16-23.56]	17.32 [12.2-45.67]	13.07 [4.85-19.78]	16.17 [11.54-33.2]
8-iso-PGF ₂	1 [0.76-1.19]	0.7 [0.53-0.88]	0.68 [0.36-0.93]	0.57 [0.29-0.85]
9a,11b-PGF ₂	0.75 [0.38-0.92]	0.66 [0.45-1.15]	0.35 [0.06-1.08]	0.66 [0.33-0.97]
PGF _{2α}	2.15 [1.23-2.6]	0.72 [0.43-1.16]	0.42 [0.11-5.19]	0.66 [0.42-0.85]

Median values [25-75 interquartile range] of eicosanoids presented as eicosanoid/palmitic acid ratio.

Abbreviations: CSS- Churg-Strauss syndrome; HES-hypereosinophilic syndrome; HC- healthy controls

* P values compared to CSS patients

* p<0.05; ** p<0.01; *** p<0.001

FIG.1

Comparison of 12-HETE in exhaled breath condensate in the studied groups.

Footnotes:

Data shown as medians with interquartile range

HC-healthy controls; CSS- Churg Strauss syndrome; HES- hypereosinophilic syndrome

*** $p < 0.001$ compared to CSS;

FIG2.

ROC curves for 12-HETE concentration in EBC as the biomarker distinguishing patients with CSS from HC, asthma and HES

Footnotes

Sensitivity and specificity for 12-HETE levels above 6.16pg/ml (dot line; chose as the point of combined best sensitivity and specificity) in differentiating CSS

CSS vs HC - sensitivity= 82.6%; specificity=94.2%

CSS vs asthma - sensitivity= 82.6%; specificity=80.7%

CSS vs HES - sensitivity= 83%; specificity=100%

HC-healthy controls; CSS- Churg Strauss syndrome; HES- hypereosinophilic syndrome

References

1. Szczeklik W, Jakiela B, Adamek D, Musial J, Cutting Edge Issues in the Churg-Strauss Syndrome. Clinical reviews in allergy & immunology 2011.
2. Guillevin L, Lhote F, Gayraud M, Cohen P, Jarrousse B, Lortholary O, Thibault N, Casassus P, Prognostic factors in polyarteritis nodosa and Churg-Strauss syndrome. A prospective study in 342 patients. Medicine (Baltimore) 1996;75: 17-28.
3. Helbig G, Kyrz-Krzemien S, Diagnostic and therapeutic management in patients with hypereosinophilic syndromes. Polskie Archiwum Medycyny Wewnętrznej 2011;121: 44-52.
4. Szczeklik W, Sokolowska BM, Zuk J, Mastalerz L, Szczeklik A, Musial J, The course of asthma in Churg-Strauss syndrome. J Asthma 2011;48: 183-7.
5. Serhan CN, Novel lipid mediators and resolution mechanisms in acute inflammation: to resolve or not? The American journal of pathology 2010;177: 1576-91.
6. Dobrian AD, Lieb DC, Cole BK, Taylor-Fishwick DA, Chakrabarti SK, Nadler JL, Functional and pathological roles of the 12- and 15-lipoxygenases. Prog Lipid Res 2011;50: 115-31.
7. Levy BD, De Sanctis GT, Devchand PR, Kim E, Ackerman K, Schmidt B, Szczeklik W, Drazen JM, Serhan CN, Lipoxins and aspirin-triggered lipoxins in airway responses. Advances in experimental medicine and biology 2003;525: 19-23.
8. Sachs-Olsen C, Sanak M, Lang AM, Gielicz A, Mowinckel P, Lodrup Carlsen KC, Carlsen KH, Szczeklik A, Eoxins: a new inflammatory pathway in childhood asthma. J Allergy Clin Immunol 2010;126: 859-67 e9.
9. Nigam S, Hepoxilins: novel enzymatic pathways and clinical significance. The FEBS journal 2007;274: 3493.
10. Jakiela B, Szczeklik W, Sokolowska B, Mastalerz L, Sanak M, Plutecka H, Szczeklik A, Intrinsic pathway of apoptosis in peripheral blood eosinophils of Churg-Strauss syndrome. Rheumatology (Oxford) 2009;48: 1202-7.
11. Simon HU, Rothenberg ME, Bochner BS, Weller PF, Wardlaw AJ, Wechsler ME, Rosenwasser LJ, Roufosse F, Gleich GJ, Klion AD, Refining the definition of hypereosinophilic syndrome. J Allergy Clin Immunol 2010;126: 45-9.
12. Bandeira-Melo C, Weller PF, Eosinophils and cysteinyl leukotrienes. Prostaglandins Leukot Essent Fatty Acids 2003;69: 135-43.
13. Roufosse F, Weller PF, Practical approach to the patient with hypereosinophilia. J Allergy Clin Immunol 2010;126: 39-44.
14. Humbles AA, Lloyd CM, McMillan SJ, Friend DS, Xanthou G, McKenna EE, Ghiran S, Gerard NP, Yu C, Orkin SH, Gerard C, A critical role for eosinophils in allergic airways remodeling. Science 2004;305: 1776-9.
15. Higashi N, Mita H, Taniguchi M, Turikisawa N, Higashi A, Ozawa Y, Tohma S, Arimura K, Akiyama K, Urinary eicosanoid and tyrosine derivative concentrations in patients with vasculitides. J Allergy Clin Immunol 2004;114: 1353-8.
16. Nakamura M, Ueda N, Kishimoto K, Yoshimoto T, Yamamoto S, Ishimura K, Immunocytochemical localization of platelet-type arachidonate 12-lipoxygenase in mouse blood cells. J Histochem Cytochem 1995;43: 237-44.
17. Masi AT, Hunder GG, Lie JT, Michel BA, Bloch DA, Arend WP, Calabrese LH, Edworthy SM, Fauci AS, Leavitt RY, et al., The American College of Rheumatology 1990 criteria for the classification of Churg-Strauss syndrome (allergic granulomatosis and angiitis). Arthritis Rheum 1990;33: 1094-100.

- Accepted Article
18. Suppiah R, Mukhtyar C, Flossmann O, Alberici F, Baslund B, Batra R, Brown D, Holle J, Hruskova Z, Jayne DR, Judge A, Little MA, Palmisano A, Stegeman C, Tesar V, Vaglio A, Westman K, Luqmani R, A cross-sectional study of the Birmingham Vasculitis Activity Score version 3 in systemic vasculitis. *Rheumatology (Oxford)* 2010.
 19. National Asthma Education and Prevention Program (National Heart Lung and Blood Institute). Third Expert Panel on the Management of Asthma., Guidelines for the diagnosis and management of asthma : summary report 2007. Bethesda, MD: U.S. Dept. of Health and Human Services, National Institutes of Health, National Heart, Lung, and Blood Institute, 2008.
 20. Nizankowska-Mogilnicka E, Bochenek G, Mastalerz L, Swierczynska M, Picado C, Scadding G, Kowalski ML, Setkowicz M, Ring J, Brockow K, Bachert C, Wohrl S, Dahlen B, Szczeklik A, EAACI/GA2LEN guideline: aspirin provocation tests for diagnosis of aspirin hypersensitivity. *Allergy* 2007;62: 1111-8.
 21. Chusid MJ, Dale DC, West BC, Wolff SM, The hypereosinophilic syndrome: analysis of fourteen cases with review of the literature. *Medicine (Baltimore)* 1975;54: 1-27.
 22. Horvath IdJ, J.C, Exhaled Biomarkers. In: K.Larsson ed. *European Respiratory monograph*: European Respiratory Society, 2010:250.
 23. Sanak M, Gielicz A, Nagraba K, Kaszuba M, Kumik J, Szczeklik A, Targeted eicosanoids lipidomics of exhaled breath condensate in healthy subjects. *J Chromatogr B Analyt Technol Biomed Life Sci* 2010;878: 1796-800.
 24. Sanak M, Gielicz A, Bochenek G, Kaszuba M, Nizankowska-Mogilnicka E, Szczeklik A, Targeted eicosanoid lipidomics of exhaled breath condensate provide a distinct pattern in the aspirin-intolerant asthma phenotype. *The Journal of allergy and clinical immunology* 2011;127: 1141-7 e2.
 25. British Thoracic Society guidelines on diagnostic flexible bronchoscopy. *Thorax* 2001;56 Suppl 1: i1-21.
 26. Bronchoalveolar lavage constituents in healthy individuals, idiopathic pulmonary fibrosis, and selected comparison groups. The BAL Cooperative Group Steering Committee. *Am Rev Respir Dis* 1990;141: S169-202.
 27. Moore WC, Bleeker ER, Curran-Everett D, Erzurum SC, Ameredes BT, Bacharier L, Calhoun WJ, Castro M, Chung KF, Clark MP, Dweik RA, Fitzpatrick AM, Gaston B, Hew M, Hussain I, Jarjour NN, Israel E, Levy BD, Murphy JR, Peters SP, Teague WG, Meyers DA, Busse WW, Wenzel SE, Characterization of the severe asthma phenotype by the National Heart, Lung, and Blood Institute's Severe Asthma Research Program. *J Allergy Clin Immunol* 2007;119: 405-13.
 28. Brusasco V, Crapo R, Viegi G, Coming together: the ATS/ERS consensus on clinical pulmonary function testing. *Eur Respir J* 2005;26: 1-2.
 29. Kuhn H, O'Donnell VB, Inflammation and immune regulation by 12/15-lipoxygenases. *Prog Lipid Res* 2006;45: 334-56.
 30. Andersson CK, Claesson HE, Rydell-Tormanen K, Swedmark S, Hallgren A, Erjefalt JS, Mice lacking 12/15-lipoxygenase have attenuated airway allergic inflammation and remodeling. *Am J Respir Cell Mol Biol* 2008;39: 648-56.
 31. Vincent C, Fiancette R, Donnard M, Bordessoule D, Turlure P, Trimoreau F, Denizot Y, 5-LOX, 12-LOX and 15-LOX in immature forms of human leukemic blasts. *Leuk Res* 2008;32: 1756-62.
 32. Piao YS, Du YC, Oshima H, Jin JC, Nomura M, Yoshimoto T, Oshima M, Platelet-type 12-lipoxygenase accelerates tumor promotion of mouse epidermal cells through enhancement of cloning efficiency. *Carcinogenesis* 2008;29: 440-7.
 33. Honn KV, Tang DG, Gao X, Butovich IA, Liu B, Timar J, Hagmann W, 12-lipoxygenases and 12(S)-HETE: role in cancer metastasis. *Cancer Metastasis Rev* 1994;13: 365-96.
 34. Wang MM, Reynaud D, Pace-Asciak CR, In vivo stimulation of 12(S)-lipoxygenase in the rat skin by bradykinin and platelet activating factor: formation of 12(S)-HETE and hepoxilins, and actions on vascular permeability. *Biochim Biophys Acta* 1999;1436: 354-62.

- Accepted Article
35. Ulfman LH, Joosten DP, van Aalst CW, Lammers JW, van de Graaf EA, Koenderman L, Zwaginga JJ, Platelets promote eosinophil adhesion of patients with asthma to endothelium under flow conditions. *Am J Respir Cell Mol Biol* 2003;28: 512-9.
 36. Arnoux B, Denjean A, Page CP, Nolibe D, Morley J, Benveniste J, Accumulation of platelets and eosinophils in baboon lung after paf-acether challenge. Inhibition by ketotifen. *Am Rev Respir Dis* 1988;137: 855-60.
 37. Herd CM, Page CP, Pulmonary immune cells in health and disease: platelets. *Eur Respir J* 1994;7: 1145-60.
 38. Coyle AJ, Page CP, Atkinson L, Flanagan R, Metzger WJ, The requirement for platelets in allergen-induced late asthmatic airway obstruction. Eosinophil infiltration and heightened airway responsiveness in allergic rabbits. *Am Rev Respir Dis* 1990;142: 587-93.
 39. Tomasson G, Lavalley M, Tanriverdi K, Finkelman JD, Davis JC, Jr., Hoffman GS, McCune WJ, St Clair EW, Specks U, Spiera R, Stone JH, Freedman JE, Merkel PA, Relationship between markers of platelet activation and inflammation with disease activity in Wegener's granulomatosis. *J Rheumatol* 2011;38: 1048-54.
 40. Szczeklik W, Sokolowska B, Mastalerz L, Grzanka P, Gorka J, Pacult K, Misalski-Jamka T, Soja J, Musial J, Pulmonary findings in Churg-Strauss syndrome in chest X-rays and high resolution computed tomography at the time of initial diagnosis. *Clin Rheumatol* 2010;29: 1127-34.
 41. Szczeklik W, Grzanka P, Mastalerz L, Sokolowska B, Musial J, Lung involvement in Churg-Strauss syndrome as related to the activity of the disease. *Allergy* 2010;65: 1484-5.
 42. Profita M, Sala A, Riccobono L, Paterno A, Mirabella A, Bonanno A, Guerrera D, Pace E, Bonsignore G, Bousquet J, Vignola AM, 15-Lipoxygenase expression and 15(S)-hydroxyeicoisatetraenoic acid release and reincorporation in induced sputum of asthmatic subjects. *J Allergy Clin Immunol* 2000;105: 711-6.
 43. Chu HW, Balzar S, Westcott JY, Trudeau JB, Sun Y, Conrad DJ, Wenzel SE, Expression and activation of 15-lipoxygenase pathway in severe asthma: relationship to eosinophilic phenotype and collagen deposition. *Clin Exp Allergy* 2002;32: 1558-65.
 44. Salari H, Schellenberg RR, Stimulation of human airway epithelial cells by platelet activating factor (PAF) and arachidonic acid produces 15-hydroxyeicosatetraenoic acid (15-HETE) capable of contracting bronchial smooth muscle. *Pulm Pharmacol* 1991;4: 1-7.
 45. Jackson AS, Sandrini A, Campbell C, Chow S, Thomas PS, Yates DH, Comparison of biomarkers in exhaled breath condensate and bronchoalveolar lavage. *Am J Respir Crit Care Med* 2007;175: 222-7.



