Egyptian Journal of Bronchology

Vol. 6, No 2, December, 2012

ORIGINAL ARTICLE

PLASMA TRANSFORMING GROWTH FACTOR BETA- 1 IN ASTHMATIC CHILDREN: A NOVEL LOOK!

Dina Shokry*, Mervat Soliman**
Pediatric* and Microbiology** Departments, Zagazig University, Zagazig, Egypt

Correspondence to: Dina Shokry, E-mail: dinaa @yahoo.com

Background*: Transforming growth factor beta 1 (TGF-B1) is a multifunctional cytokine that has been implicated in the pathogenesis of asthma. It is important in transformation, tissue repair, fibrosis and modulation of inflammatory immune responses. It is postulated to perform a critical role in asthma; however it is not clear especially for growing children.*

Objective: *Our aim was to evaluate TGF-B1 levels in asthmatic children (during and between attacks) in relation to asthma severity, their atopic status and asthma inflammatory milieu.*

Subjects and methods: *This study included 70 children. They were divided into Group (1): Fifty asthmatic children (21 atopic and 29 non atopic asthmatics) and Group (2): Twenty healthy children. Estimation of TGFB-1, leucocyte, neutrophil, eosinophil counts and total IgE. Skin prick test and spirometry were done.*

Results: *TGF-B1 level was high in all asthmatic children. Non- atopic asthmatics showed higher levels than atopic ones (either during or between asthma attacks). TGFB-1 was higher inbetween attacks than during acute asthmatic attacks. Leucocyte , neutrophil , eosinophil counts and IgE level were higher in asthmatics than the control group (either during or between asthma attacks). Absolute eosinophilic count and IgE were higher in the atopic group. TGFB-1 showed significant negative correlations with both FEV1 and age of children. Nevertheless, it showed significant positive correlations with neutrophilic and eosinophilic counts.*

Conclusion: *TGF-B1 plasma level is a marker of asthma severity in younger age. TGFB-1 increase means that more fibroblasts and fibrocytes will be activated, leading more airway injurious effects in younger children.*

INTRODUCTION

TRANSFORMING GROWTH FACTOR-B1 (TGF-β1) is a multifunctional cytokine that is involved in many important biological functions.[1] Many studies have shown over expression of TGF-β1 in the airways of asthmatic individuals.[2-7] In asthmatic airways, TGF-β1 is localized to airway smooth muscle cells, as well as mucous glands, endothelial cells, and other cells infiltrating the submucosa.[8] TGF-β1 induces hypertrophy in human primary airway smooth muscle cells.[9] and both hypertrophy and hyperplasia in bovine airway smooth muscle cells.[10] These data suggest that airway smooth muscle cell may be a physiological target of TGF-β1 that might play a role in the pathogenesis of asthma. During tidal breathing, the airway smooth muscle within airway walls is continuously exposed to mechanical strain.
In particular, the airway walls in patients with asthma are exposed to excessive mechanical strain during bronchoconstriction.[11]

Thus, the cellular properties of airway smooth muscle such as contraction, stiffness, reorganization of the actin cytoskeleton, and alteration in gene expressions are influenced by mechanical strain in both normal and asthmatic conditions.[12,13] Therefore, it is important to characterize how mechanical strain triggers signal transduction in lung cells to fully understand the pathogenesis of asthma. Human airway smooth muscle cells (HASMCs) are particularly responsive to mechanical stretch as evident by observations from in vitro experiments documenting that airway smooth muscle cells respond to cyclic mechanical strain with increased proliferation, cell reorientation, protein production, reorientation of stress fibers, and additional recruitment of focal adhesions.[12,13]. However, the precise cellular and molecular signaling mechanisms that underlie TGF-β1 expression in response to stretch in HASMCs remain unknown. TGF-β is a pleiotropic cytokine with diverse effects on T-cell differentiation and immune regulation and potent anti-inflammatory functions.[14-17] In the lung microenvironment TGF-β inhibits cell proliferation, induces mucus secretion, and regulates airway fibrosis and remodeling,[18-25] all of which are hallmarks of chronic asthma. Specifically, it has been reported that TGF-β expression is elevated in bronchoalveolar lavage fluids and lung tissue of asthmatic patients.[26,27] The aim of this study was to evaluate TGF-B1 levels in asthmatic children in relation to asthma serverity, the atopic status and control of asthma whether during acute attacks or in-between attacks.

SUBJECTS AND METHODS

Study design: This case control study was carried out at Chest Unit of Pediatric Department and at Allergy and Immunology Unit of Microbiology Department at Zagazig University Hospital from September 2008 to March 2010. The study was approved by the Pediatric Department of Zagazig University Hospital Ethics Committee. Written informed consents were obtained from all participants.

Subjects: The study included 70 children; their ages ranged between 4-11 years. They were divided into 50 asthmatics and 20 healthy children as a control group. The diagnosis and severity of asthma were based on clinical symptoms and the criteria of the Global Initiative for Asthma (GINA 2010)[28]. Bahaie et al and Hoffian et al [29-30] diagnosed atopy if any of the following was found; positive family history, history of atopy to a specific allergen, positive skin prick test to one or more allergens, eosinophilia and elevated IgE level.

The asthmatic children were divided into Group (1): Thirty asthmatic children between attacks and Group (2): Twenty asthmatic children during attack. As regards to atopy they were classified into 21 atopic and 29 non-atopic asthmatic children.

Exclusion criteria were children with any significant medical conditions especially bronchial or respiratory tract infection during the month preceding the test or those receiving immunotherapy or anti-histamines in the last 3 days (for the efficacy of the skin prick test)

Interventions: The following were done to all children, history taking to find if there was positive family history of asthma, assess asthma severity and differentiate between atopic and non-atopic asthmatics. Thorough clinical examination was performed to exclude any other cause of distress as chest infection. Pulmonary function tests to prove the diagnosis and assess the severity of asthma as FEV1 ≥ 80%: intermittent or mild asthma, 60-80%: moderate asthma, <60%: severe asthma. [31] The apparatus used was master screen Viasys Health Care Gmph, Germany.

Blood investigations: total leucocytic count, neutrophil count, absolute eosinophilic count, IgE level and estimation of serum transforming growth factor beta 1 (TGF-B1). All were done in the same setting.

Allergy skin test:

The allergens used were: house dust, human hair, smoke, wool, cotton, mixed fungi, mixed pollens and hay dust.

The procedure of skin intradermal test: The skin of the volar surface of the forearm was cleaned and marked, 0.1 ml of 1/1000 dilution of each extract, negative and positive controls were injected intradermally; after15 minutes the tested sites were inspected. Fully developed erythema and wheal reactions were remeasured after 30 minutes. The mean wheal size was represented as $(X + x)/2$ (X is the maximum diameter, and x is the perpendicular diameter). Mean wheal size of greater than or equal to 3 mm was regarded as positive [32]. No antihistamines were taken in the last 48 hours prior to the test, so as not to affect the result.

Measurement of active human transforming growth factor-β1: Blood samples were taken to the serum separator tubes and stored for 30 minutes at 26°C to clot. The samples were incubated overnight at 4°C before centrifugation (1,000 x g/10 minutes). Serum levels of TGF-β1 were measured with enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (Quantikine; R & D Systems Inc., Minneapolis, MN, USA). Before the assay, latent TGF-β1 was activated to immunoreactiveTGF-β1, detectable by the Quantikine TGF-β1 immunoassay.

Aliquots of standard or activated samples were added to each well of an assay plate and the plate was incubated at 26°C for 3 hours. After aspirating/washing three times, 200 µl of conjugate was added to each well and the plate was incubated again at 26°C for 1.5 hours. Again, after aspiration and washing, 200 µl of substrate solution was added and the plate was incubated at 26°C for 20 minutes. Finally, 50 µl of stop solution were added to each well. The optical density in each well was measured within 30 minutes by using a microplate reader set to absorbance 450 nm. The minimum detectable level of TGF-β1 is 2 pg/ml [(33)].

Statistical Analysis:

Descriptive data were presented as mean ± standard deviation (X± SD),median and range or percentage (%). All statistical comparisons were performed by using the student's t test, Chi-Square test X2, Kruskal-Wallis H test, Mann-Whitney (Z test), Fisher's exact test and a Pearson correlation test. Data were checked and analyzed using Statistical Package of Social Science (SPSS) for windows version 11. P values less than 0.05 were considered significant.

RESULTS

The studied groups were age and sex matched. Mean TGFB-1 level was higher in asthmatics more than healthy children (table 1). It was higher in non-atopic asthmatics more than atopic ones (either during or between attacks) (table 2,3). The highest TGFB-1 level was measured in non-atopic between asthma attacks. Total leucocytic, neutrophilic, absolute eosinophilic count and IgE levels were statistically higher in asthmatic children than the healthy group (table 1). IgE level and absolute eoinophilic count were higher in atopic asthmatics (both during and between asthma attacks). Neutrophilic count showed the highest level in non-atopics between attacks (table 3). Severe asthma was found more in the non-atopic group during attack (FEV1 <60%) with a P value < 0.05 (table 2), but it showed no difference between atopic and non-atopic asthmatics between attacks (table 3). TGFB-1 was negatively correlated with both FEV1 and age of children. TGFB-1 showed a significant positive correlation with neutrophilic and eosinophilic count. Total WBC count showed a non-sgnificant positive correlation with TGFB-1.

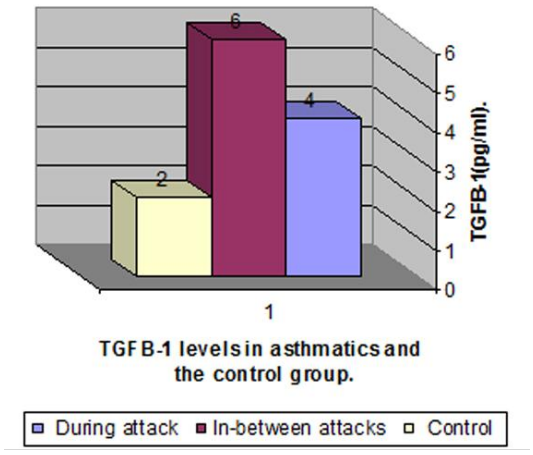

Fig 1. TGFB-1 Levels in Asthmatic Children (during and in-between attacks) and the Control Groups

Table 1. Clinical and Laboratory Data of the Studied Groups.

	Asthmatic children (n= 50)	Control group (n=20)	P
Age (yr)$	7.12 ± 2.4	6.8 ± 1.9	>0.05
Sex: Female*	21 (42%)	9 (45.5%)	>0.05
Male	29 (58%)	11 (54.5)	
TLC /mm³ $	7540 ± 690	6370 ± 960	>0.05
Absolute eosinophilic count /µl $	420 ± 45	110 ± 32	<0.05
IgE level ng/ml #	300 (100-2000)	50 (20-100)	<0.05
Neutrophilic count /mm³ #	2500 (900-5000)	1600 (435-2500)	<0.05
TGFB-1 pg/ml $	5.2 ± 1.3	2.6 ± 0.7	<0.05

TLC: total leucocyte count IgE: immunoglobulin E TGFB-1: transforming growth factor beta 1.
$: t test *: X2 test #: Z test

Table 2. Clinical and Laboratory Data in Atopic, Non-atopic Asthmatics (during attacks) and Healthy Children.

	Atopic asthmatics (n=8)	Non-atopic asthmatics (n=12)	Control group (n=20)	P
TGFB-1 (pg/ml) ¿	2.32 ± 1.4[a]	6.25 ± 1.75[b]	2.4 ± 0.25 [c]	<0.05
Neutrophilic count (/mm³) #	2100 (900-4000)[a]	4200 (2500-5000)[b]	1600 (435-2500)[c]	<0.05
Absolute eosinophilic count (/µl) ¿	450 ±35[a]	235 ± 20[b]	110 ± 32[c]	<0.05
IgE (ng/ml) #	1500 (300-2000)[a]	500 (105-920)[b]	50 (28- 96)[c]	<0.05
FEV1 < 60% »	2/8	5/12		<0.05

¿: F test (compare means ± SD) #: Z test (compare median values and ranges) »: Fisher's exact (compare proportions)
TGFB-1: transforming growth factor beta-1. IgE: immunoglobulin E FEV1: forced expiratory volume 1st second.
ab, ac, bc: significant (P < 0.05).

Table 3. Clinical and Laboratory Data in Atopic, Non-atopic Asthmatics (between attacks) and Healthy Children.

	Atopic asthmatics (n=13)	Non-atopic asthmatics (n=17)	Control group (n=20)	P
TGFB-1 pg/ml ¿	3.32 ± 2.1[a]	7.25 ± 1.95[b]	2.4 ± 0.25 [c]	<0.05
Neutrophilic count /mm³ #	2505 (900-4000)[a]	4420 (2500-5000)[b]	1600 (435-2500)[c]	<0.05
Abssolute Eosinophilic count/µl ¿	327 ±46[a]	196 ± 22[b]	110 ± 32[c]	<0.05
IgE ng/ml #	1240 (300-2000)[a]	390 (100-920)[b]	50 (20- 100)[c]	<0.05
FEV1< 60% »	2/13	2/17		>0.05

¿: F test (compares means ± SD) #: Z test (compares median values and ranges) »: Fisher's exact (compares proportions)
TGFB-1: transforming growth factor beta-1. IgE: immunoglobulin E FEV1: forced expiratory volume 1st second.
ab, ac, bc: significant (P < 0.05).

Table 4. Correlation between TGFB-1 and (FEV1, Age, Total Leucocytic, Eosinophil and Neutrophil Counts).

TGFB-1	R	P
FEV1(%)	-0.65	<0.05
Age (yr)	-0.57	<0.05
Total leucocytic count /mm³	0.15	>0.05
Eosinophilic count /µl	0.29	<0.05
Neutrophil count /mm³	0.32	<0.05

TGFB-1 : transforming growth factor beta1 FEV1 : forced expiratory volume 1st second.
P >0.05 : non-significant P <0.05 : significant

DISCUSSION

Asthma is a chronic inflammatory disease of the airways characterized by Th2 type inflammation. Pathological repair of chronic inflammation may lead to structural alteration in the airways that are called airway remodeling. TGF-β belongs to a family of growth factors that has an important role in wound healing and fibrogenesis. It promotes differentiation of fibroblasts into myofibroblasts.(34) Our study was conducted on 50 asthmatic children (20 during attack and 30 between attacks), according to atopy, they were (21 atopic and 29 non-atopic) and 20 healthy children as control group. All the groups were age and sex matched. In our study mean TGFB-1 level was higher in asthmatics than the control group. Another study on asthmatics reported that TGF-β was increased and associated with submucosal inflammatory cells, including fibro-blasts, smooth muscle cells, eosinophils, macro-phages and the connective tissue of the airway, with variable expression in the epithelial cells. (35) Non-atopic group showed the highest TGF-B1 level compared to the atopic and the control groups. We can explain this by the theory which suggests that all asthmatics are atopic, and the non-atopic group are atopic to unknown allergen, so they show a more severe form of asthma, as they do not avoid the causative agent,(36) so more remodeling occurred in those patients, and consequently higher TGFB-1 level. Lommatzsch et al.(37) have found that levels of serum TGF-β1 in atopic asthmatic adults were not different from control subjects. This may be due to weaker and less remodeling process in adults than children. (36) The ability of the tissues for reforming, healing and synthesizing in adults is less than children. Another study found that TGF-β1 was increased and participated in the pathogenesis of asthma. (37) In our study TGFB-1 was increased between asthma attacks, this may be due to continuous airway remodeling after the acute attack of asthma which is a part of the inflammatory reaction secondary to poor asthma control.(38) The same was reported by many other studies.(21,23,24) Neutrophilic count was increased in asthmatics more than healthy children. Non-atopic asthmatics had the highest neutrophilic count than atopic ones and than the control group. Chu et al. (19) reported higher level of neutrophils and TGF-B1 positive cells in airway tissue of asthmatics compared to normal subjects. They concluded that higher levels of TGF-B1 expression-release from asthmatic neutrophils indicated that neutrophils might be involved in the airway remodeling process of asthmatic subjects. Other studies reported increased neutrophilic count in asthma and its relation to TGF-B1 production.(39,40) In our study, absolute eosinophilic count and IgE were significantly increased in atopic asthmatics during the attack of asthma than between attacks. These results agreed with Ohno et al. (4) who reported that atopic asthma showed eosinophilia and high IgE level. TGFB-1 levels were higher in severe asthmatics. TGFB-1 showed higher levels with a decrease in FEV1. Expression of TGF-β has been shown to correlate with fibroblast numbers, sub-epithelial thickening and severity of asthma. (35) Boxall et al. (41) reported that TGFB-1 was positively correlated with asthma severity. In our study a negative correlation between age and TGFB-1 was found. This means that, the younger the age of the child, the higher the level of TGFB-1 level. Herr et al. (42) reported that asthma is less severe in young age due to better remodeling process. We also found a positive correlation between eosinophilic count and TGFB-1 level. Yap et al. (43) reported the same results. Bacci et al. (44) found a correlation between the increased expression of TGF-B1 in asthmatic airways and an increased number of infiltrated eosinophils. On the other hand, Ricciardolo et al.(45) found that eosinophilic count showed no significant correlation to TGFB-1 level. In our study, neutrophil count showed a positive correlation with TGFB-1. Choi et al.(39) reported that neutrophils had a role in production of TGFB-1. Uddin et al.(40) found a close relation between TGFB-1 and neutrophils and described neutrophils as the precursors of TGFB-1.

CONCLUSION

In conclusion, TGFB-1 is increased in asthmatic children. It is significantly associated with asthma severity and young age. As it has a role in remodeling process of the airways seen in bronchial asthma after the acute attack, increased

TGFB-1 levels reflect injurious effects on the airways, especially in younger children. This result spotlights a novel robust marker for destructive effects of airway remodeling in younger asthmatic children.

REFERENCES

1. Blobe GC, Schiemann WP, Lodish HF. Role of transforming growth factor-β1 in human disease. N Engl J Med. 2000;342:1350–8.

2. Minshall EM, Leung DY, Martin RJ, et al. Eosinophil-associated TGF-beta1 mRNA expression and airways fibrosis in bronchial asthma. Am J Respir Cell Mol Biol. 1997;17:326-33.

3. Nomura A, Uchida Y, Sakamoto T, et al. Increases in collagen type I synthesis in asthma: the role of eosinophils and transforming growth factor-β. Clin Exp Allergy. 2002;32:860–5.

4. Ohno I, Nitta Y, Yamauchi K, , et al. Transforming growth factor beta 1 (TGF beta 1) gene expression by eosinophils in asthmatic airway inflammation. Am J Respir Cell Mol Biol. 1996;15:404–9.

5. Redington AE, Madden J, Frew AJ, et al. Transforming growth factor-beta 1 in asthma. Measurement in bronchoalveolar lavage fluid. Am J Respir Crit Care Med. 1997;156:642–7.

6. Takizawa H, Tanaka M, Takami K, et al. Increased expression of transforming growth factor-beta1 in small airway epithelium from tobacco smokers and patients with chronic obstructive pulmonary disease (COPD). Am J Respir Crit Care Med 2001; 163: 1476–1483.

7. Tillie-Leblond I, Pugin J, Marquette CH, et al. Balance between proinflammatory cytokines and their inhibitors in bronchial lavage from patients with status asthmaticus. Am J Respir Crit Care Med. 1999;159:487–94.

8. Vignola AM, Chanez P, Chiappara G, et al. Transforming growth factor-beta expression in mucosal biopsies in asthma and chronic bronchitis. Am J Respir Crit Care Med. 1997; 156:591–9.

9. Goldsmith AM, Bentley JK, Zhou L, et al. Transforming growth factor-beta induces airway smooth muscle hypertrophy. Am J Respir Cell Mol Biol. 2006;34:247–54.

10. Black PN, Young PG, Skinner SJ. Response of airway smooth muscle cells to TGF-β1: effects on growth and synthesis of glycosaminoglycans. Am J Physiol Lung Cell Mol Physiol. 1996;271:L910–7.

11. Wiggs BR, Hrousis CA, Drazen JM, et al. On the mechanism of mucosal folding in normal and asthmatic airways. J Appl Physiol. 1997;83:1814–21.[PubMed]

12. Seow Chun Y, Schellenberg RR, Pare Peter D. Structural and functional changes in the airway smooth muscle of asthmatic subjects. Am J Respir Crit Care Med. 1998;158:179S–86S.

13. Smith PG, Janiga KE, Bruce MC. Strain increases airway smooth muscle cell proliferation. Am J Respir Cell Mol Biol. 1994;10:85–90.

14. Izcue, A., J. L. Coombes, and F. Powrie**.** Regulatory T cells suppress systemic and mucosal immune activation to control intestinal inflammation. Immunol. Rev.2006;212**:**256-71.

15. Li, M. O., and R. A. Flavell. TGF-beta: a master of all T cell trades. Cell. 2008;134:392-404.

16. Moore, B., R. F. Murphy, and D. K. Agrawal. Interaction of TGF-beta with immune cells in airway disease. Curr. Mol. Med. 2008;8:427-36.

17. Taylor, A., J. Verhagen, K. Blaser, et al. Mechanisms of immune suppression by interleukin-10 and transforming growth factor-beta: the role of T regulatory cells.Immunology. 2006;117:433-42.

18. Boland, S., E. Boisvieux-Ulrich, O. Houcine, et al. TGF beta 1 promotes actin cytoskeleton reorganization and migratory phenotype in epithelial tracheal cells in primary culture. J. Cell Sci. 1996;109:2207-19.

19. Chu, H. W., S. Balzar, G. J. Seedorf, et al. Transforming growth factor-beta2 induces bronchial epithelial mucin expression in asthma. Am. J. Pathol. 2004;165:1097-106.

20. Cohn, L., J. A. Elias, and G. L. Chupp. Asthma: mechanisms of disease persistence and progression. Annu. Rev. Immunol. 2004;22:789-815.

21. Howat, W. J., S. T. Holgate, and P. M. Lackie. TGF-beta isoform release and activation during in vitro bronchial epithelial wound repair. Am. J. Physiol. Lung Cell. Mol. Physiol. 2002;282:L115-23.

22. Kelly, M., M. Kolb, P. Bonniaud, et al. Re-evaluation of fibrogenic cytokines in lung fibrosis. Curr. Pharm. Des 2003;9:39-49.

23. Moses, H. L., E. Y. Yang, and J. A. Pietenpol. TGF-beta stimulation and inhibition of cell proliferation: new mechanistic insights. Cell 1990;63:245-7.

24. A. B. Roberts, M. B. Sporn, R. K. Assoian, et al. Transforming growth factor type beta: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. Proc. Natl. Acad. Sci. USA.1986;83:4167-71.

25. Ten Dijke, P., M. J. Goumans, F. Itohet, al. Regulation of cell proliferation by Smad proteins. J. Cell Physiol. 2002;191:1-16.

26. Doherty, T., and D. Broide. Cytokines and growth factors in airway remodeling in asthma.Curr. Opin. Immunol. 2007;19:676-80.

27. Minshall, E. M., D. Y. Leung, R. J. Martin, et al. Eosinophil-associated TGF-beta1 mRNA expression and airways fibrosis in bronchial asthma. Am. J. Respir. Cell Mol. Biol. 1997;17:326-33.

28. GINA 2010, p. 3. www.ginasthma.org.

29. Bahaie NS, Hosseinkhani MR, Ge XN, et al. Regulation of eosinophil trafficking by SWAP-70 and its role in allergic airway inflammation. J Immunol. 2012;1;188(3):1479-90.

30. Hoffjan S, Nicolae D, Ober C. Association Studies for Asthma and Atopic Diseases: A Comprehensive Review of the Literature. Respir Res.2003;4;4:14.

31. Reddel HK, Vincent SD, Civitico J. The need for standardization of peak flow charts Thorax. 2005;60(2):164-7.

32. Llanora GV, Ming LJ, Wei LM, et al. House dust mite sensitization in toddlers predict persistent wheeze in children between eight to fourteen years old Asia Pac Allergy. 2012;2(3):181-6.

33. Fedulov AV, Ses TP, Gavrisheva NA, et al. Serum TGF-beta 1 and TNF-alpha levels and cardiac fibrosis in experimental chronic renal failure. Immunol Invest. 2005;34:143-52.

34. Tomasek JJ, Gabbiani G, Hinz B, et al. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev Mol Cell Biol. 2002;3:349-63.

35. Howell JE, McAnulty RJ. TGF-beta: its role in asthma and therapeutic potential. Curr Drug Targets. 2006;7:547-65.

36. Chen Y, Rennie DC, Pahwa P, et al. Pulmonary function in adults with recent and former asthma and the role of sex and atopy.. BMC Pulm Med. 2012;29;12:32.

37. Lommatzsch M, Schloetcke K, Klotz J, et al. Brain-derived neurotrophic factor in platelets and airflow limitation in asthma. Am J Respir Crit Care Med. 2005;171:115-20.

38. Wiparat Manuyakorn, Wasu Kamchaisatian, Kalayanee Atamasirikul, et al. Asian Pacific Journal of Allergy and Immunology. 2008;26:185-9.

39. Choi JS, Jang AS, Park JS, et al. Role of neutrophils in persistent airway obstruction due to refractory asthma. Respirology. 2012;17(2):322-9.

40. Uddin M, Nong G, Ward J, et al. Prosurvival activity for airway neutrophils in severe asthma. Thorax. 2010 Aug;65(8):684-9.

41. Boxall C, Holgate ST, Davies DE. The contribution of transforming growth factor-beta and epidermal growth factor sig-nalling to airway remodelling in chronic asthma. Eur Respir J 2006;27:208-29.

42. Herr M, Just J, Nikasinovic L, et al. Risk factors and characteristics of respiratory and allergic phenotypes in early childhood. J Allergy Clin Immunol. 2012;130(2):389-96. e4. doi: 10.1016/j.jaci.2012.05.054.

43. Yap E, Chua WM, Jayaram L, et al. Can we predict sputum eosinophilia from clinical assessment in patients referred to an adultasthma clinic? Intern Med J. 2011 Jul 25. doi: 10.1111/j.1445-5994.2011.02565.x. [Epub ahead of print]

44. Bacci E, Latorre M, Cianchetti S, et al. Transient sputum eosinophilia may occur over time in non-eosinophilic asthma and this is not prevented by salmeterol. Respirology. 2012 Aug 10. doi: 10.1111/j.1440-1843.2012.02242.x. [Epub ahead of print]

45. Ricciardolo FL, Di Stefano A, Silvestri M, et al. Exhaled nitric oxide is related to bronchial eosinophilia and airway hyperresponsiveness to bradykinin in allergen-induced asthma exacerbation. Int J Immunopathol Pharmacol.2012;25(1):175-82