Exhaled 15-HETE and thromboxin-B2 are associated with therapeutic outcome in childhood asthma

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Abstract

Background: Dysregulation of eicosanoids is associated with asthma and a composite of oxylipins, including exhaled LTB4, but their potential utility in monitoring the therapeutic outcomes has not been comprehensively assessed. Objectives: We aimed to examine the levels of major eicosanoids representing different metabolic pathways in exhaled breath condensates (EBCs) of children with asthma during exacerbation and after treatment. Methods: Levels of 6 exhaled eicosanoid species in asthmatic children and healthy subjects were evaluated using ELISA. Results: In addition to those previously reported, including LTB4, LTE4, LXA4 and PGE2, the levels of exhaled 15-HETE, but not TXB2, showed significant difference between asthmatics (N=318) and healthy controls (N=97). When the asthmatic population was stratified into different severity groups, the severe group was characterized by significantly lower levels of 15-HETE and 15-HETE/LTB4 ratio, as compared to the mild and control groups. Receiver Operating Characteristic (ROC) analyses revealed similar distinguishing power for the level of exhaled 15-HETE and those of FEV1 and FeNO. Analysis of asthmatics (N=75) during exacerbation and convalescence showed significant improvement in lung function (FEV1; $p<0.001$), but not FeNO, concomitant with significantly increased levels of 15-HETE ($p<0.001$) and reduced levels of TXB2 ($p<0.05$) after therapy, particularly for those who at the top 30% level during exacerbation. Further, decreased LTB4 and LXA4 at convalescence were noted only in those at the top 30 percentile during exacerbation. Conclusion: The exhaled 15-HETE was found to discriminate childhood asthma while decreased levels of exhaled TXB2 and increased levels of 15-HETE were prominent after treatment.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests

AUTHOR CONTRIBUTIONS

It was the responsibility of Li-Chen Chen to conceive, organize, and implement the research protocol, as well as to interpret the data, guide discussions of the results, and draft the manuscript. Chih-Yung Chiu, Sui-Ling Liao, Kuan-Wen Su, Ming-Han Tsai, Man-Chin Hua, ShenHao Lai, Tsung-Chieh Yao, and Kuo-Wei Yeh were responsible for the recruitment of study subjects and clinical data evaluation. AiHsuan Wu, Hsu-Min Tseng, and Ming-Ling Kuo participated in the experiment and data analysis. With regards to the corresponding authors, Jing-Long Huang coordinated the study and interpreted the data, while Shau-Ku Huang supervised the research program and contributed to the design and manuscript writing. All authors had an opportunity to edit the manuscript and all approved of its submission.

Abstract (249 words)

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Results: In addition to those previously reported, including LTB_4, LTE_4, LXA_4 and PGE_2, the levels of exhaled 15-HETE, but not TXB_2, showed significant difference between asthmatics (N=318) and healthy controls (N=97). When the asthmatic population was stratified into different severity groups, the severe group was characterized by significantly lower levels of 15-HETE and 15-HETE/LTB_4 ratio, as compared to the mild and control groups. Receiver Operating Characteristic (ROC) analyses revealed similar distinguishing power for the level of exhaled 15-HETE and those of FEV_1 and FeNO. Analysis of asthmatics (N=75) during exacerbation and convalescence showed significant improvement in lung function (FEV_1; $p<0.001$), but not FeNO, concomitant with significantly increased levels of 15-HETE ($p<0.001$) and reduced levels of TXB_2($p<0.05$) after therapy, particularly for those who at the top 30% level during exacerbation. Further, decreased $LTB_4$and LXA_4 at convalescence were noted only in those at the top 30 percentile during exacerbation.

Conclusion: The exhaled 15-HETE was found to discriminate childhood asthma while decreased levels of exhaled $TXB_2$and increased levels of 15-HETE were prominent after treatment.

Keywords: childhood asthma, exhaled breath condensates (EBCs), 15-HETE, TXB_2

Introduction (2897 words)

Asthma is a chronic inflammatory disorder of the airways and is characterized by airway hyperresponsiveness and reversible airflow obstruction that fluctuates over time. It is also increasingly recognized as a disease with multiple phenotypes that differ in clinical severity, response to therapy, and long-term outcome[1]. Eicosanoids are a family of bioactive lipid mediators that regulate a wide variety of inflammatory processes[2]. Eicosanoid species are generated from ω-6- and ω-3-derived polyunsaturated fatty acids (PUFAs), such as arachidonic acid (AA) and eicosapentaenoic acid (EPA), respectively. AA can be converted into prostaglandins (PGs), leukotrienes (LTs) and hydroxyeicosatetraenoic acids (HETEs)[3] by cyclooxygenases (COXs), lipoxygenases (LOXs) and cytochrome P450 epoxygenases (CYP450). 5-LOX-derived LTA_4 can be converted to lipoxins in the presence of 15-LOX, while 15-LOX generates 15-HETE[3]. Lipoxins and 15-HETE have been reported to exert anti-inflammatory activity via reducing activation and recruitment of inflammatory cells and modulating the expression of adhesion molecules; for example, $LXA_4$and 15-HETE inhibit LTB_4-induced chemotaxis of neutrophils in vitro and in vivo[4-6]. Thromboxane B_2 (TXB_2) is non-enzymatically hydrolyzed from COX-derived TXA_2, a potent bronchial smooth muscle spasmogen formed by platelets[7], is known to reduce T cell secretion of the Th1 cytokine, interferon-γ in vitro, which may favor T cell differentiation toward a Th2 cytokine profile[8].

Together, these functionally diverse classes of eicosanoids are thought to play a critical role in maintaining homeostasis and have been an active area of investigation in assessing the mechanism underlying the pathogenesis of asthma and their potential utility as the inflammatory indicators in disease progression and treatment outcome. Indeed, several studies have suggested their roles as the biomarkers for screening, diagnosis, and, to a limited extent, monitoring the treatment outcome. For example, several independent studies have shown elevated levels of eicosanoids in the EBC of patients with asthma[9,10], but due, perhaps in part, to the limited sample sizes and the heterogeneity of the study patient populations, unified evidence remains to be obtained. As the result, knowledge about eicosanoids in disease progression and therapeutic outcome remains incomplete, and their relationship with the disease status has yet to be comprehensively explored and their clinical utility as biomarkers remains to be defined. We have previously reported that in a pediatric study population in Taiwan, the levels of exhaled LTB_4, LTE_4, LXA_4, and PGE_2 in asthmatic children were significantly different from those of healthy controls, and the combination of exhaled LTB_4 and LXA_4, together with FeNO and FEV_1, best characterized childhood asthma[11]. We described herein an analysis of the levels of exhaled eicosanoids differed at the time of acute exacerbation and convalescence and reported that the levels of TXB_2 and 15-HETE were the most responsive to therapy.

Material and Method

Study subject

A total of 393 bronchial asthmatic children aged between 5 and 12 years, consisting of 318 stable asthmatics (205 males and 113 females) and 75 acute asthma attack sufferers (47 males and 28 females), were recruited from the pediatric clinics of the Chang Gung Memorial Hospital, Taiwan, as a part of the ongoing PATCH study (Prediction of Allergies in Taiwanese Children). A total of 97 (59 males and 38 females) age-matched healthy subjects with no history of bronchial asthma, allergic, or immunological diseases) were enrolled from an elementary school in Taoyuan City, Taiwan. The diagnosis and classification of the clinical severity of asthma followed the published guidelines[12]. Asthma severity was categorized as mild (intermittent), moderate and severe. The mild-asthma group exhibited a symptom frequency of less than once a day and nocturnal symptoms of less than once a week, with an $FEV_1 > 80\%$ of predict as well as those experiencing minor limitations in their daily activities. The inclusion criteria for the children with severe asthma consisted of frequent daytime symptoms and night waking maybe every night, an FEV_1 <60% of predicted and experienced major limitations in their daily activities. Patients with moderate asthma had features between these two extremes. An acute asthma attack was defined as a patient with dyspnea symptoms and audible expiratory wheeze accompanied by a 20% reduction in FEV_1. Levels of EBC eicosanoids, FEV_1, and FeNO

were measured during acute asthma attack episodes and at two weeks after the acute asthma attacks. For the management of acute asthma exacerbation, all of the asthmatic children received terbutaline inhalation and oral prednisolone 1 mg/kg/day for 3 days. In the two weeks prior to EBC collection, none of the patients took medication containing antipyretics or anti-platelet agents that would have suppressed platelet function. The healthy, non-asthmatic, and non-allergic subjects served as normal controls. This study was approved by the Humane Research Committee and informed consents were obtained from patients' parents or guardians prior to the start of the study. Additional phenotypic characterization for participants with asthma included assessment for BMI and measurement of serum IgE levels (UniCap 100, Pharmacia, Uppsala, Sweden), FEV_1, and fraction of exhaled nitric oxide (FeNO) levels.

Exhaled breath condensate (EBC) collection and exhaled nitric oxide measurement

EBC was collected after rinsing the mouth with distilled water using Turbo-Deccs system (Medivac, Parma, Italy). All participants wearing a nose clip were asked to breathe at tidal volume for 8 mins through a mouthpiece connected with a one-way valve, which also served as a saliva trap. Approximately 1 ml of breath condensate was collected and immediately stored at -80 for further analysis. Following EBC collection (after 30 minutes of rest), fractional exhaled nitric oxide (FeNO) was measured according to published standards[13]by using NIOX MINO Airway Inflammation Monitor (Aerocrine, Solna, Sweden) equipped with an electrochemical sensor.

Lung function tests and methacholine challenge tests

Following FeNO measurement, lung function tests were performed with the spirometer-Lungest 1000 (MES, Krakow, Poland) according to ERS/ATS[14] standards. These children had not received any anti-asthmatic medication, including oral β-2 agonists, theophylline, steroids, or antihistamines, for at least two days.

Measurement of eicosanoid metabolites

Utilizing established solid phase extraction approach for the collection and purification of eicosanoids in EBCs, a panel of 6 eicosanoid species derived from arachidonic acids, representing products from major enzymatic pathways, was selected for initial discovery phase of the study population consisting of 60 asthmatics and 20 healthy controls, who were randomly selected from among the study populations. Each of the selected eicosanoid species was then measured, depending upon the availability of the EBC samples. Eicosanoids were extracted from EBC with C18 Sep-Pak cartridges (Waters, Milford, Massachusetts) and concentrated as previously described[15]. The levels of eicosanoids were measured with the respective enzyme immunoassay kit (Cayman Chemical, Ann Arbor, Michigan and Neogen, Lexington, Ky) as recommended by the manufacturer. The lower limits of detection for the assays were 3.9 pg/ml for LTB_4, 7.8 pg/ml for LTE_4, 7.8 pg/ml for PGE_2, 20 pg/ml for LXA_4, 1.6 pg/ml for TXB_2, and 39 pg/ml for 15-HETE. When the level in samples was below the detection limit, the detection limit for the respective measurement was then assigned to each sample.

Statistical analysis

Statistical calculations were performed using the SPSS 19.0 (IBM Inc., Chicago, IL) software. The significance of differences between the asthmatic and healthy children in their categorical variables was estimated by the χ^2 test and continuous variables (for example, age, BMI, $\Delta\%FEV_1$) by the t test or ANOVA analyses. Receiver operating characteristic (ROC) curve with analysis of differences in the area under curves (AUC) was used to estimate the diagnostic accuracy. Furthermore, asthmatic subjects with repeated data were further divided into three groups according to the levels of eicosanoid species during exacerbation, i.e. the top 30%, middle 40%, and bottom 30%. A two-way mixed-design analysis of variance (i.e. split-plot ANOVA) was performed for analyzing the effect of stratified eicosanoid levels (top 30% vs. middle 40% vs. bottom 30%) and phases (active exacerbation vs. convalescence).

Results

Analysis of exhaled eicosanoid species for differentiating asthma from normal controls

In the discovery phase of the study, in addition to those previously reported[11], including LTB_4, LTE_4, LXA_4 and PGE_2, the level of 15-HETE, but not TXB_2, in EBCs of subjects with asthma (N=60) was significantly lower than that noted in the control group (N=20) (Data not shown). To confirm the validity of these eicosanoid species in differentiating asthma patients from normal subjects, a total of 415 children were included in the validation phase, which consisted of 318 stable asthmatic and 97 healthy subjects. The demographics of these asthmatic children and healthy children are summarized in Table 1. Significant differences were noted for age, gender, serum total IgE, FEV_1 and FeNO between the subjects in the asthmatic and the control groups (all had $p<0.001$ except for age and gender with $p<0.05$ and $p<0.01$, respectively; Table 1). No significant difference was found between these two groups for BMI. In the expanded case-control design, the levels of exhaled 15-HETE were significantly lower for asthmatic subjects than for healthy subjects ($p<0.0001$; Table 2), while the level of TXB_2 was similar between the two groups. Correlation analysis revealed that in asthmatic children, there was a significant positive correlation between the levels of TXB_2 and those of LTB_4 and PGE_2 (Supplementary Figs. S1A and S1B) in the exhaled condensate. Moreover, among the asthmatic subjects, negative correlations were found for TXB_2 and FEV_1, and also for 15-HETE and LTB_4, ($r=-0.13$, $p<0.05$; $r=-0.11$, $p<0.05$, respectively; Supplemental Figs. S1C and S1D).

When the asthmatic population was stratified into different severity groups (Table 2), it was noted that in comparison to the mild group, the moderate group was characterized with lower levels of exhaled 15-HETE, and the severe group exhibited even lower levels. The difference in 15-HETE levels between healthy subjects and all three asthmatic severity groups was significant, but no significant difference was found between groups for TXB_2. Further, as 15-HETE is known to exert inhibitory effect on 5-LOX–derived pro-inflammatory leukotrienes, the ratios of exhaled 15-HETE/LTB_4 were calculated, and the results showed that the ratio of 15-HETE/LTB_4 was significantly lower in subjects with severe asthma ($p<0.01$; Table 2). We then utilized the data of Table 2 to generate the ROC curves and calculated the AUC values for each eicosanoid species. Figure 1 shows the ROC curves and the AUC values of the analyzed eicosanoids in differentiating asthma from healthy controls. Results showed a similar discriminating power for exhaled 15-HETE, FEV_1 and FeNO (Fig. 1).

Assessment of the relationship between the levels of exhaled eicosanoids, FEV_1, and FeNO with steroid responsiveness

To assess whether the levels of exhaled eicosanoids varied during exacerbation and after convalescence, the levels of the exhaled eicosanoids, FeNO and FEV_1 in asthmatic children (N=75; Supplementary Table S1) at acute exacerbation and convalescence stages were measured. As shown in Fig. 2, while the level of FeNO was not at variance between these two stages (Fig. 2A), there was a significant enhancement in the level of FEV_1 (Fig. 2B; $p<0.001$), and 15-HETE (Fig. 2C, $p<0.001$), and a significant reduction in the TXB_2 (Fig. 2D, $p<0.05$) level, while, as a group, the levels of LTB_4, LTE_4, LXA_4 and PGE_2 did not reveal significant difference (Supplemental Fig. S2) before and after prednisone treatment. Furthermore, when the respective levels of each eicosanoid species were stratified into those at the 30th percentile, significant changes were particularly noted for those with higher initial levels of both 15-HETE and TXB_2 at the exacerbation phase (Fig. 3); also, significant decreases for exhaled LTB_4, LTE_4, LXA_4, and PGE_2were noted (all with $p<0.001$; Supplemental Fig. S3) for those at the top 30 percentile.

Discussion

In a study population consisting of 318 children with asthma, lower levels of 15-HETE were found. ROC analysis of individual parameters demonstrated similar levels of the sensitivity and specificity between exhaled 15-HETE and two commonly used parameters in monitoring asthma, FEV_1 and FeNO[16]. Further, positive correlations were found between the levels of TXB_2 and those of LTB_4 and PGE_2 in the exhaled condensate of asthmatic children. Also, among the asthmatic subjects, negative correlations were found for TXB_2 and FEV_1, and for 15-HETE and LTB_4. Among those parameters analyzed, reduced levels of TXB_2, but increased levels of 15-HETE, were noted after 3 days of oral prednisolone treatment, concomitant with the improvement of lung function in asthmatic children. When the asthmatic population was stratified into

different severity groups, it was noted that the ratio of 15-HETE/LTB_4 was significantly lower in subjects with severe asthma. Furthermore, when we investigated changes in the levels of 15-HETE and TXB_2 during exacerbation and convalescence in subjects according to the top 30%, middle 40%, and bottom 30% (as determined at the exacerbation levels), it was found that higher initial exacerbation would have responded well to prednisone treatment. These results, collectively, suggest their potential utility as a new set of lipid markers for monitoring asthma and its therapeutic outcome.

The family of eicosanoids is the most prevalent lipid mediators which contribute to inflammation providing both pro-inflammatory signals and terminating the inflammatory process. Eicosanoid profiling in the exhaled breath condensate is complementary to the cellular phenotyping of asthmatic inflammation[17]. Our findings revealed that the levels of 15-HETE were significantly reduced in the EBCs of asthmatic subjects as compared to that of healthy controls, but was increased after treatment. Kowal et al also reported that the mean concentration of 15-HETE in asthma patients was significantly lower than in healthy subjects[15]. Song et al demonstrated that 15-HETE regulated MUC5AC expression via modulating MMP-9, MEK/ERK/Sp-1, and PPARγ/PTEN/ Akt signaling pathways in PMA-treated respiratory epithelial cells[18]. While these results appear to be contradictory to those suggesting that high 12/15-LOX activity and 15-HETE levels are mainly indicative of pro-inflammatory responses in asthma [19,20]. However, 15-LOX-1 preferentially metabolizes linoleic acid to 13-hydroperoxyoctadecadienoic acid[21]. Moreover, 15-LOX1 has been reported to be less efficient than 15-LOX-2 in the production of 15-HETE[22]. Therefore, 15-HETE has been reported to be synthesized mainly by 15-LOX-2 rather than 15-LOX-1[23]. In addition, further study will be required for the identification of the role of 15-LOX-2 in airway inflammation. Besides the anti-inflammatory effects, 15-HETE has been shown to be an endogenous ligand for PPARγ (peroxisome proliferator-activated receptor gamma), which has anti-inflammatory effects such as regulating inflammatory cytokines[24], neutrophil migration and mucin secretion[18] underlying many airway diseases[25,26]. For instance, the PPARγ agonist rosiglitazone has been shown to display bronchodilator effects in a group of patients with glucocorticoids-resistant asthma[27]. The reduction of 15-HETE may, therefore, suggest its close relationship with asthma and warrant further investigation.

Moreover, as 15-HETE may exert their anti-inflammatory effect through inhibiting 5-LOX–derived pro-inflammatory leukotrienes[28,29], we also calculated the ratio of exhaled 15-HETE:LTB_4 and found significantly lower in subjects with severe asthma. The mean 15-HETE:LTB_4 ratio was 79% lower in patients with severe asthma when compared with that in patients with moderate asthma ($P < 0.01$). These findings regarding 15-HETE in EBC support data suggesting that 15-HETE biosynthetic capacity might be defective in patients with severe asthma and thus contribute to the perpetuation of airway inflammation in these patients. An additional key message derived from this study is the finding that the level of exhaled TXB_2 was significantly reduced during convalescence. TXA_2 is a lipid mediator and a bronchoconstrictor contributing to the pathophysiology of asthma[7], while TXB_2 is a stable metabolite of TXA_2. The reduction of TXB_2 levels might be indicative of steroid's effect and a marker responsive to the intervention, concomitant with improvement of lung function.

While, consistent with a previous report[30], but not the others[13,31-33], we did not find difference in the level of exhaled TXB_2 (and its metabolite, 11-dihydro-TXB_2; data not shown) between asthmatic and healthy children, but the level of TXB_2 showed significant reduction after 3 days of oral prednisolone treatment. Further, Dworski et al. found that prednisone was able to reduce the synthesis of eicosanoids, including TXB_2 level, in macrophage-rich BAL-fluid cells from 14 atopic asthmatic volunteers at baseline and after allergen instillation[34]. It is also worth noting that in double-blind, placebo-controlled trials, the thromboxane receptor antagonist, seratrodast, and the thromboxane synthase inhibitor, ozagrel, were proven efficacious in the treatment of patients with asthma[35]. However, the effect of TXA_2 inhibitors in asthma has not been widely used because no statistically significant difference was observed, but it has been suggested that it might be a good disease marker of asthma only in a certain ethnic group[36]. One explanation for these conflicting results could be phenotypically different in the study population. Nevertheless, while the level of TXB_2 may be dependent on the stage of asthma and its severity, the reduction in TXB_2 after therapy appears to be consistent. Further independent studies are needed to confirm these results. The finding

that the level of exhaled TXB_2 was significantly reduced during convalescence is significant in and of itself, providing a basis for further exploring its clinical utility in monitoring the therapeutic outcome in place of FeNO.

Furthermore, it is worth noting that LTB_4, LTE_4, PGE_2 and LXA_4 also showed reduction in patients with the respective levels at the 30% percentile, and, in fact, only in those who had higher levels of exhaled eicosanoids. This could be related to the stages of asthma progression during exacerbation, and to the phenotypic heterogeneity of asthma in the study population in terms of its etiology and pathogenic mechanism. Further investigation into this possibility is clearly required. In conclusion, these results provided insight into the measurements of exhaled eicosanoid profiles in our study population, and showed that there was a significant difference between the levels of TXB_2 and 15-HETE during acute asthma exacerbation and convalescence. Additional prospective studies are necessary to evaluate the utility of the proposed discriminator in the context of the diagnosis and monitoring of childhood asthma.

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Table 1. Demographics of study subjects.

Parameter	Asthmatic(N=318)	Healthy(N=97)	P value
Age	8.58±0.16	8.93±0.2	P<0.05
Gender (boy/girl)	205/113	59/38	P<0.01
BMI category, N(%)			
Underweight	11 (3.5%)	7 (7.2%)	
Normal	203 (63.8%)	56 (57.7%)	
Overweight	52 (16.4%)	20 (20.6%)	
Obesity	52 (16.4%)	14 (14.4%)	
Asthma severity, N(%)			
Mild	227 (71.4%)	ND	
Moderate	62 (19.5%)	ND	
Severe	29 (9.1%)	ND	
Serum total IgE	561.1±39.5	68.2±5.1	P<0.001
FEV 1%	72.3±1.0	85.2±1.2	P<0.001
FeNO,ppb	23.5±1.2	10.3±0.7	P<0.001
PC_{20}	8.6±0.6	ND	
ECP(ng/L)	30.4±10.9	ND	

Table 2. Levels of 15-HETE and TXB_2 in subjects in the validation phase and asthmatic subjects stratified by severity.

	N	15-HETE[a] mean±S.E.	P value	15-HETE/LTB_4[a] ratio	P value	TXB_2 mean±S.E.	P value
Healthy	97	209.7 ± 22.93		144.63 ± 17.93		8.13 ± 1.92	

	N	15-HETE[a] mean±S.E.	P value	15-HETE/LTB_4[a] ratio	P value	TXB_2 mean±S.E.	P value
Asthma[b]	318	72.71 ± 7.67	<0.0001	35.17 ± 4.13	<0.01	8.16 ± 1.06	NS
Mild	227	76.19 ± 9.03	<0.0001	38.92 ± 5.04	<0.01	8.21 ± 1.25	NS
Moderate	62	71.02 ± 17.4	<0.0001	34.88 ± 7.06	<0.01	8.60 ± 2.4	NS
Severe	29	49.50 ± 25.44	<0.0001	6.92 ± 2.58	<0.01	6.81 ± 3.5	NS

[a] Asthma group or each of its severity groups versus healthy control group.

[b] Among the subgroups of asthma, there are no significant differences from each other.

NS, not significant.

Figure legends

Figure 1. ROC analysis of exhaled 15-HETE, TXB_2, FeNO and Δ%FEV1.

Figure 2. Changes in the levels of FeNO, $FEV_1$and eicosanoid species during exacerbation and convalescence. The levels of (A) FeNO, (B) FEV_1, (C) 15-HETE and (D) TXB_2 during acute exacerbation (AE) and after treatment (C, convalescence) in a total of 75 children with asthma. Each line represents each individual sample. Note: All p-values were adjusted for multiple testing by Holm methods.

Figure 3. Changes in the levels of TXB_2 and 15-HETE during exacerbation and convalescence in subjects according to the top 30%, middle 40%, and bottom 30% (as determined at the exacerbation levels). F_A denotes the between-subjects main effect of stratified eicosanoid levels during exacerbation; F_B denotes the within-subjects main effect of phasic change; F_{AXB} denotes the interaction of F_A and F_B variables.

Supplemental Figure legends

Supplemental Table S1. Demographic comparison of acute asthmatics.

Supplemental Figure S1. Correlation analysis of TXB_2 with (A) FEV_1, (B) LTB_4, (C) PGE_2 and of (D) 15-HETE and LTB_4.

Supplemental Figure S2. Levels of (A) LTB_4, (B) LTE_4, (C) Lipoxin A_4 , and (D) PGE_2 in EBCs of asthmatic children at acute exacerbation and convalescence stages.

Supplemental Figure S3. Levels of LTB_4 (A), LTE_4 (B), Lipoxin A_4 (C), and PGE_2 (D) during acute exacerbation (AE) and two weeks after oral prednisolone treatment (C, convalescence) in asthmatic subjects with the respective eicosanoid levels at the 30th percentile at exacerbation. Each line represents each individual sample. Note: All p-values are adjusted for multiple testing by Holm methods. F_A denotes the between-subjects main effect of stratified eicosanoid levels during exacerbation; $F_B$denotes the within-subjects main effect of phasic change; F_{AXB} denotes the interaction of F_A and F_B variables.

Figure 1. ROC analysis of exhaled 15-HETE, TXB_2, FeNO and Δ%FEV1.

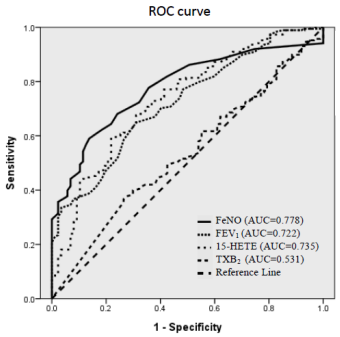

Figure 2. Changes in the levels of exhaled 15-HETE, TXB_2, FeNO and FEV_1 during exacerbation and convalescence

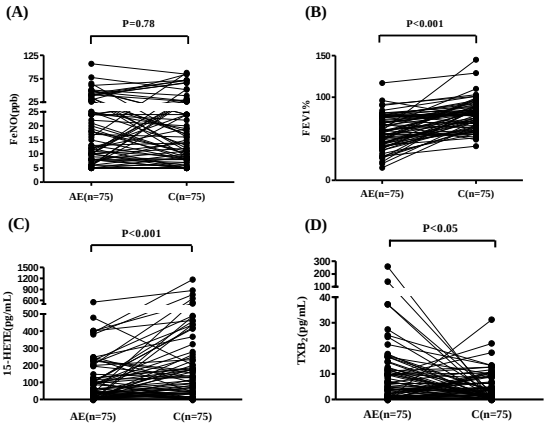

Figure 3. Changes in the levels of TXB_2 and 15-HETE during exacerbation and convalescence phases in subjects stratified by their respective levels during exacerbation.