Received: 1 April 2021 | Revised: 4 June 2021 | Accepted: 18 June 2021

DOI: 10.1111/pai.13587

ORIGINAL ARTICLE

WILEY

# Levels of 15-HETE and $TXB_2$ in exhaled breath condensates as markers for diagnosis of childhood asthma and its therapeutic outcome

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**Funding information**
Chang Gung Memorial Hospital, Grant/Award Number: CMRPG3G2051 and CMRPVVK0161; the Ministry of Science and Technology, Taiwan, Grant/Award Number: 108-2314-B-182A-088; National Health Research Institutes, Taiwan, Grant/Award Number: EO-109-PP-10

**Editor:** Ömer Kalaycı

## Abstract

**Background:** Dysregulation of eicosanoids is associated with asthma and a composite of oxylipins, including exhaled leukotriene $B_4$ ($LTB_4$), characterizes childhood asthma. While fractional exhaled nitric oxide (FeNO) has been used as the standard for monitoring steroid responsiveness, the potential utility of eicosanoids in monitoring the therapeutic outcomes remains unclear. 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 $LTB_4$, the levels of exhaled 15-hydroxyeicosatetraenoic acid (15-HETE), but not thromboxane $B_2$ ($TXB_2$), showed significant difference between asthmatics ($N$ = 318) and healthy controls ($N$ = 97), particularly the severe group showed the lowest levels of exhaled 15-HETE. Receiver operating characteristic (ROC) curve analyses revealed similar distinguishing power for the levels of 15-HETE, $FEV_1$ (forced expiratory volume in the first second), and FeNO, while the 15-HETE/$LTB_4$ ratio was significantly lower in subjects with asthma

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as compared to that of healthy controls ($p < 0.0001$). Analysis of asthmatics ($N = 75$) during exacerbation and convalescence showed significant improvement in lung function ($FEV_1$, $p < .001$), but not FeNO, concomitant with significantly increased levels of 15-HETE ($p < .001$) and reduced levels of $TXB_2$ ($p < .05$) at convalescence, particularly for those who at the top 30% level during exacerbation. Further, decreased $LTB_4$ and lipoxin $A_4$ ($LXA_4$) 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 at convalescence.

**KEYWORDS**
15-HETE, childhood asthma, exhaled breath condensates, $TXB_2$

## 1 | INTRODUCTION

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 recognized as a heterogeneous disease with varying severity, responsiveness to therapy, and appropriate treatment in early childhood can determine more positive outcomes in later life.[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 $LTA_4$ (leukotriene $A_4$) can be converted to lipoxins in the presence of 15-LOX, while 15-HETE can be generated by the 15-LOX activity.[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.[4–6] $TXB_2$ is non-enzymatically hydrolyzed from COX-derived $TXA_2$ (thromboxane $A_2$), a potent bronchial smooth muscle spasmogen,[7] and is known to inhibit the secretion of the Th1 cytokine, interferon-γ, in vitro, which may favor Th2 cell differentiation.[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 asthma and their potential utility in monitoring disease progression and treatment outcome. Indeed, several studies[6,9,10] 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 is currently lacking. 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 determined. We have previously reported that in a pediatric study population in Taiwan, the levels of exhaled $LTB_4$, leukotriene $E_4$ ($LTE_4$), $LXA_4$, and prostaglandin $E_2$ ($PGE_2$) 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 a case-control analysis of the levels of exhaled $TXB_2$ and 15-HETE and showed that their levels differed at the time of acute exacerbation and convalescence.

**Key Message**

Aberrant generation of eicosanoids is associated with childhood asthma, but their relationship with the disease status and therapeutic outcome remains to be defined. To this end, the present study discovered that as compared to the healthy controls, asthmatic patients, particularly the severe cases, showed significantly lower levels of exhaled 15-HETE during exacerbation, but its levels were significantly increased at convalescence, concomitant with reduced levels of $TXB_2$, and significantly improved $FEV_1$, but not FeNO. These findings suggest the potential utility of exhaled 15-HETE and $TXB_2$ in monitoring disease progression, therapeutic outcome, and as a target for modulation.

## 2 | MATERIALS AND METHODS

### 2.1 | Study subject

A total of 393 bronchial asthmatic children aged between 5 and 12 years, consisting of 318 stable asthmatics 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 (Prediction of Allergies in Taiwanese Children) study. 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] The diagnosis of asthma was based on the evidence of airflow obstruction such as coughing, wheezing, or shortness of breath, especially if the symptoms became worse at night or with exertion and improvement with short-acting bronchodilator; also, objective assessment consisting of the evidence of reversible airway obstruction on spirometry was made, and if any obstruction is found through spirometry, the patient was retested after 2 puffs of a short-acting bronchodilator. An improvement in $FEV_1$ > 12% was considered to be a reversible obstruction. Other causes of chronic or recurrent coughing, wheezing, or respiratory distress were excluded through a consideration of the patient's history and a physical examination. Asthma severity was categorized as mild (intermittent), moderate, and severe, based on the previously described criteria.[11] 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 during convalescence when the patients had fully recovered from the symptoms and lung function had returned to normal. 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 of the Chang Gung Memorial Hospital, and informed consents were obtained from patients' parents or guardians prior to the start of the study. Additional parameters included body mass index (BMI), serum IgE levels, $FEV_1$, FeNO, and absolute eosinophil counts measured as previously described.[13]

### 2.2 | Exhaled breath condensate collection and exhaled nitric oxide measurement

Exhaled breath condensate (EBC) was collected after rinsing the mouth with distilled water using Turbo-Deccs system (Medivac). Approximately 1 ml of breath condensate was collected and immediately stored at −80°C for further analysis. Following EBC collection (after 30 minutes of rest), FeNO was measured according to published standards by using NIOX MINO Airway Inflammation Monitor (Aerocrine).

### 2.3 | Lung function tests, methacholine challenge tests, and analysis of eicosanoids

The lung function tests were performed with the spirometer-Lungest 1000 (MES) according to ERS/ATS[14] standards. For stable asthmatics, these children had not received any anti-asthmatic medication, including oral $\beta_2$ agonists, theophylline, steroids, or antihistamines, for at least two days. The exacerbation group, unlike the stable asthmatic group, was not expected to temporarily stop anti-asthma medication. Utilizing established solid-phase extraction approach for the collection and purification of eicosanoids in EBCs, a panel of 6 eicosanoid species derived from arachidonic

**TABLE 1** Demographics of study subjects

| Parameter | Asthmatic (N = 318) | Healthy (N = 97) | t test or $\chi^2$, p value |
|---|---|---|---|
| Age (mean ± SE) | 8.58 ± 0.16 | 8.93 ± 0.2 | t = 1.3, $p = .196$ |
| Gender (boy/girl) | 205/113 | 59/38 | $\chi^2 = 7.18$, $p < .01$ |
| BMI category, *N* (%) | | | $\chi^2 = 2.57$, $p = .47$ |
| 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[a] | |
| Moderate | 62 (19.5%) | ND | |
| Severe | 29 (9.1%) | ND | |
| Comorbidity, *N* (%) | | | |
| Allergic rhinitis | 239 (75.2%) | ND | |
| Atopic dermatitis | 49 (15.4%) | ND | |
| Atopic conjunctivitis | 30 (9.4%) | ND | |
| Serum total IgE (mean ± SE) | 561.1 ± 39.5 | 68.2 ± 5.1 | $t = 12.0$, $p < .001$ |
| $FEV_1$% (mean ± SE) | 72.3 ± 1.0 | 85.2 ± 1.2 | $t = 7.9$, $p < .001$ |
| FeNO (ppb; mean ± SE) | 23.5 ± 1.2 | 10.3 ± 0.7 | $t = 9.8$, $p < .001$ |
| $PC_{20}$ (mean ± SE) | 8.6 ± 0.6 | ND | |
| ECP (μg/L; mean ± SE) | 30.4 ± 10.9 | ND | |
| Absolute eosinophil count/μL | 407.0 ± 24.9 | ND | |

*Note:* $PC_{20}$, the provocation concentration of methacholine causing a 20% $FEV_1$ fall; ECP, eosinophil cationic protein.

[a]ND, not determined.

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. Eicosanoids were extracted from EBCs[15] and measured with the respective enzyme immunoassay kit (Cayman Chemical, Ann Arbor, Michigan and Neogen) as described previously.[11]

## 2.4 | Statistical analysis

The significance of differences between the asthmatic and healthy children in their categorical variables was estimated by the chi-square test and continuous variables (eg, age, BMI, $\Delta\%FEV_1$) by the t test or ANOVAs. Receiver operating characteristic (ROC) curve with analysis of differences in the area under curves (AUC) was used to estimate the diagnostic accuracy. The Kruskal-Wallis *H* test followed by Mann-Whitney *U* test for post hoc analysis and the trend test were used for nonparametric testing of significance among healthy and asthmatics with varying severities. *p* value less than .05 was considered statistically significant. Furthermore, asthmatic subjects with repeated data were further divided into three groups according to the levels of eicosanoid species during exacerbation, that is, the top 30%, middle 40%, and bottom 30%. A two-way mixed-design analysis of variance (ie, 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).

# 3 | RESULTS

## 3.1 | Analysis of exhaled eicosanoid species for differentiating asthma from normal controls

In the discovery phase of the study, 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 and disease-related variables of these asthmatic children and healthy children are summarized in Table 1, while the allergen sensitization results (MAST or ImmunoCAP scores) and common sensitizing allergens are provided in Table S1. Among the most frequently encountered comorbid conditions associated with asthma was allergic rhinitis. Significant differences were noted for gender, serum total IgE, $FEV_{1,}$ and FeNO between the subjects in the asthmatic and the control groups (all had $p < .001$ except for gender with $p < .01$; Table 1). No significant difference was found between these two groups for age and BMI. In the expanded case-control design, the levels of exhaled 15-HETE were significantly lower for asthmatic subjects than for healthy subjects

**TABLE 2** Levels of 15-HETE and $TXB_2$ in subjects in the validation phase and asthmatic subjects stratified by severity

| | | 15-HETE | | | | | | 15-HETE/$LTB_4$ ratio | | | | | | $TXB_2$ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *p* value | | | | | | *p* value | | | | | | *p* value | | |
| Group (*N*) | | Median | Q1 | Q3 | K-W test[b] | Mann-Whitney *U* test[c] | Trend test[d] | Median | Q1 | Q3 | K-W test | Mann-Whitney *U* test | Trend test | Median | Q1 | Q3 | K-W test | Mann-Whitney *U* test | Trend test |
| Control (97) | | 168.4 | 61.2 | 260.1 | $p < .0001$ | NS[e], Mi vs. Mo | $p < .0001$ | 99.8 | 19.8 | 220 | $p < .0001$ | NS, Mi vs. Mo | NS | 4 | 0.3 | 11.6 | NS | NS, Mi vs. Mo | NS |
| Asthma severity | Mi[a] (227) | 39.8 | 0 | 120.1 | | NS, Mi vs. S | | 5.2 | 0 | 22.1 | | NS, Mi vs. S | | 2.2 | 0 | 8.9 | | NS, Mi vs. S | |
| | Mo[a] (62) | 44.4 | 0 | 110.8 | | NS, Mo | | 4.8 | 0 | 53.2 | | NS, Mo vs. S | | 2.4 | 0 | 10 | | NS, Mo vs. S | |
| | S[a] (29) | 3 | 0 | 70 | | vs. S | | 1.6 | 0 | 6.4 | | | | 1.9 | 0 | 9 | | | |

[a]Mi, mild; Mo, moderate; S, severe.
[b]K-W test, Kruskal-Wallis H test was used for nonparametric testing of significance among 4 groups, healthy control and 3 asthma severity subgroups.
[c]Mann-Whitney U test was used for post hoc analysis of difference between the severity subgroups as indicated.
[d]Trend test was used for testing of dose-response among 4 groups, healthy control and 3 asthma severity subgroups.
[e]NS, not significant.

($p < .0001$; 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 $PGE_2$ (Figure. 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 $LTB_4$, ($r = -.13$, $p < .05$; $r = -.11$, $p < .05$, respectively; Figure S1C and S1D).

When the asthmatic population was stratified into different severity groups (Table 2), results of Kruskal-Wallis test followed by Mann-Whitney U test showed that the differences in 15-HETE levels and 15-HETE/$LTB_4$ ratios between healthy subjects and all three asthmatic severity groups were significant. Also, while no significant difference in 15-HETE levels was found between different severity groups, results from the trend test showed a significant decreasing trend (normal healthy control >mild > moderate >severe asthma groups). In these analyses, no significant difference was found for $TXB_2$ among different severity groups. 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 the lowest in the severe group (Table 2), but in comparison with mild and moderate groups, no significant difference was found. 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 (Figure 1).

### 3.2 | Assessment of the relationship between the levels of exhaled eicosanoids, $FEV_1$, and FeNO during acute exacerbation and at convalescence

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 ($N = 75$; Tables S2) at acute exacerbation and convalescence stages were measured. As shown in Figure 2, while the level of FeNO was not at variance between these two stages (Figure 2A), there was a significant enhancement in the level of $FEV_1$ (Figure 2B, $p < .001$) and 15-HETE (Figure 2C, $p < .001$), and a significant reduction in the $TXB_2$ (Figure 2D, $p < .05$) level, while, as a group, the levels of $LTB_4$, $LTE_4$, $LXA_{4,}$ and $PGE_2$ did not reveal significant difference (Figure S2) during acute exacerbation and at convalescence. 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 $TXB_2$ at the exacerbation phase (Figure 3), while the levels of 15-HETE were increased in all three quartiles; also, significant decreases for exhaled $LTB_4$, $LTE_4$, $LXA_{4,}$ and $PGE_2$ were noted (all with $p < .001$; Figure S3) for those at the top 30 percentile.

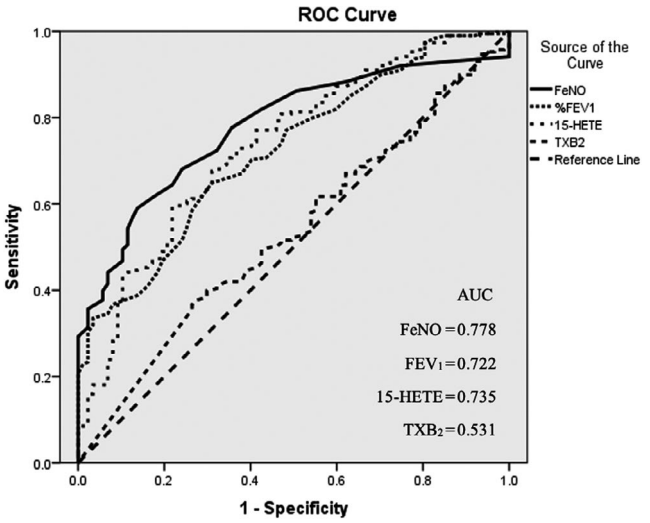


**FIGURE 1** Receiver operating characteristic (ROC) curve analysis of exhaled 15-HETE, $TXB_2$, FeNO, and Δ%FEV1. The area under curve (AUC) was generated from each curve as indicated

## 4 | DISCUSSION

In a study population consisting of 318 children with asthma, lower levels of 15-HETE were found in comparison with those in the healthy sample. In differentiating asthma from healthy controls, ROC curves 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 at convalescence, 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 level of 15-HETE was the lowest 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 those with higher initial levels during exacerbation showed significant reduction in their relative levels at convalescence. These results, collectively, suggest their potential utility as a new set of lipid markers for monitoring asthma and its therapeutic outcome, particularly considering the collection of EBCs is a relatively non-invasive means.

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 EBC is complementary to the cellular phenotyping of asthmatic inflammation.[17] Our findings revealed that the levels of

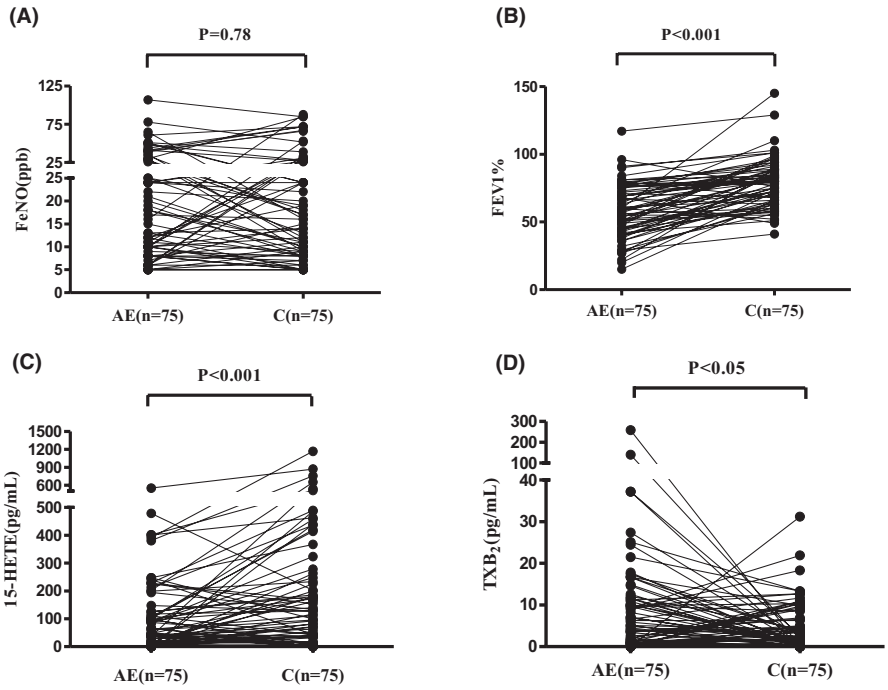


FIGURE 2 Changes in the levels of FeNO, $FEV_{1,}$ and eicosanoid species during exacerbation and convalescence. The levels of (A) FeNO, (B) $FEV_1$, (C) 15-HETE, and (D) $TXB_2$ during acute exacerbation (AE) and convalescence (C) in a total of 75 children with asthma. Each line represents each individual sample. All *p* values (paired t test) were adjusted for multiple testing by Holm methods

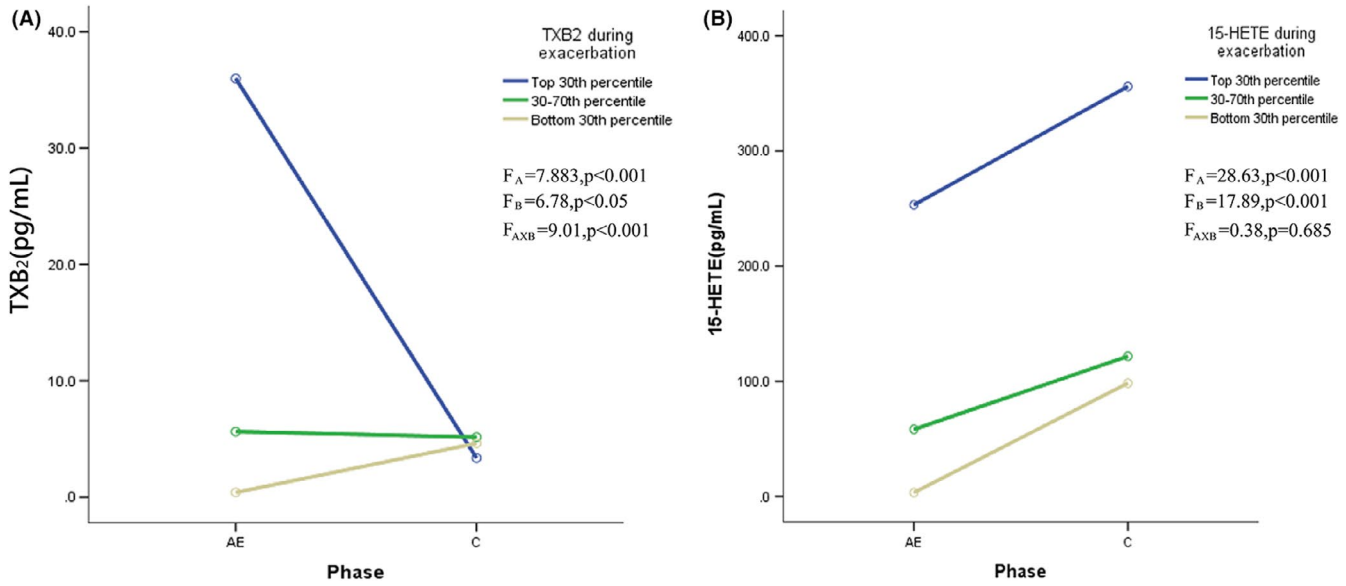


FIGURE 3 Changes in the levels of (A) $TXB_2$ and (B)15-HETE during exacerbation and convalescence in subjects according to the top 30%, middle 40%, and bottom 30% (as determined at the exacerbation levels). $F_A$ denotes the between-subjects main effect of stratified eicosanoid levels during exacerbation;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

15-HETE were significantly reduced in the EBCs of asthmatic subjects as compared to that of healthy controls, but were increased after treatment. Kowal et al also reported that 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] Also, high 12/15-LOX activity and 15-HETE levels have been suggested to be indicative of pro-inflammatory responses in asthma.[19,20] 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,[21,22] neutrophil migration, and mucin secretion.[18] For instance, the PPARγ agonist rosiglitazone has been shown to display bronchodilator effects in a group of patients with glucocorticoids-resistant asthma.[23] 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,[5] 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 ($p < .01$). These findings suggest 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. Moreover, $TXA_2$ 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 in $TXB_2$ levels might be indicative of recovery after intervention during convalescence.

While, consistent with a previous report,[24] but not the others,[13,25,26] 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 at convalescence. Further, Dworski et al. found that prednisone was able to reduce the synthesis of eicosanoids, including $TXB_2$ level, in macrophage-rich bronchoalveolar lavage fluid cells from 14 atopic asthmatic volunteers at baseline and after allergen instillation.[27] 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.[28] 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.[29] 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 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 diagnosis and monitoring of childhood asthma.

## ACKNOWLEDGMENTS

This work was supported by Chang Gung Memorial Hospital (CMRPG3G2051, CMRPVVK0161), the Ministry of Science and Technology, Taiwan (MOST 108-2314-B-182A-088), and, in part, by a grant from National Health Research Institutes, Taiwan (EO-109-PP-10).

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

## AUTHOR CONTRIBUTIONS

**Li-Chen Chen:** Conceptualization (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (equal); Project administration (equal); Resources (equal); Writing-original draft (lead); Writing-review & editing (equal). **Hsu-Min Tseng:** Data curation (lead); Formal analysis (lead); Methodology (equal); Software (lead). **Ming-Ling Kuo:** Data curation (supporting); Methodology (supporting); Writing-review & editing (equal). **Chih-Yung Chiu:** Resources (equal). **Sui-Ling Liao:** Resources (equal). **Kuan-Wen Su:** Resources (equal). **Ming-Han Tsai:** Resources (equal). **Man Chin Hua:** Resources (equal). **Shen-Hao Lai:** Resources (equal). **Tsung-Chieh Yao:** Resources (equal). **Kuo-Wei Yeh:** Resources (equal). **Ai-Hsuan Wu:** Methodology (equal); Resources (equal); Software (supporting). **Hsiu-Yueh Yu:** Methodology (equal); Resources (equal); Software (supporting). **Jing-Long Huang:** Supervision (equal); Writing-review & editing (equal). **Shau-Ku Huang:** Data curation (equal); Supervision (equal); Writing-review & editing (lead).

## PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/pai.13587.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Chen L-C, Tseng H-M, Kuo M-L, et al. Levels of 15-HETE and $TXB_2$ in exhaled breath condensates as markers for diagnosis of childhood asthma and its therapeutic outcome. *Pediatr Allergy Immunol*. 2021;32:1673–1680. https://doi.org/10.1111/pai.13587