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## FUNCTIONAL EICOSANOID TEST AND TYPING (FET) OF PERIPHERAL BLOOD CELLS IN EICOSANOID RELATED DISEASES

Monitoring of eicosanoid synthesis in peripheral blood cells has significant potential for improving the diagnosis and therapy of many human diseases. The quantitative relation between concentrations of prostaglandins and leukotrienes is central to the physiologic function of the eicosanoid network. Here we show that this regulation, which we call the functional eicosanoid typing (FET), fluctuates dynamically in individual living blood cells from patients, thereby limiting the accuracy with which concentration circuits of eicosanoids can transfer metabolic information. Using living cells in functional cell testing, we characterised the eicosanoid pattern score (EPS). A novel technique based on binomial errors on lipid mediator partitioning enabled calibration of *in vivo* biochemical parameters in molecular units. We found that eicosanoid production rates fluctuate over a time scale of about twenty minutes, while intrinsic noise decays rapidly. Thus, biochemical eicosanoid parameters, noise, and slowly varying cellular states together determine the effective FET. These results can form a basis for quantitative modelling of natural eicosanoid circuits in diagnosis of eicosanoid related diseases and design of synthetic ones for the prediction other diseases.

key words: *eicosanoid pattern, Functional Eicosanoid Typing, FET, in vitro test, inflammatory diseases*

### INTRODUCTION

Medicine is undergoing an evolution: Diseases are increasingly being redefined in terms of underlying molecular and functional abnormalities, as opposed to signs and symptoms of the patient. This new definition may be referred to as "functional signature" of a diseases and the current developments in biomedical research should accordingly be termed "functional medicine". In the case of asthma, urticaria, or intestinal cancer, we know that this diseases result from genetic alterations of cells - most likely induced by exogenous factors, which may involve over-expression or under-expression of normal genes, or

mutations that generate abnormal gene products. This may affect any of the molecules within the cell and cell membrane modifying the microenvironment, including stromal, vascular, and blood cells, which is most relevant for the persistence, growth, and attenuation of the disease.

As diseases are associated with altered gene expression and gene products, "functional medicine" is looking on altered cellular function. Considering diseases of the respiratory system (RS), the skin, or the gastro-intestinal (GI) tract, like analgesic induced asthma bronchiale, urticaria, gastric ulcer, or intestinal cancer, you will find modifications of the lipid metabolism, i.e. of the eicosanoid metabolism. Recent publications mostly focus on a single eicosanoid which is altered in the diseases (2, 6, 11, 19, 20, 22, 26, 27, 34, 32). "Functional" studies on eicosanoid metabolism of diseases are missing. Also, suitable *in vitro* methods, especially those using peripheral blood cells and analysis of eicosanoid patterns, are not described or were not available for an application as a diagnostic tool in the diseases mentioned, except those from our group.

Therefore, during the last decade our group established and validated functional tests based on living cells gained from different tissue biopsy specimens as well as blood for monitoring normal and altered eicosanoid patterns from unaffected persons and patients suffering from eicosanoid related diseases (3, 4, 8, 9, 10, 13, 16, 29, 30, 31).

### *General Considerations on Eicosanoids*

Our present understanding of eicosanoid interactions, and our ability to exploit their properties in building synthetic circuits, relies on modelling what goes on inside individual cells. In this study we use a synthetic eicosanoid network - the eicosanoid pattern score (EPS) - obtained from a functional eicosanoid test using living blood cells to model how eicosanoid signals are transmitted, heralding a welcome quantitative turn in the field of functional networks of "functional medicine", the "Functional Eicosanoid Typing" (FET) of eicosanoid related disease. Among the eicosanoids prostaglandin E<sub>2</sub> and peptido-leukotrienes (pLT; i.e. LTC<sub>4</sub>, LTD<sub>4</sub>, and LTE<sub>4</sub>) are known to play a crucial fundamental role in maintaining cellular integrity of organ and body function (5, 25, 28, 33). Furthermore, the amount of eicosanoids released depend on the cell type (7, 14, 17) as well as on the complex interaction of the eicosanoids itself (1, 4, 12, 18, 21, 23, 24, 29, 30).

Reduced to its bare bones, eicosanoid synthesis in mammalian involves arachidonic acid as predominantly substrate of the initial enzyme-catalysed metabolic steps, interaction of enzymatic products, and modification by endogenous as well as exogenous enzyme modulators, eventually leading to the production or suppression of eicosanoids. But how we describe the dynamic properties of this simple relationship? Also, what type of "noise" affects these eicosanoid balance, and how can it be mathematically integrated?

To address these questions, we quantified the concentration of prostaglandin  $E_2$  and peptido-leukotrienes synthesised and released in vitro by peripheral blood cells at base line and upon experimentally induction by addition of exogenous substrate and enzyme inhibitor. Thereafter, the values of the differentially induced eicosanoids were calculated as functional eicosanoid pattern score based on physiologically considerations followed by an integration revealing a "functional eicosanoid type" classified in four main groups. This was performed for patients suffering from gastric ulcer, intestinal cancer and analgesic intolerant asthmatics compared to unaffected controls.

## METHODS AND MATERIALS

### *Controls*

Sixty-one healthy individuals (28 females, mean 49.3 years; and 33 males, mean 47.2 years) were investigated. The healthy individuals had no history of sensitivity to COX-inhibitors, asthma, or allergy to airborne or food allergens. Furthermore, none of the individuals reported a known clinical history of intolerance to COX-inhibitors (e.g. nasal polyps, asthma upon treatment with NSAID, gastrointestinal cancer, or gastric ulcer). Controls had no previous nasopharyngeal, bronchial, gastrointestinal intervention and no treatment interfering with the eicosanoid metabolism. Allergic diseases were ruled out by with a prick test. Sinusitis was ruled out by endoscopy. Eight subjects were smoker and none were atopic. No regular medication, other than oral contraceptives were taken.

The studies on controls and patients were performed in accordance with the ethical standards of the Helsinki Declaration 1975 (revised 1983). The protocol of the study was approved by Institutional Ethical Committee of Erlangen-Nuremberg University. The subjects gave their written consent before participating in this study.

### *Analgesic-intolerant asthmatics*

Sixty-five patients (43 female and 22 male) aged 23-78 years with classic symptoms of an aspirin triad ("syndrom Widal") were studied. They were selected on the basis of a previous severe asthma attack after ingestion of non-steroidal anti-inflammatory drugs and a decrease of forced expiratory volume in one second (FEV1) > 25% after bronchial provocation. All patients had recurrent chronic polypous sinusitis on nasoendoscopy. All of the patients had undergone previous surgery for polyposis. None of the patients received topical or systemic corticosteroid therapy during the last 14 days before the study. Allergic diseases were ruled out by with a prick test. Therapy with anti-histamines or cromolyn sodium was withheld for at least 24 hours prior to the study. Inhalative provocation was performed as described before (30).

### *Patients suffering from gastric ulcer*

Forty-one patients (18 females and 22 males, mean 62.1.5 and 53.6 years, respectively) aged 34-75 years suffering from gastric ulcer were investigated. Gastric ulcer was objectivated by endoscopy and histology of biopsy-specimens. Patients taking drugs interfering with the eicosanoid metabolism (e.g. corticosteroids, NSAIDs, leukotriene antagonists) were excluded. In addition no patient had a history of intolerance to COX-inhibitors, asthma, or allergy to airborne or food

allergens. Patients were checked by standard techniques. Infection with helicobacter pylori (Hp) was confirmed by endoscopic and histologic examination. Allergic diseases were ruled out by with a prick test. Blood eosinophilia was excluded by standard microscopic cytology technique. More details on study design and patient recruitment had been published recently (4).

### *Patients suffering from gastrointestinal cancer*

Seventy patients with gastrointestinal cancer (31 female; 39 male; mean 68 and 63 years, respectively) aged 67.4 years) were investigated. Diagnosis was assessed by endoscopy and histology. There was one patient with gastric cancer, 24 with colonic cancer, 18 with sigmoid cancer and 22 with rectal cancer. All patients underwent surgical therapy and chemotherapy according to the actual guidelines. There was a complete remission of at least two years at the moment of investigation omitting any therapy in this concern. Individuals suffering from other gastrointestinal diseases or disorders basing on eicosanoid imbalance were excluded as well as those taking drugs interfering with the generation or metabolism of eicosanoids like NSAID or steroids. Allergic diseases were ruled out by with a prick test. Blood was drawn during control-examination.

### *Functional-Eicosanoid-Test (FET) using living peripheral blood cells*

Venous blood (5 ml) was collected using S-monovette® (Sarstedt, Hannover, Germany), including heparin to prevent clotting. All samples were anonymous. All individuals gave their informed consent. Peripheral blood cells were further processed as published (4, 30) with slight modifications using LiPiDoC®-AIT and LiPiDoC®-FET (SIAT, Bad Essen, Germany) according to the instruction of the manufacture. Briefly, collected cells were diluted in vitro using cell incubation buffer. Diluted cells were incubated with diluent, arachidonic acid, or acetylsalicylic at room temperature for 20 minutes. Reaction was stopped by storing the samples at -20°C for up to 6 weeks until further procession for analysis of eicosanoids using competitive enzyme-immuno-assays (EIA). For control experiments parallel samples were prepared and processed as described. But before storage at -20°C samples were centrifuged (900 g, 4°C, 7 min), supernatant was collected in separate tubes. Integrity of centrifuged cells was checked microscopically by trypan-blue exclusion test and live-dead test. The live-dead-test was performed according to the instructions of the manufacture (Molecular Probes, Göttingen, Germany). There were neither conspicuous results concerning cell morphology nor cell integrity comparing blood samples stored immediately upon incubation and those of separates supernatant. The same was true for measurement of eicosanoids measured in supernatant of the samples.

### *Measurement of eicosanoids*

The stored samples were defrosted and centrifuged (900 g, 4°C, 7 min). Thereafter, aliquots of the supernatants were transferred to of highly specific and sensitive competitive PGE<sub>2</sub>- or pLT- EIA plates (SPI-bio, Paris, France / SIAT, Bad Essen, Germany), which was specifically produced und validated for use in combination with LiPiDoC®-kits. The LiPiDoC®-kits were performed according to instructions of the manufacture. In brief, standards and samples were analysed in duplicates in parallel for PGE<sub>2</sub> and pLT. Results are presented as mean ±SD in pg/ml. The intra- and inter-assay variances were 7.8% and 12.4% for PGE<sub>2</sub> and pLT, respectively.

### *Statistical Analysis*

For each sample the individual arithmetic mean ±SD of PGE<sub>2</sub> and pLT was calculated. The differentially manipulated samples of each patient were compared using the two-tailed U-test. The

same procedure was done comparing patients and controls. A continuity correction of 0.5 was included because of some paired scores. A comparison of EPS values was done using the paired t-test. The t-test of Satterthait was performed if homogeneity of variance of values of both groups was not achieved. The linear regression model was used for controlling the integrated eicosanoid pattern score. Furthermore, other clinical and pathological variables were compared using the Chi-squared test. The statistical analysis was performed using the SHS statistic package version 8.1E. The levels of significance were established at  $p < 0.05$  (significant) and  $p < 0.001$  (highly significant).

## RESULTS AND DISCUSSION

### *Derivation of the Eicosanoid-Pattern-Score (EBS) based Functional-Eicosanoid-Typing (FET)*

As outlined before, there is a complex network of eicosanoids in inflammatory diseases (33) like analgesic-induced asthma (8, 10, 13, 16, 30, 31), gastric ulcer (4), and intestinal cancer (Baenkler & Schäfer in this issue), which can be allegorised by prostaglandin  $E_2$  ( $PGE_2$ ) and peptide-leukotrienes (pLT, i.e.  $LTC_4$ ,  $LTD_4$ , and  $LTE_4$ ).

Because of their complex biological function (5, 12, 17, 21, 23, 24, 26, 29) several actions and interactions of  $PGE_2$  and pLT have to be taken in consideration when modelling a network of eicosanoid-related inflammatory diseases. The altered eicosanoid pattern, as analysed in former (3, 29, 30) and recent studies (4, Baenkler & Schäfer in this issue) pointed on  $PGE_2$  and pLT as potential markers of diagnostic value characterising diseases of different origin, like analgesic-induced asthma associated with polypous nasal mucosa and asthmatic complication on the one side and gastric ulcer associated with lesion of the gastric mucosa or intestinal cancer associated with malign intestinal mucosal tissue on the other side. Because of the known bronchoconstrictive effects leukotrienes (6, 18, 19, 28, 33) these eicosanoids are of high functional-physiological interest. But also prostaglandins are of special functional-physiological interest, as to the one side they have bronchoprotective effects and over the other respect they are ailment appointing enhanced in intestinal diseases (26, 27, 32). Furthermore, taking everything into account, the transcellular network of eicosanoids (21) has to be considered.

Therefore, the derivation of an eicosanoid pattern scores (EPS) -based characterisation of AIA, UC, IC, will outlined stepwise reflecting the functionally-physiologically founded model of EPS-based inflammatory diseases.

**(1)** First of all, for characterising the eicosanoid network, the eicosanoid synthesis as to be analysed and quantified.

**(A)** Therefore, the basal eicosanoid synthesis ( $B_x$ ,  $B_y$  with  $x = PGE_2$  and  $y = pLT$ ) was quantified, demonstrating the intrinsic performance of cellular eicosanoid synthesis.

As seen in *figure 1A*, there was a wide distribution of basal PGE<sub>2</sub> and pLT synthesis in controls as well as AIA, GU, and IC over four powers (~1 to 10,000 pg/ml PGE<sub>2</sub> and 0.2 to ~2,000 pg/ml pLT), without revealing clearly structured differences of the groups analysed. Even though, IC-patients tended to synthesize more PGE<sub>2</sub> than the others - as outlined in the literature mentioned above.

- (B)** Thereafter the (physiologically) maximal inducible eicosanoid synthesis of PGE<sub>2</sub> (A<sub>X</sub>) and pLT (A<sub>Y</sub>) should be recorded, getting insight to the dynamic flexibility of the cellular eicosanoid metabolism. At this *in vitro* approach of the Functional-Eicosanoid-Test (FET) arachidonic acid, the natural substrate of the involved key enzymes cyclooxygenases (COX) and 5-lipogenases (LOX) was provided.

Again, there was a wide distribution of PGE<sub>2</sub>- and pLT-values over three to four powers (~10 to 20,000 and ~10 to 100,000 pg/ml, respectively). As expected, the arachidonic acid-induced eicosanoid synthesis was ~2 to 10 fold higher than the basal eicosanoid synthesis, pointing to a physiologically relevant *in vitro* model causing predictable biochemical effects. Even though PGE<sub>2</sub> and pLT values of controls are less distributed, and IC-patients tended to elevated eicosanoid values, whereas AIT-patients tended to be characterised by lowered PGE<sub>2</sub> and pLT-values, there was no clear differentiation of those groups compared to controls (*s. figure 1B*). But there were some first hints supporting the implication of these eicosanoids in the relevant diseases as outlined and hypothesised before.

- (C)** Furthermore, the most prominent enzyme-complex, i.e. cyclooxygenases, should be modified, introducing a non-competitive inhibitor, i.e. acetylsalicylic acid, (a) to get some insight on the effect of the modification of the enzymatic COX- and LOX-capacity, because of the know interaction of these two pathways during generation of PGE<sub>2</sub> (S<sub>X</sub>) and pLT (S<sub>Y</sub>); and (b) because COX-inhibitors are known elicitors of those diseases investigated in this study.

As expected from the design of a functional-physiological *in vitro* model, there was a clear tendency of reduced PGE<sub>2</sub> synthesis compared to the basal synthesis (~20 to 5000, mean ~200 pg/ml, compared to ~80 to 10.000, mean ~300 pg/ml, respectively). Also pLT-synthesis tended to be elevated by incubation of cells with acetylsalicylic acid as generally accepted by pharmacological considerations, even though these "shifting / shunting effect" (15) depends on the group and is lowest expressed in controls, but highest in AIA-patients (*s. figure 1C*). But to our astonishment, there was again no differentiation of groups introducing an enzymatic inhibitor eliciting the diseases investigated.

The analysis of these basic eicosanoid synthesis data supported the functional-physiological design of the *in vitro* model in all three aspects analysed. But to our surprise, there was no clear differentiation of the groups

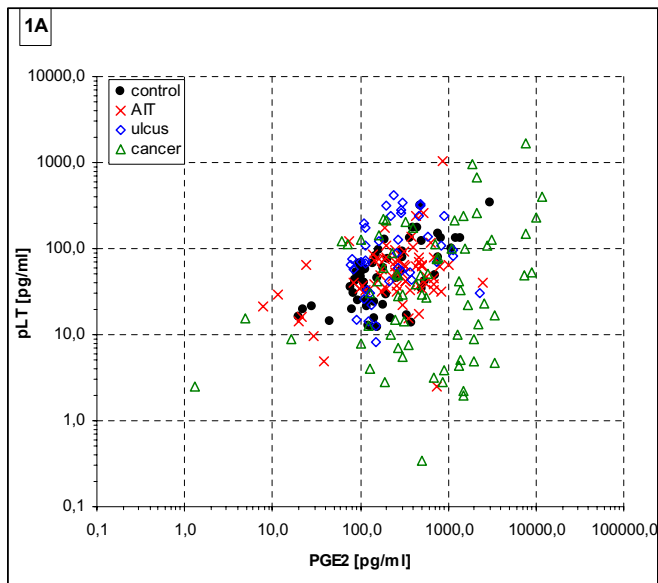


Figure 1A: Basal eicosanoid synthesis. AIA: analgesic-induced asthma, ucluc: gastric ulcer, cancer: intestinal cancer dots represent the arithmetic mean of each individual for the relevant group.

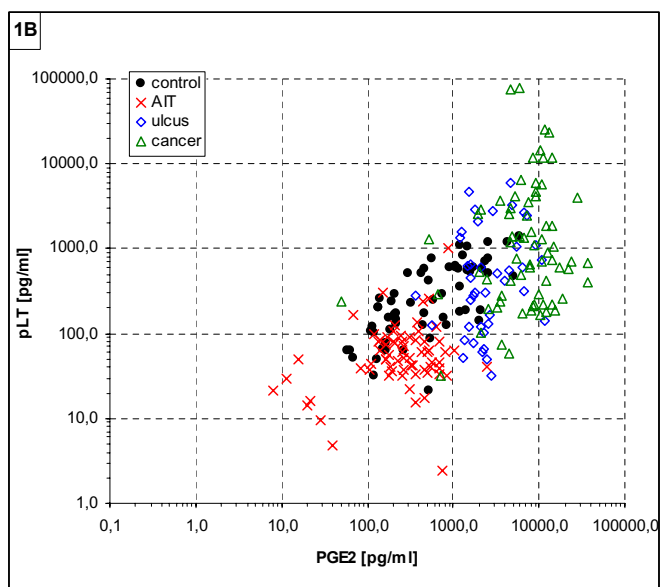


Figure 1B: Arachidonic acid-induced eicosanoid synthesis. abrev. s. figure 1A

investigated, even though there were some clues that e.g. IC-patients or AIA-patients tended to be characterised by an altered eicosanoid pattern as hypothesised in the beginning and deduced from some data in literature.

(2) Taking in account the fundamental role of prostaglandins, deduced from the data in the literature and from the results of the analysis outlined

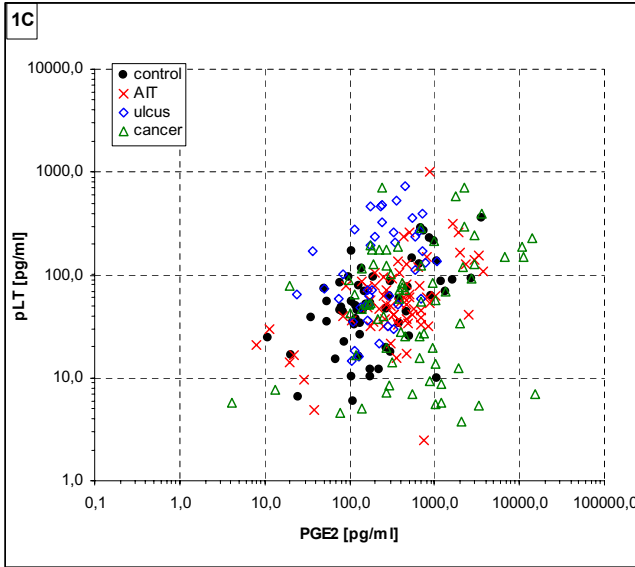


Figure 1C: Acetylsalicylic acid-mediated eicosanoid synthesis. abbrev. s. figure 1A

above, the further analysis of data was focused on the enzymatic capacity of COX ( $EC_{COX}$ ), calculated according to equation (1).

$$(1) EC_{COX} = A_X / B_X, \text{ with } EC_{COX} = \text{enzymatic capacity of cyclooxygenases,}$$

$$A_X = \text{arachidonic acid-induced PGE}_2 \text{ synthesis,}$$

$$B_X = \text{basal PGE}_2 \text{ synthesis}$$

But for getting some insight into LOX enzymatic capacity,  $EC_{LOX}$  was calculated according to equation (2).

$$(2) EC_{LOX} = A_Y / B_Y, \text{ with } EC_{LOX} = \text{enzymatic capacity of 5-lipoxygenase,}$$

$$A_X = \text{arachidonic acid-induced pLT synthesis,}$$

$$B_Y = \text{basal pLT synthesis}$$

Furthermore, considering also the interaction of eicosanoids causing a (naturally) given balance of  $PGE_2$  and pLT during cellular eicosanoid metabolism, the arachidonic acid-induced eicosanoid balance ( $EB_A$ ) and the acetylsalicylic acid-mediated eicosanoid balance ( $EB_S$ ) were calculated according to equation (3) or (4).

$$(3) EB_A = A_X / A_Y, \text{ with } EB_A = \text{arachidonic acid-induced eicosanoid balance of PGE}_2 \text{ and pLT,}$$

$$A_X = \text{arachidonic acid-induced PGE}_2 \text{ synthesis,}$$



- AY = arachidonic acid-induced pLT synthesis
- (4)  $EB_A = A_X / A_Y$ , with  $EB_S =$  acetylsalicylic acid-mediated eicosanoid balance of PGE<sub>2</sub> and pLT,  
 $S_X =$  acetylsalicylic acid-mediated PGE<sub>2</sub> synthesis,  
 $S_Y =$  acetylsalicylic acid - mediated pLT synthesis

The individual data from these calculations were correlated revealing *figure 2A* ( $EC_{COX}$  and  $EB_A$ ) and *figure 2B* ( $EC_{COX}$  and  $EB_S$ ).

The correlation of functional-biochemical combination of the 'most implicated enzymatic capacity'  $EC_{COX}$  and the 'maximal dynamic activity-balance'  $EB_A$  revealed some strong evidence for the differentiation of AIA-patients from controls, GU- and IC-patients. Slight hints of differentially an altered eicosanoid patterns were also revealed from IC-patients, compared to controls. But GU-patients obviously did not differ from controls (s. *figure 2A*).

When correlating  $EC_{COX}$  and  $EB_S$ , again AIT-patients were highly significantly ( $P < 0.001$ ) different from controls, GU, and IC. There was also a weak differentiation of GU- and IC-patients from controls, whereas GU- and IC-patients revealed no significantly altered eicosanoid patterns (s. *figure 2B*).

- (3) The results revealed from these functionally based calculations of eicosanoid interactions pointed to a potentially relevant implication of the enzymatic capacities of COX and/or LOX. Therefore, the individual enzymatic capacities,  $EC_{COX}$  and  $EC_{LOX}$ , were calculated according equation (1) and (2) and analysed in more detail according to the different groups investigated.

As lined out in *figure 3A* the  $EC_{COX}$  value highly significantly ( $P < 0.001$ ) differentiated AIT-patients ( $EC_{COX}$ :  $<0.2$  to  $\sim 1$ ) from controls ( $EC_{COX}$ :  $\sim 1$  to  $10$ ), GU- ( $EC_{COX}$ :  $\sim 2$  to  $20$ ), and IC-patients ( $EC_{COX}$ :  $\sim 0.8$  to  $110$ ). Also  $EC_{LOX}$  differentiated only AIT-patients ( $EC_{LOX}$ :  $\sim 0.02$  to  $2$ ) significantly ( $P < 0.001$ ) from controls ( $EC_{LOX}$ :  $\sim 1$  to  $60$ ), UC- ( $EC_{LOX}$ :  $\sim 0.8$  to  $40$ ), and IC ( $EC_{LOX}$ :  $\sim 2$  to  $1000$ ), neither differentiating GU- and IC-patients from controls nor from each other.

- (4) Because of the limited information deduced from the above mentioned results, the further approach focused on the 'maximal dynamic activity-balance' induced by arachidonic acid ( $EB_A$ ) and the acetylsalicylic acid-mediated eicosanoid balance ( $EB_S$ ), in respect to the groups investigated; this approach takes the PGE<sub>2</sub>-pLT interaction in stronger consideration.

As shown in *figure 4A*, also  $EB_A$  did not differentiate IC- and GU-patients ( $EB_A$ :  $\sim 0.1$  to  $100$  and  $\sim 6$  to  $100$ , respectively), which had also no altered eicosanoid pattern compared to controls ( $EB_A$   $\sim 0.6$  to  $9$ ).

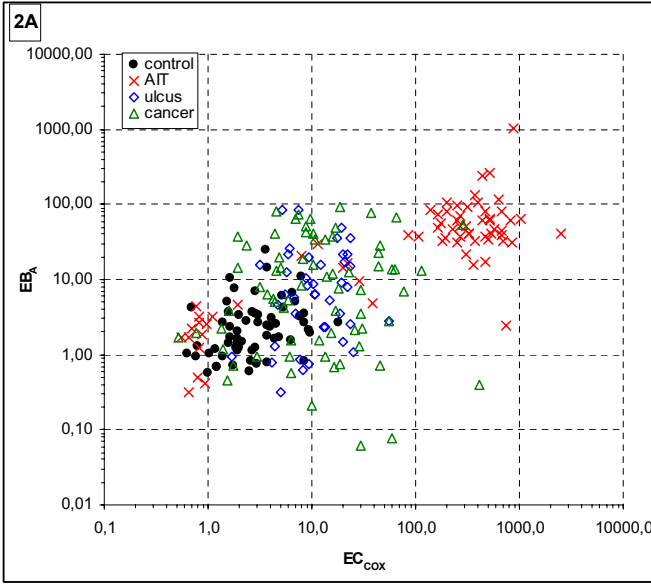


Figure 2A: COX-enzyme capacity in relation to arachidonic acid-induced eicosanoid balance. brev. s. figure 1A

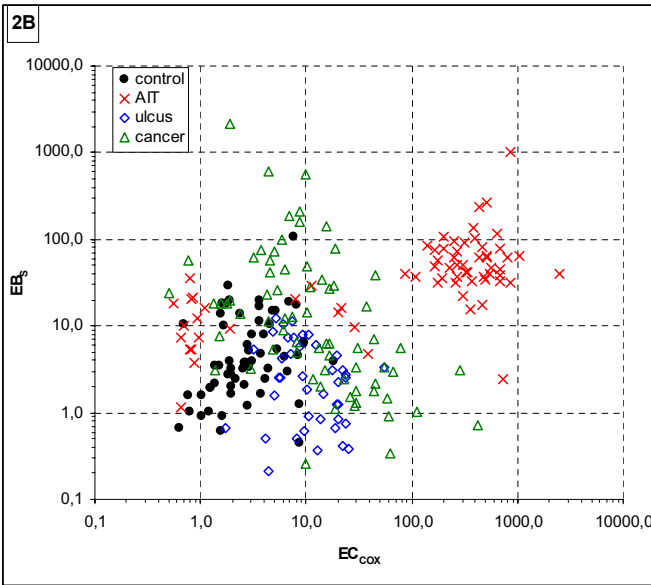


Figure 2B: COX-enzyme capacity in relation to acetylsalicylic acid-mediated eicosanoid balance. brev. s. figure 1A

Only AIA-patients presented a tendency of diminished COX-activity ( $EB_A \sim 0.2$  to 6). This was in line with the postulated pathomechanism (34, 30).

When looking on  $EB_S$ , there was a tendency of differentiation of the three patient groups investigated ( $EB_S$  of AIA: 0.02 to 3; GU: 0.3 to 10;

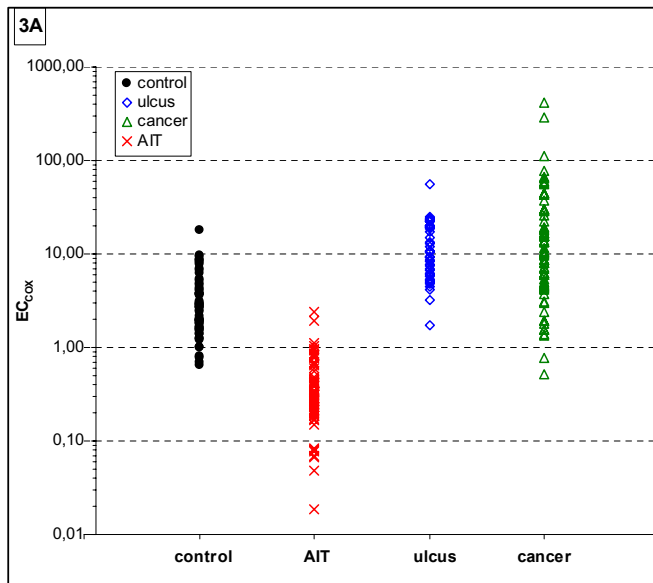


Figure 3A: COX-enzyme capacity. rev. s. *figure 1A*

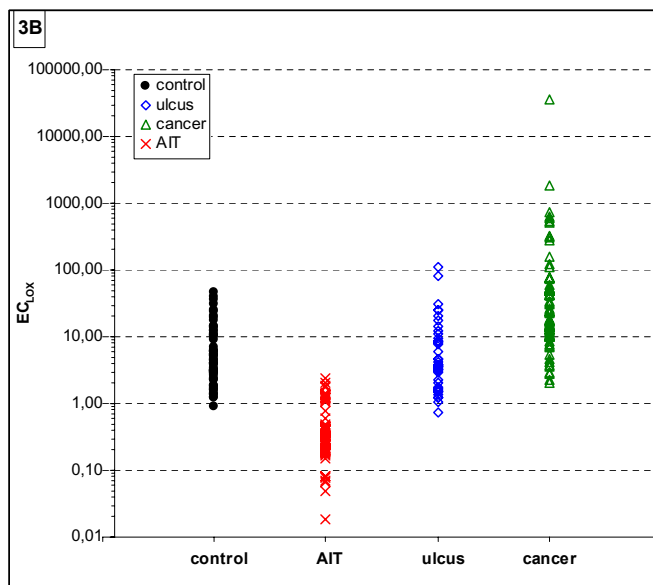


Figure 3B: LOX-enzyme capacity. rev. s. *figure 1A*

IC: 0.8 to 100), but again there was no significant differentiation according to controls ( $EB_S$ : 0.7 to 20) (s. *figure 4B*).

- (5) Adapted from the results described above, and again reclaiming the complex interaction of the (naturally) given eicosanoid network described before, the separate results have to be integrated in a more complex

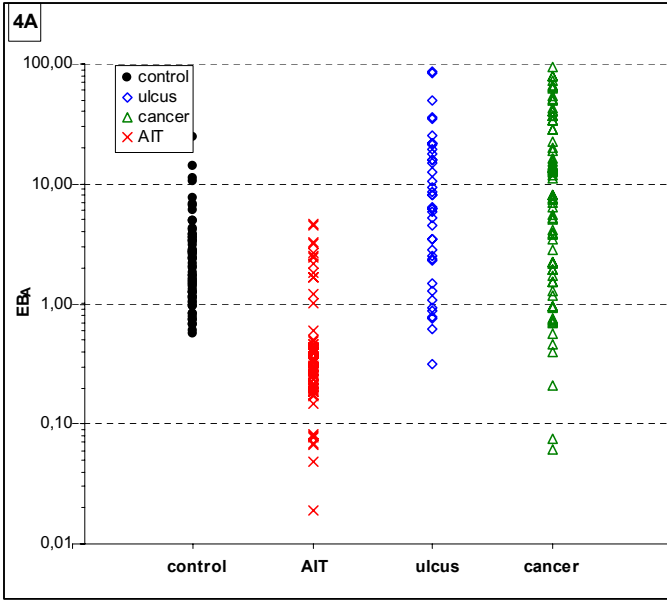


Figure 4A: Arachidonic acid-induced eicosanoid balance. rev. s. figure 1A

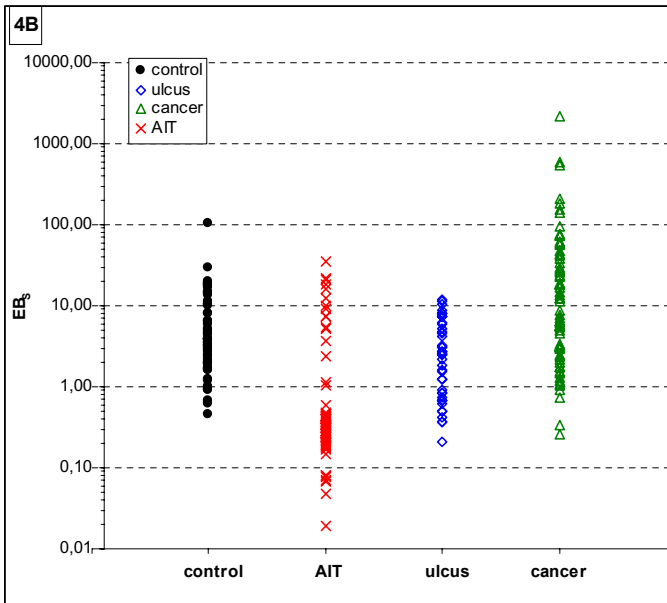


Figure 4B: Acetylsalicylic acid-mediated eicosanoid balance. rev. s. figure 1A

manner, which might better reflect the fractal-like eicosanoid network interaction of the presented functional-physiologically based in vitro model. Therefore, the allometric scaling relationship, relating enzymatic capacity of cyclooxygenases ( $EC_{COX}$ ) to arachidonic acid-induced and

acetylsalicylic acid-mediated eicosanoid balance ( $EB_A$  and  $EB_S$ , respectively), were integrated according to equation (5) using the scaling exponent ( $k$ ). The single parameters were selected according to [a] their most relevant implication in the eicosanoid network (as outlined in the evaluation done before), [b] representing fundamental biochemical mediator parameters causing disease relevant eicosanoid patterns (deduced from the evaluation done above), and [c] the scaling exponent was chosen as it best reflects the wide range of individual eicosanoid parameters.

$$(5) \text{ EPS} = [EC_{\text{COX}} * EB_A / EB_S]^k, \text{ with } \begin{aligned} \text{EPS} &= \text{eicosanoid pattern score,} \\ EC_{\text{COX}} &= \text{enzymatic capacity} \\ &\quad \text{of cyclooxygenases,} \\ EB_A &= \text{arachidonic acid-} \\ &\quad \text{induced eicosanoid} \\ &\quad \text{balance,} \\ EB_S &= \text{acetylsalicylic acid-} \\ &\quad \text{mediated eicosanoid} \\ &\quad \text{balance,} \\ k &= \text{scaling exponent } 0.75 \end{aligned}$$

The resulting individual "Eicosanoid Pattern Score" (EPS) were summarised in figure 5.

Controls were characterised by EPS-values from 0.34 to 3.4. AIA-patients revealed EPS-values from  $<0.02$  to  $\sim 0.5$ . GU-patients demonstrated EPS-values from  $>3.4$  to  $\sim 100$ , and IC-patients mostly had

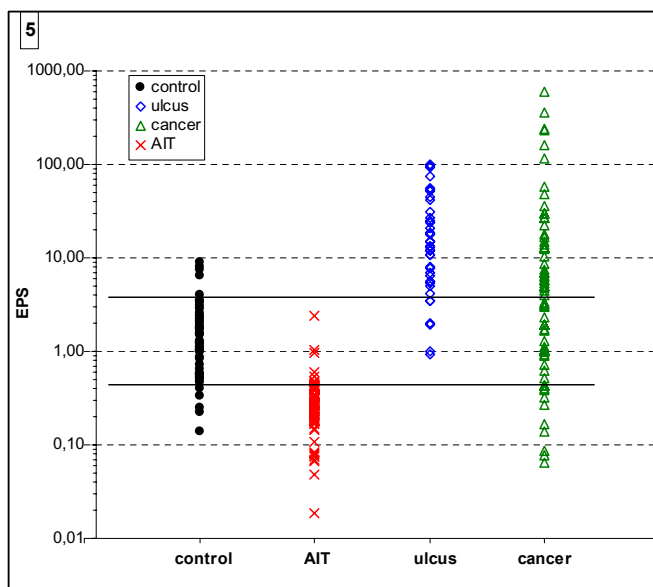


Figure 5: Eicosanoid Pattern Score. AIA: analgesic-induced asthma, uclus: gastric ulcer, cancer: intestinal cancer dots represent the arithmetic mean of each individual for the relevant group; bars indicate the upper and lower cut-off of the control group.

EPS-values  $>3.4$  to 800, but there were also some with a low EPS of 0.07 to 0.34 and some IC-patients investigated revealed EPS-values not discriminating from controls (s. *figure 5*). The integrated eicosanoid values using the EPS-equation revealed values differentiating patients suffering from inflammatory diseases from healthy controls. Furthermore, clinically and morphologically distinguished patients were characterised by different EPS-values. In addition, the EPS-value reflects the most likely pathomechanism in AIA-patients, characterised by reduced COX-pathway metabolites an association with elevated LOX-pathway metabolites, versus GU- and IC-patients, characterised by elevated COX-pathway products and possibly normal or reduce LOX-pathway metabolites. IC-patients were typed here as "hybrid-types" showing AIA-like as well as GU-like altered eicosanoid patterns.

In summary, here the first time a comparative study, using a human blood cell based functional eicosanoid test (FET), was presented, which was evaluated by modelling *in vitro* and *in silico* inflammatory processes, based on functionally and physiologically considerations.

This functional *in vitro* model was suitable for differentiating inflammatory diseases from controls and to separate inflammatory diseases, even though they are characterised by a common elicitor - acetylsalicylic acid - they show different clinical symptoms, causing asthma and nasal polyposis and otherwise mucosal lesion or malign cellular alteration. The functional eicosanoid typing (FET) derived from the altered eicosanoid pattern pointed to at least two promising aspects:

- [1] there are inflammatory processes, which are linked and characterised by a common altered metabolism, i.e. an altered eicosanoid metabolism;
- [2] the inflammatory diseases investigated are linked by a common elicitor, acetylsalicylic acid, causing different clinical symptoms and demanding different medical intervention. As the eicosanoid pattern score (EPS) differs depending on the disease, this EPS most likely reflects the presumed underlying pathomechanism, yielding an individual FET ("functional eicosanoid typing").

The latter aspects gives some very promising options, as the FET might have even more implication than only "typing" a disease, but perhaps also gives some hints for an improved therapeutic option. A more detailed analysis might differentiate the EPS-value in "functional eicosanoid types", which might be assigned as: FET-0 = normal; FET-1= modified; FET-2 = abnormal; FET-3 = severe abnormal eicosanoid pattern. Further studies have to be carried out focusing on those aspects, also revealing some possibly prognostic FET-data.

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