BRONCHOALVEOLAR LAVAGE FLUID CYTOKINES AND CHEMOKINES CHANGES AFTER BRONCHIAL THERMOPLASTY

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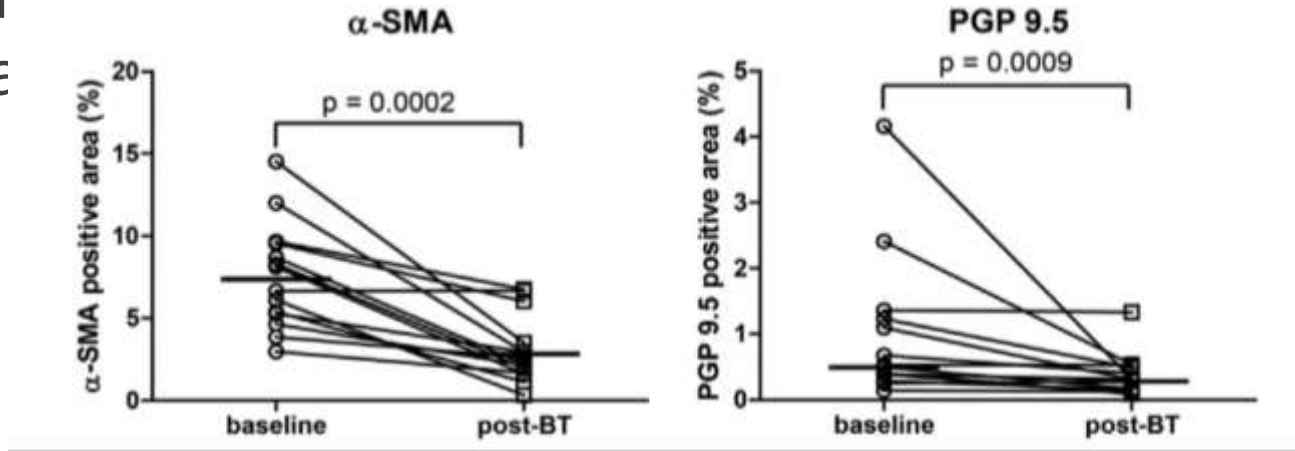
BACKGROUND AND RATIONALE

- BT had been proven effective in treating moderate-severe asthma patients based on several non-randomized trial and randomized trials including AIR, RISA, and AIR2 trial
- Despite its well-known effect of ASM reduction in airway remodeling, other potential mechanisms of action involved in extracellular matrix, airway epithelium, neural innervation could also underlie its effectiveness in controlling airway inflamma



BT reduced **neural innervation** in the airways

Cox G et al. N Engl J Med. 2007;356:1327-37
Pavord ID et al. Am J Respir Crit Care Med. 2007;176:1185-91
Castro M, et al. Am J Respir Crit Care Med. 2010;181:116-24
Ichikawa et al. Respiratory Medicine 150 (2019) 165-172

BACKGROUND AND RATIONALE

- Only few studies, that investigated the effect of BT on inflammatory mediators, have provided conflictive results
- Denner et al.
  - Only acute effect on local airway inflammation was examined
  - Should be carefully interpreted because of the potential effect of corticosteroids
- Our study aimed to investigate the immediate and sustained effects of BT on local airway inflammation in terms of the changes in concentration of BALF cytokines/chemokines over time after BT

Denner et al. Ann Am Thorac Soc 2015

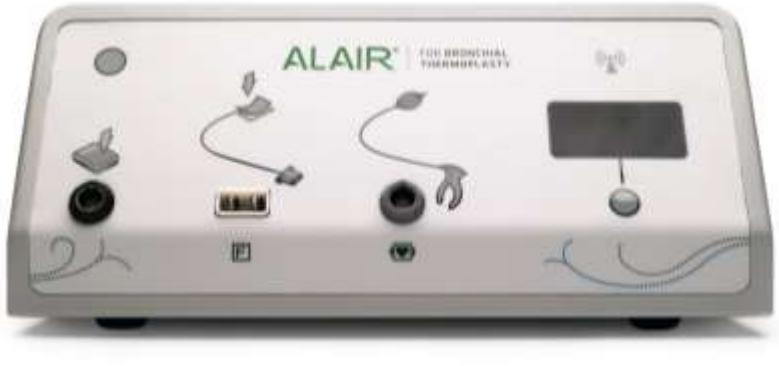
RESEARCH QUESTION

- **Primary research question**
  - Can BT alter the BALF cytokines and chemokines in moderate to severe uncontrolled asthma in acute and long-term period ?

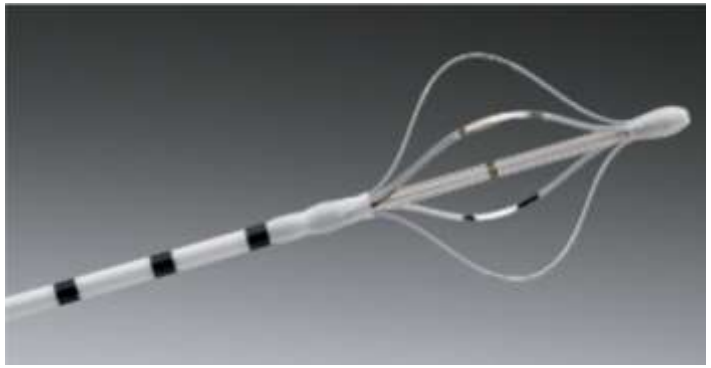
- **Secondary research questions**
  - Can BT improve asthma symptom score in moderate to severe uncontrolled asthma ?
  - Can BT improve lung funtion test in moderate to severe uncontrolled asthma ?

METHODS

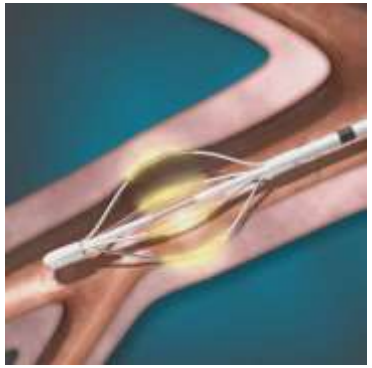
- **Design**: prospective observational study
- **Population**: severe uncontrolled asthma characterized by step 4 & 5 based on GINA
- **Intervention**: Bronchial thermoplasty*
- **Comparison**: Pre- (baseline) VS post-bronchial thermoplasty (3 weeks, 15



Alair Bronchial Thermoplasty controller



Alair BT catheter



BT

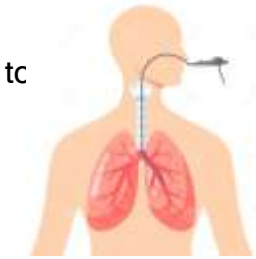
*according to current recommendation with Alair Bronchial Thermoplasty System (Boston Scientific, Natick, MA, USA)

METHODS

2-4 weeks before BT		Before 2nd BT		12 weeks after BT
1st BALF	5-7 weeks	2nd BALF	15 weeks	3rd BALF

Prednisolone 50 mg/day 3 days prior to BT

BALF collection*
ACT, AQLQ
Lung function test
FeNO



BALF collection*
ACT, AQLQ
Lung function test
FeNO

BALF collection*
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FeNO

*BAL was performed with a total volume of 100-150 ml of NSS and then collect the BALF return by suction specimen trap

Each BALF sample from the different timepoint was collected from the same location (lateral segment of right lower lobe)

STUDY OUTCOMES

Primary outcome

- The change in level of cytokines/chemokines in BALF* before and after BT

Secondary outcomes

- The change in asthma symptom questionnaire before and after BT
  - Asthma Control Test (ACT) (higher score represents better asthma control)
  - Asthma Quality of Life Questionnaire (AQLQ) scores (higher score represents better QOL)
- The change in lung function test before and after BT

***BALF processing: Bio-Plex Pro Human Cytokine T3-plex Assay kit and TGF-β (BioRad) using Luminex assay**

IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13. IL-17, RANTES, TNF)-α, TRAIL, TGF-β1, GM-CSF, and IFN-γ

Fifty microliters of 4-fold diluted samples were used for the assay according to the manufacturer's instructions
Samples were measured by Bio-Plex 200 Systems (BioRad)

STATISTICAL ANALYSIS

- Continuous variables were reported as mean (SD) and median(IQR)
- Categorical variables were reported as number of patients and percentages
- Non-normal distribution outcomes at various time points: **GEE model** to estimate the parameters of a generalised linear regression (natural log transformation to adjust the non-normally distributed data for GEE analysis)
- GEE was computed with mean values, SE, and Wald test for each independent variable
- Variables with a p-value of less than 0.05 were considered statistically significant

RESULT: BASELINE CHARACTERISTICS

Characteristics	Variables
Number of patients (n)	10
Age	56 (46.75-63)
Sex (Male/Female) (n)	5/5
Medication use, % (n)	
ICS /LABA	10 (100%)
LAMA	8 (80%)
OCS	4 (40%)
Leukotriene receptor antagonist	9 (90%)
Omalizumab	1 (10%)
Blood eosinophil count, cells/μL	230 (87.5-365)
Serum IgE level, kU/L†	187 (64-282)

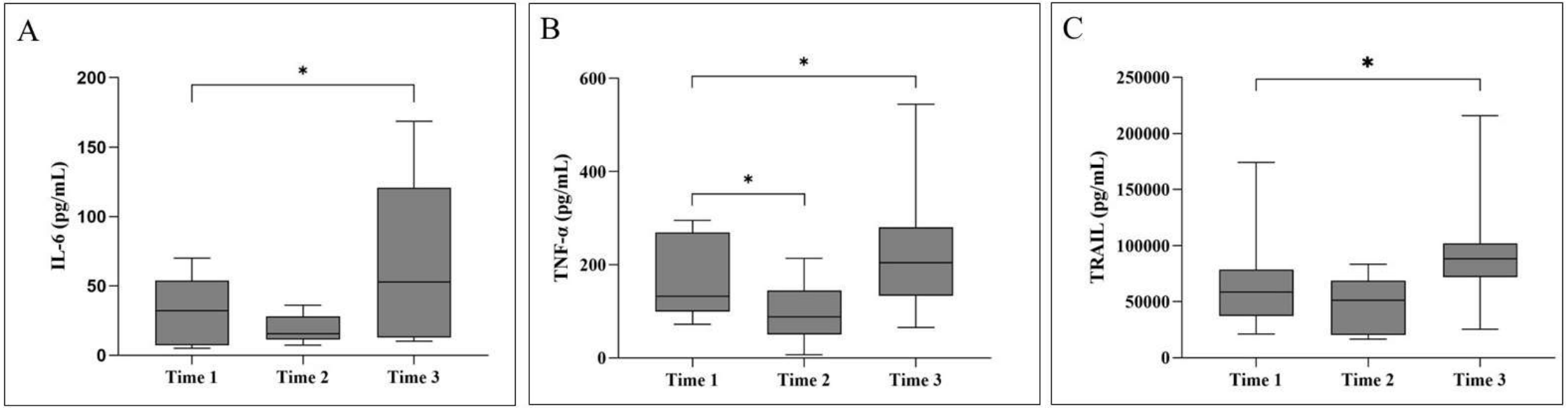
Definition of abbreviations: ICS = inhaled corticosteroids; LABA = long-acting b2-agonist; LAMA = long-acting muscarinic antagonist; OCS = oral corticosteroids. Data are presented as median (interquartile range) unless otherwise noted. †data were available in 7 patients.

RESULT: PRIMARY OUTCOME

BAL	Pre-BT	After 1st BT	12 weeks after 3rd BT
IL-2	ND	ND	ND
IL-4	ND	ND	ND
IL-5	ND	ND	ND
IL-6	31.858 (23.35)	18.226 (9.59)	66.43 (59.52)
IL-10	ND	ND	ND
IL-12	ND	ND	ND
IL-13	ND	ND	ND
IL-17	ND	ND	ND
GM-CSF	ND	ND	ND
IFN-γ	ND	ND	ND
RANTES	4,546.33 (6216.28)	5,261.98 (6376.22)	5,485.87 (8802.91)
TNF-α	162.712 (84.57)	94.7 (59.98)	224.06 (135.38)
TRAIL	66,450.37 (44921.39)	47,233.97 (26324.87)	93,711.29 (49382.72)
TGF-β1	22,287.588 (24205.75)	24,178.73 (13594.48)	23,012.66 (20290.91)

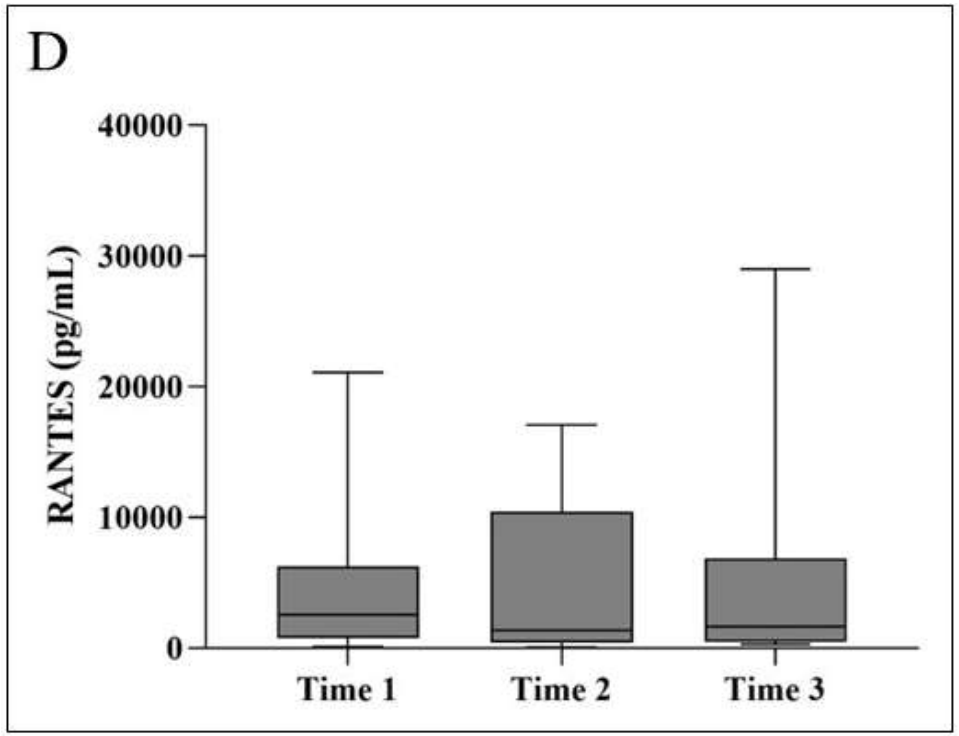
Definition of abbreviations: ND = not detected. Data are presented as mean ± SD.

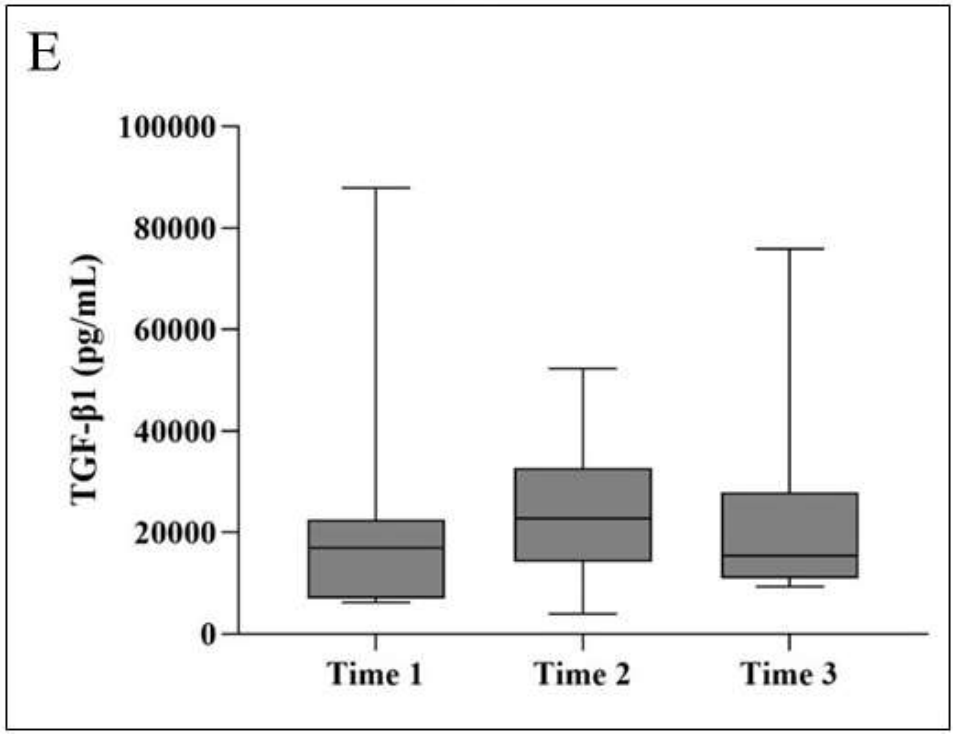
RESULT: PRIMARY OUTCOME



- GEE model to estimate the parameters of a generalised linear regression and determine the interaction between 2 groups over 3 timepoints, while adjusting for possible effects of age, sex, eosinophil, and IgE level
- Natural log transformation to adjust the non-normally distributed data for GEE analysis

RESULT: PRIMARY OUTCOME





- GEE model to estimate the parameters of a generalised linear regression and determine the interaction between 2 groups over 3 timepoints, while adjusting for possible effects of age, sex, eosinophil, and IgE level
- Natural log transformation to adjust the non-normally distributed data for GEE analysis

RESULT: SECONDARY OUTCOMES

Values	Before BT	12 weeks after BT	p-value
ACT	13.6 (3.27)	19 (4.44)	0.004
AQLQ	3.93 (0.88)	5.3 (0.99)	0.002
Pre-BD FEV1 (% pred)	74.6 (21.59)	78.8 (18.57)	0.385
Post-BD FEV1(% pred)	80.7 (21.93)	83.5 (16.91)	0.529
RV/TLC	40.78 (8.8)	41.88 (7.30)	0.503
FeNO, ppb†	30.78 (21.93)	44.44 (41.14)	0.358
ICS (BDP or equivalent)	1180 (502.88)	1160 (556.17)	0.937
LABA (formoterol or equivalent)	25.8 (8.96)	26.4 (7.58)	0.678

Data are presented as mean ± SD unless otherwise noted. †data were available in 9 patients.

DISCUSSION

- BT improved asthma symptoms but **had no significant effect on long-term local airway inflammation** before and 12 weeks after BT based on …
  - Significant improvement of ACT and AQLQ
  - Non-significant change of TNF-α, IL-6, TRAIL, RANTES, TGF- β1 level in BALF

- Previous studies
  - Denner et al. : significant reduction of RANTES and TGF- β1 in BALF
  - **Wijsman et al.** : no differences in cytokines before and after treatment
    (Only ECP, MPO, VEGF-a, Eotaxin 2/3, GRO-α, CXCL-10, CXCL-8 and MIF were consistently detectable)
  - **Ichikawa et al.** : BT reduces ASM mass and airway innervation without affecting production of inflammatory mediators in airways
    (TGF-β1 and IL-17 expressions in airway)

Ichikawa et al. Respiratory Medicine 2019

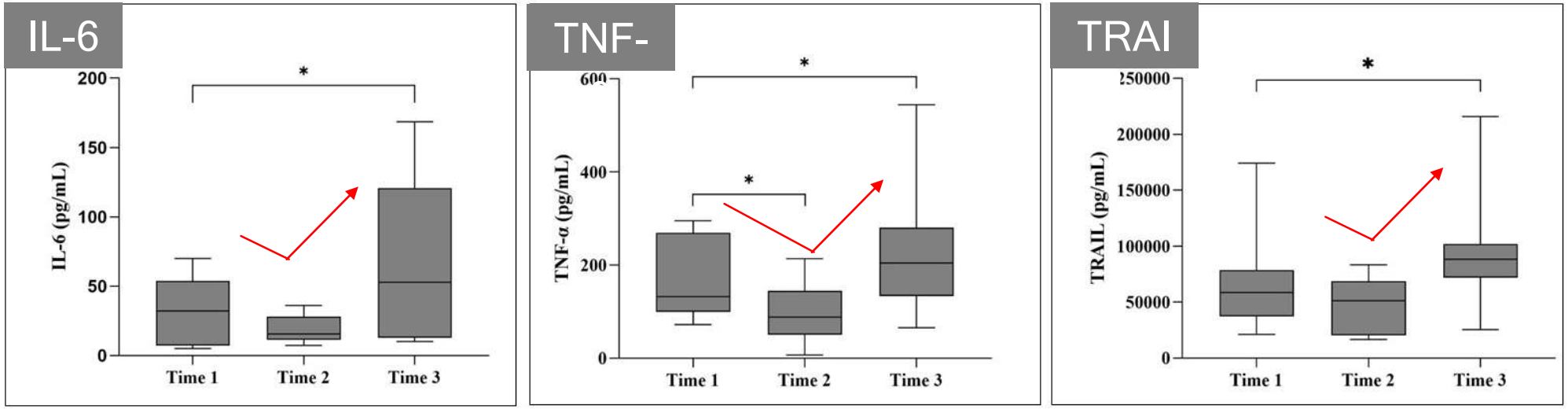
Wijsman et al. Journal of Asthma and Allergy 2022

DISCUSSION

- Denner et al. study
  - Significantly reduced level of RANTES at week 3 and 6 (with pretreatment OCS)

Denner et al. Ann Am Thorac Soc 2015

- In our study, the change of cytokines level in BALF…
  - The effect of pretreatment OCS
  - The early effect of BT



DISCUSSION

- Denner et al. study: BT significantly reduced TGF-β1 in BALF after 6 weeks suggests the potential to down-regulate inflammation and fibrosis in the first weeks after BT

  Denner et al. Ann Am Thorac Soc 2015

- Our findings of non-significant change of TGF-β1 suggests that
  - TGF-β1 in BALF might not correlate with ASM reduction and airway remodeling
  - The source of TGF-β1 in BALF could be from other structures within the airways

- In my opinion, the reduction in TGF-β1 in BALF could not clearly indicate the reduction of airway fibrosis but should concern the improvement of airway

LIMITATIONS

- Single-centre study and a small number of participants
  - It is believed that the majority of asthma patients were non-Th2 phenotype
- The heterogeneity of the asthma phenotype in our patients could affect a high variability of the cytokines and chemokines level in BALF in our data set
- Only five analytes had consistently been detectable during the study period, which may be limited in interpreting the effect of BT on overall airway inflammation

CONCLUSIONS

- BT did not have a significant effect on local airway inflammation, even a significant clinical improvement could be demonstrated in our study

- The clinical benefit of BT may not be clearly explained by the effect on local airway inflammation, and other mechanisms should be further investigated